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Poster Presentations

P-1A-1

Historical evolution of weight at birth in a primary health attention unit in Sao Paulo, Brazil

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Objectives: Comparing exclusive maternal nursing time of children who were born between 2000 and 2008 with individuals who were born between 1977 and 1987 (current adults) seen at a School Health Center (Centro Saúde Escola –CSE) of FMUSP since birth.
Methods: Information was obtained from two flows of data collection. The retrospective data on adults who were users of the CSE enrolled and supervised by the service since childhood were obtained from the charts. Information of the children who were enrolled in the service was obtained from the electronic data bank of the local pediatric service. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: 631 charts of adults and 884 of children were evaluated. The results obtained both on the adults and the children groups are described on the table below.
Conclusions: When comparing the groups, it was noticed that the same profile of weight at birth could be observed on the individuals studied in the 70's and 80's and on the first 8 years of this century, which is the same as saying that it has been stable for approximately 30 years, maybe due to the influence of similar external environment. Weight at birth epidemiological studies are used in health evaluation of newborns and adults, being related to cardiovascular diseases, hypercholesterolemia, obesity, diabetes mellitus, arterial hypertension, and depression[1,2]. Weight at birth is the result of a series of environmental, genetic and socioeconomics factors. Aggravation that may lead to an alteration of pregnancy duration, and velocity of inside of uterus growth may determine fetus alterations that will lead to permanent metabolic alterations and their consequences in adult life.

Weight at birth	Adults n (%)	Children n (%)
<2 kg	17 (2.7)	28 (3.2)
2–2.5 kg	53 (8.4)	48 (5.4)
2.5–4 kg	538 (85.3)	774 (87.5)
>4 kg	23 (3.6)	34 (3.6)
Total	631 (100)	884 (100)

Chi Square: 5.411, $p > 0.05$.

1. D.J. Baker *et al.*, *Lancet,* 341:938–41, 1993.
2. C.N. Hales, D. Barker. *Br Med Bull.,* 60:5–20, 2001.

P-1A-2

Historical evolution of exclusive maternal nursing time in a primary health attention unit in Sao Paulo, Brazil

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Objectives: Comparing exclusive maternal nursing time of children that were born during 2007 and 2008, to individuals born between 1977 and 1987 (current adults) seen at a School Health Center (Centro Saúde Escola –CSE) of FMUSP since birth.
Methods: Information was obtained from two flows of data collection. The retrospective data of adults who were users of CSE enrolled and supervised by the service since childhood were obtained from the charts. Information on children who were enrolled in the service was obtained from the electronic data bank of the local pediatric service. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: 405 charts of adults and 436 of children were evaluated. Values of duration of exclusive maternal nursing were evaluated on both groups. The results obtained both on the adults and children groups are described on the table below.

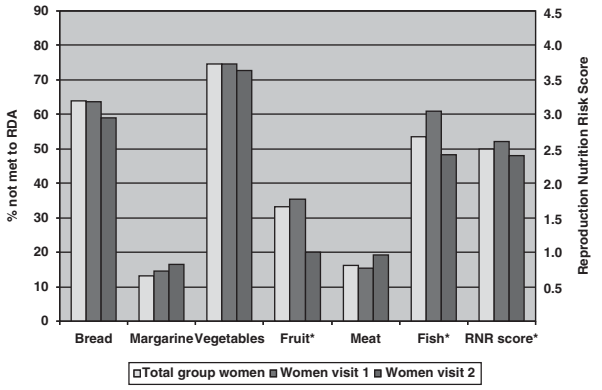
Nursing time	Adults n (%)	Children n (%)
<1 month	193 (47.7)	58 (13.3)
1 to 3 months	148 (36.5)	197 (45.1)
3 to 6 months	56 (13.8)	88 (20.2)
>6 months	8 (2)	93 (21.3)
Total	405 (100)	436 (100)

Chi Square: 157.2858, $p < 0.05$.

Conclusions: When comparing the groups, it was noticed that there has been a significant improvement on exclusive maternal nursing time in studied individuals in the same Primary Attention service on Sao Paulo city that were born in two distinctive decades, namely the 70's and 80's and the years 2007–2008. Therefore, it is observed that there was an important improvement in health orientations given to mothers, which certainly contributed to health promotion, especially in countries with deprived population. Exclusive maternal nursing time influences ideal growth, child's cognitive development and protection against infections;

reduces child mortality, the risk of allergies development, sudden death and protects against adult chronic diseases[1,2]. It is necessary that we assist women and children's health, approaching the various factors that lead to current low nursing index: women having a job, contraception and new family structures. Above all, especially in developing countries, there is the need to improve the approach of said problem with new public policies for maternal nursing.

1. D.A. Lawor *et al.*, *Arch Dis Child.,* 90: 582–588, 2005.
2. S. Arenz *et al., Int J Obes.,* 10:1247–1256, 2004.



P-1A-3

Preconception counselling on nutrition and folic acid use

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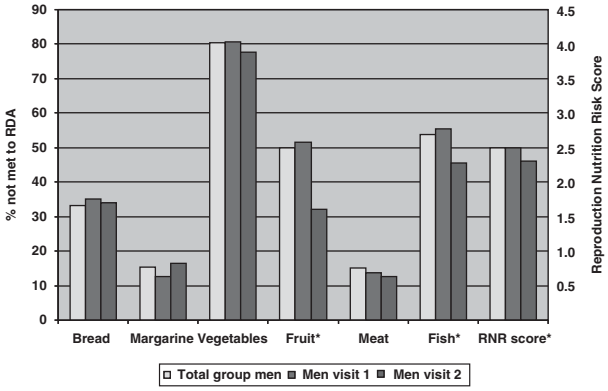

Objective: Maternal malnutrition is implicated in subfertility, miscarriages, congenital malformations, and intrauterine growth restriction (IUGR). These reproductive failures all originate in the periconceptional period when gametes and embryonic tissues are most vulnerable to environmental factors. Therefore, changing unhealthy nutritional behaviours preconceptionally in parents-to-be is important to improve general health, reproductive performance and pregnancy outcomes. Our objective was to evaluate the preconceptional nutritional intake of couples planning pregnancy and to investigate whether preconception nutritional counselling increases healthy nutritional behaviours.

Methods: Between October 2007 and April 2009 couples planning pregnancy were invited for two preconception counselling visits for screening and advice on nutrition and folic acid use at the outpatient clinic of Obstetrics and Gynaecology, Erasmus MC, Rotterdam, in the Netherlands. Questionnaires were filled out by the couple at home and checked during the visit. We defined a reproduction nutritional risk score (RNR-score) with a maximum total RNR-score of 6, based on 6 questions about the intake of food groups according to the recommended daily intakes. The Wilcoxon signed rank test was used to analyse the significance between paired continous variables, the Mc Nemar test for paired dichotomous variables and the Chi-Square for non-paired categorical variables.

Results: 419 couples visited the preconception counseling clinic once and a subgroup of 110 couples came twice with a fixed time interval of 3 months in between. At the first visit women and men had a mean age of 31 years (range 19–44) and 32 (22–63), 53.2% and 58.5% were from Dutch origin and 22.2% and 28.2% were low educated, respectively. The low compliance of 26% for the second visit was due to the fact that couples were already very satisfied about the first counseling and therefore less motivated to come a second time. In 99.1% of the women and 97.3% of the men the nutritional intakes were not according to the recommendations. An improvement, however, could be established in the couples who came for the second visit, i.e., women 93.6%, men 94.5%. Women significantly increased the consumption of fruit from 64.5% to 80.0% ($p < 0.001$) and fish from 39.1% to 51.8% ($p < 0.001$), and folic acid use increased from 67.3% to 84.5% ($p < 0.001$). Men only significantly increased fruit consumption from 48.5% to 68.0% ($p < 0.001$). The RNR-score decreased significantly from 2.6 to 2.4 in women and from 2.5 to 2.2 in men (both $p < 0.05$) in men.

Conclusion: This data confirms the very high prevalence of unhealthy nutritional behaviors in couples planning pregnancy in a Western country. We emphasize that the period of planning pregnancy should be used as 'a window of opportunity' to change unhealthy nutritional behaviors by individualized preconception counseling. Future studies should corroborate on the predictive value of the RNR-score for reproductive performance, and beyond.

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P-1A-4

Secular trends of birth outcomes in Southern Brazil

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Objective: Low birth weight (LBW) and prematurity have been considered risk factors for infant morbidity and mortality, affecting patterns of health and disease during the life course. The aim of this study was to evaluate secular trends of LBW, preterm births and their contributions for intrauterine growth restriction rate in the city of Porto Alegre, a large city in a developed area in Southern Brazil during 15 years.
Methods: This is a registry-based study. Data were obtained from birth certificates of all live births in the city from 1994 to 2008. The birth weight and gestational age were analyzed among the total of newborns. Linear regression was performed in order to determine secular trends of LBW and preterm birth rates. Appropriate institutional ethics committee clearance was obtained.
Results: A total of 312,662 singleton newborns were delivered in the city during the period, with a steady reduction in the total number of live-births of 23.5%. The results showed significant increase in LBW and preterm births rates from 8.7% to 9.2% and from 7.0% to 9.8%, respectively ($P = 0.015$; $P < 0.001$). Meanwhile, there were significant reductions in LBW rates among term births from 4.8% to 3.8% and among preterm births from 60.7 to 58.4%.
Conclusions: These results show that Southern Brazil is going through a demographic and epidemiologic transition characterized by a significant decrease in the number of live-births. We also observed an increase in LBW due to a significant rise in preterm birth rates associated with a reduction in the intrauterine growth restriction rate, mainly among term newborns. This scenario may lead to changes in adult patterns of health and disease in the future. Supported by CNPq.

P-1A-5

Nutrient supplementation in pregnancy: development of evidence-based best-practice guidelines

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Background: Nutrient supplementation in lead up to, and during, pregnancy is common in both Western and developing countries. However, evidence-based guidelines on the use of nutritional supplements to optimise pregnancy, birth and postpartum outcomes are needed.
Objectives: 1. To derive from published literature data on the safety and efficacy of nutrient supplementation in lead up to, and during, pregnancy. This includes single and combination supplements, containing macro- and/or micro-nutrients, 2. To construct a set of evidence-based national nutritional guidelines on an appropriate use of supplements for pregnancy in Australia, and 3. To disseminate the findings to the relevant interest groups including health professionals and consumer groups, primarily women of childbearing age.
Methods: The Cochrane Library and PubMed were searched for previously published meta-analyses and recent systematic reviews (from 1999 onwards) using the key search terms 'pregnancy', 'supplement', and 'nutrient'. Hand searching of reference lists was also undertaken. The review of reviews was then translated into evidence-based best-practice guidelines, using the Appraisal of Guidelines for Research and Evaluation Instrument[1].
Results: To date, 16 meta-analyses for a range of macro- and micro-nutrients have been identified, and the data extracted. These include data for energy and protein, long-chain polyunsaturated fatty acids (marine oil), combination micro-nutrient preparations, the antioxidants (vitamins A, C, E, β-carotene, lycopene, and selenium, either singularly or in combination), vitamins D, folate, vitamin B6 (pyridoxine), iron, calcium, magnesium and zinc. Of these, folate remains the only ubiquitously recommended nutrient for supplementation before and during pregnancy, because of the major relative risk (RR) reduction in neural tube defects (0.28; 95% confidence intervals (CI) 0.13, 0.58)[(2)]. This is despite possible risks associated with an increased incidence of multiple births (RR 1.4; 95% CI 0.93, 2.11)[2]. Prophylactic calcium supplementation may offer benefits in high-risk cases of preeclampsia (RR 0.48; 95% CI 0.33, 0.69), particularly where dietary intake is poor[3]. Iron supplements improve haematological markers, but users also frequently experience adverse gastrointestinal disturbances (RR 1.90; 95% CI 1.09, 3.33), and other clinically important outcomes are not improved[4].
Conclusions: With the exception of folate, nutrient supplementation before and/or during pregnancy, is not generally recommended, unless dietary intake is inadequate. This is because of significant deficits in the available evidence for their safety and efficacy. Further work is needed to synthesise the best of the available evidence and to then develop a set of clear guidelines for appropriate use. Given that this is a widespread practise, advice on nutrient supplementation should be integrated into standard antenatal care.

1. The Agree Research Trust, http://www.agreetrust.org/instrument.htm, accessed 18 June 2008.

2. J. Lumley *et al., Cochrane Database Syst Rev*., 3:CD001056, 2001.
3. G.J. Hofmeyr *et al., Cochrane Database Syst Rev,* 3:CD001059, 2006.
4. L. Reveiz *et al., Cochrane Database Syst Rev.,* 2:CD003094, 2007.

P-1A-6

Risk factors for low birth weight at a School Health Center in Sao Paulo, Brazil

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Objective: This study aimed to identify the following variables related to pregnancy : maternal age, education, desire of pregnancy, parity, prenatal care (including time of onset and number of consultations); associated morbidity, weight gain and smoking, besides the type of delivery and sex of children and their relationship with the incidence of low birth weight (less than 2500 g).
Methods: All children under one year of age who were attended in the Health Center School since January to December 2007, were included in the study. SPSS version 16.0 was used for statistical analysis. Chi-Squared and Mann-Whitney tests had been employed to select the composing variables of the final statistics model. Subsequently, the multi-alternated Logistic Regression model was applied to the analysis of the final results to compose a predictive model of risk of low birth weight. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The casuistic was composed by 383 children. The low birth weight rate was 6.3%. Factors associated to low birth weight were: under five prenatal appointments, maternal smoking, weight gain under 8 kg and hypertension during pregnancy. Variables of study present during pregnancy: maternal age, maternal educational level, pregnancy desire, moment in which the mother began the prenatal follow up and parity didn't show any significant statistical relationship with low birth weight. Variables related to the conditions surrounding these children's births: type of childbirth and gender didn't present a significant statistic relationship with low birth weight. Using the technique of Logistic Regression, a predictive model for low birth weight was composed. For example: in a mother who held up to 5 pre-natal consultations, who smoked during pregnancy, won less than 8 kg during pregnancy and is hypertensive, the probability of a child with low weight is 87.8%.
Conclusions: The identification of risk factors for low birth weight is very important to determine health and public actions directed to improve maternal conditions, and to children at risk, aiming to reduce the morbidity rates related to low birth weight. The birth weight is a complex process; the result of a series of factors of biological, social and environmental origin. Identifying those risk factors that intervene significantly in birth weight means one step further towards implementing actions directed at the prophylaxis of such events, especially when it comes to maternal conditions and to the development of strategies aiming the prevention and controlling of future illnesses to the level of health assistance services and regarding children in risk situations[1].

1. B.F. Araújo, A.C. Tanaka. *Cad Saúde Pública*. 23:2869, 2007.

P-1A-7

Weight at birth and maternal nursing time in a primary health attention unit in Sao Paulo, Brazil

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Objective: Comparing total maternal nursing time on newborns with proper and low birth weight and supervised at *Centro de Saúde da Faculdade de Medicina da Universidade de São Paulo.*
Methods: The casuistic was composed of 263 children that were born between January 2008 and September 2009, being excluded from the study those who were still been nursed by their mothers or the ones who were never breast fed. All data concerning the children are kept in an electronic data bank. Data regarding the children's weight at birth and maternal nursing time were obtained from this data bank. Mothers' answers to questioning about the total in months (m) of maternal nursing time and child's weight at birth were extracted for analysis. Children with weight $\leqslant$2.500 grams were considered newborns of low birth weight (LBW). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The information found in the 263 analyzed charts has shown 24 LBW (9.1%). Total maternal nursing time and weight at birth are described on the table below.
Conclusions: LBW nursing time was significantly different from the nursing time of children born with proper weight. Low birth weight newborns breast feed for a shorter period of time. Health policies must, therefore, direct efforts to encourage maternal nursing, especially low birth weight newborns. Nursing during the first months of life is fundamental for the health and well-being of the individual during all their lives, for it protects against many diseases, promotes growth and child development and prevents adult chronic diseases. In this sense, health professionals should

engage in the support programs, encouraging parents of LBW in the nursing process. Weight at birth is the result of complex processes of biological, social and environmental order that interfere in the genetic potential of the individual. Low birth weight newborns' vulnerability demands maternal milk as the best nutritional condition possible to provide factors that may benefit them on the medium and long term consequences[1].

	0 to 3 m	> 3 m to 6 m	> 6 m to 12 m	12 m or more
< or = 2500 g	10	4	9	1
> 2500 g	117	45	30	47
Total (263)*	127	49	39	48

*Chi-Square: 9.45, $p < 0.05$.

1. M.T. Joca *et al.*, *Escola Anna Nery Revista de Enfermagem.*, 9:356–364, 2005.

P-1A-8

Historical evolution of birth body mass index on users of a School Health Center in Sao Paulo, Brazil

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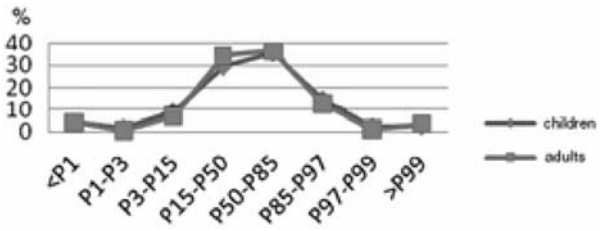
Objectives: Comparing body mass index (BMI) at birth of children who were born between 2007 and 2008 with individuals who were born between 1977 and 1987 (current adults) seen since birth at the School Health Center (Centro de Saúde Escola – CSE) of FMUSP, according to the referential proposed by the World Health Organization (WHO)[1].
Methods: Data collection related to the identification of the individual, date of birth, gender, weight and length. Such information was obtained according to two flows of data collection. Retrospective data for adults who were users of the CSE and were enrolled and supervised since childhood was obtained from the charts. Information on the children who were enrolled on the service was extracted from an electronic data bank of the local pediatric service. The Quetelet index or BMI (Weight at birth(kilograms)/Length(meters)^2) of both groups of users was done, with the results of each group distributed according to the percentiles (P) described in the BMI curve of the World Health Organization, stratified in: <P1; P1-P3; P3-P15; P15-P50; P50-P85; P85-P97; P97-P99 and >P991. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Data were obtained on weight and length at birth of 168 adults, 118 women and 51 men, and 787 children, 401 girls and 386 boys. The values of BMI at birth were verified on the groups of adults and children, respectively, being distributed as follows: <P1[7(4.2%) and 33(4.2%)]; P1-P3[1(0.6%) and 14(1.9%)]; P3-P15[12(7.13%) and 75(9.5%)]; P15-P50[58(34.5%) and 230(29.2%)]; P50-P85[61(36.3%) and 279(35.4%)]; P85-P97[21(12.5%) and 115(14.6%)]; P97-P99[2(1.2%) and 19(2.4%)], and >P99[6(3.57%) and 22(2.8%)], according to the graphic below.



Conclusions: When comparing both studied groups (adults and children), a similarity was noticed on the BMI at birth profiles, with the curves dislocated to the right, for more elevated percentiles, in relation to the WHO curve. Therefore, during the thirty years that were studied there was no significant alteration on the weight and length pattern of the studied population. The BMI epidemiologic study, recently validated for children between zero and 36 months of age, defines the nutritional state and proportionality of the newborn, with the possibility of being related to adult chronic diseases[2,3].

1. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards. World Health Organization, 2006.
2. J. Karlberg *et al.*, *Acta Paediatr.*, 92:648–652, 2003.
3. D.J. Baker *et al.*, *Lancet,* 341:938–941, 1993.

P-1A-9

Preconception counselling on modifiable lifestyle risk factors The Rotterdam Reproduction Risk Score (R^3 Score)

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Objective: Periconceptional lifestyle factors play a significant role in reproduction[1–3]. Here, we show the first intervention results of a special clinic for preconception counselling in terms of modified lifestyle risk factors in couples planning pregnancy.
Methods: One hundred and ten couples planning pregnancy visited twice the preconception counselling clinic for screening

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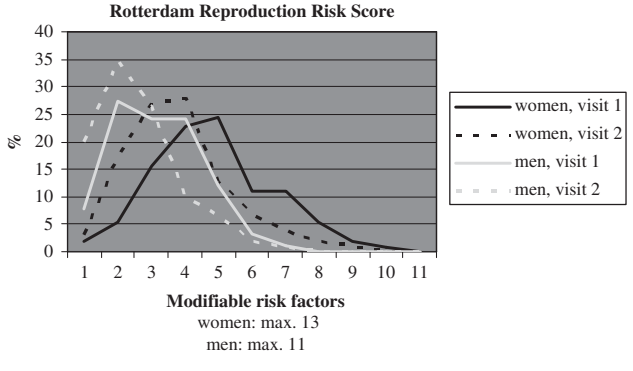
and advice on healthy and harmful lifestyle factors at the outpatient clinic of Obstetrics and Gynaecology, Erasmus MC, Rotterdam, in the Netherlands. Questionnaires and the open accessible website www.zwangerwijzer.nl (in Dutch) were filled out by the couple at home. During the counselling visit the questionnaires were checked, a physical examination was performed and advises were provided to modify lifestyle risk factors, to improve healthy behaviors and to increase health literacy. The risk factors considered were the use of medication, tobacco, recreational drugs, alcohol, absent folic acid use (women only), lack of physical activity, caffeine intake ⩾6 cups/day, BMI <20 or ⩾30, hypertension (systolic ⩾160 mmHg and/ or diastolic ⩾90 mmHg), waist circumference (women ⩾88 cm, men ⩾102 cm), waist-to-hip ratio (>0.8) and risk of infection by toxoplasmosis and listeriosis. Each risk factor was scored with one or two points (literature based) and a total score was computed, i.e., the Rotterdam Reproduction Risk Score (R^3 Score, score in women 0–13 and men 0–11). The individual risk factors and R^3 Score were assessed at the two visits and compared and tested using Wilcoxon signed rank test.

Results: In women and men a reduction of around 30% in the R^3 Score was observed at the second visit 3 months after the first visit for preconception counseling (women 3.9 vs. 2.8; $p = 0.000$, men 2.3 vs. 1.6; $p = 0.000$). Major reductions were observed in alcohol consumption (women 35.5% vs. 20.9%; $p = 0.0001$, men 60.2% vs. 46.6%; $p = 0.003$), increased folic acid use (women 76.3% vs. 84.5%; $p = 0.0001$), increased physical activity (women 24.5% vs. 38.8%; $P = 0.002$, men not significant) and reduced infection risk (women 41.8% vs. 7.3%; $p = 0.000$).

Conclusion: The preconception period is a window of opportunity for counselling on modifiable lifestyle risk factors in couples planning pregnancy. Future studies should corroborate on the predictive value of the R^3 Score on reproductive performance, and beyond.



1. M.A.M. Hassan, S.R. Killick. *Fertil Steril.,* 81:384–392, 2004.
2. G.F. Homan *et al., Hum Reprod Update*, 13:209–223, 2007.
3. R.E. Chapin *et al., Environ Health Perspect*., 112:69–78, 2004.

P-1A-9B

Relation of maternal hypertension with infant growth: the ABCD study

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Objective: To investigate the association of pre-existent and pregnancy-induced hypertension with the offspring's weight and length gain in the first 14 months of life. We hypothesized that hypertension during pregnancy is an independent determinant of growth acceleration in the offspring.

Methods: Prospective community-based cohort study of 8266 pregnancies (Amsterdam Born Children and their Development, ABCD study). 3994 women from the original cohort completed questionnaires (both prenatal and postnatal) obtaining information about pre-existent and pregnancy-induced hypertension which was replenished by information from the obstetric caregiver. Anthropometry in the offspring was followed during the first 14 months of life. The main outcome measures were presence or absence of growth acceleration in weight or length, respectively (growth acceleration: $\Delta SDS > 0.67$ vs. absence of growth acceleration: $\Delta SDS \leq 0.67$). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Pre-existent hypertension was significantly related to growth acceleration in weight and length. After correction for the intermediating variables birth weight and pregnancy duration, the association remained significant for growth acceleration in weight (OR 1.89; 95%CI 1.21–2.97; $p < 0.01$). Pregnancy-induced hypertension showed similar results, although correction for birth weight and pregnancy duration rendered the associations non significant. In stratified analyses by birth weight (standardised birth weight ratio below vs. above average), the aforementioned relations were established in the below average birth weight group. This is illustrated in the figure presenting raw data. It demonstrates the modifying effect of standardized birth weight on the association between maternal hypertension and accelerated weight gain: only in the below average birth weight group a synergistic effect of maternal hypertension on accelerated weight gain was present.

Conclusions: Infants of women with pre-existent hypertension more frequently have growth acceleration in weight and length during pregnancy, yet the mechanisms acting on postnatal growth in weight and length appear to be different. In contrast to pre-existent hypertension, the association between pregnancy-induced hypertension and accelerated

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growth appears to work entirely through a reduction in birth weight and pregnancy duration. Financial support was granted by ZonMw.

P-1B-10

Systemic leptin antagonist inhibits hypothalamic leptin signal transduction in new born rat pups

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Objective: Leptin, produced by the adipose tissue, plays a pivotal role in hypothalamic appetite regulation. Leptin mediates central anorexigenic signalling responses via JAK-STAT3 pathway by binding to its receptor (ObRb) with subsequent phosphorylation of STAT3 with negative feed-back inhibition by endogenous inhibitor, SOCS-3. Using a rat model, we have shown that maternal food restriction results in growth restricted newborns that develop hyperphagia and hyperleptinemia prior to adult obesity. It is likely that inhibition of increased leptin mediated effects early in life may alter the sensitivity of the JAK-STAT3 signal transduction and prevent obesity. Thus, we investigated the effects on hypothalamic leptin signaling using a recombinant rat pegylated leptin antagonist (L39A/D40A/F41A).
Methods: 1 day old male control pups, matched for body weights received either (i) saline, (ii) leptin (10 μg/g, s.c), (iii) pegylated leptin antagonist (PEG-MLA, 20 μg/g, s.c) or (iv) leptin plus PEG-MLA. Hypothalamus was dissected from individual pups at 30, 45 and 60 minutes. Protein expression of ObRb, STAT3, pSTAT3 and SOCS3 was analyzed (Western Blot). 4 pups per group were studied and data is compared to saline treatment.
Results: As expected, leptin treatment up regulated JAK-STAT3 signalling pathway at 30 mins: ObRb (2-fold), STAT3 (2-fold), pSTAT3 (1.5-fold) and SOCS3 (3-fold). Further at 45 and 60 mins, pSTAT3 was down regulated (0.5-fold) whereas all other signal molecules continued to be up regulated. In contrast, leptin plus PEG-MLA showed no change in any of the signal molecules except for pSTAT3 which was down regulated at 45 and 60 mins (0.4 and 0.5-fold, respectively). PEG-MLA treatment showed similar changes as PEG-MLA with leptin.
Conclusions: The findings suggest that systemically administered PEG-MLA effectively blocks leptin signal induction of hypothalamic JAK-STAT. It is likely that PEG-MLA impairs binding of leptin to its ObRb receptor and inhibits leptin signaling. Leptin-specific antagonists may assist in understanding the underlying mechanisms contributing to a programmed or diet induced obesity and provides a potential therapeutic intervention strategy.

P-1B-11

Ethnic differences in overweight at age 2: The role of prenatal factors, birth outcome and postnatal factors

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Objective: Childhood overweight/obesity is a major public health problem worldwide which disproportionally affects specific ethnic groups. So far, little is known whether these differences already exists at early age and what factors contribute to these ethnic differences. Therefore, the present study assessed possible ethnic differences in overweight at the age of 2 years and the explanatory role of prenatal factors, birth outcome and postnatal factors.
Methods: Data were derived from a large population-based cohort study: the Amsterdam Born Children and their Development (ABCD) study. Pregnant women filled out an extensive, multi-lingual, questionnaire around the 16th week of pregnancy. Pregnancy outcome and growth data (weight and height) during the first 2 years of life of 3120 singleton infants were available from the Youth Health Care Registration in Amsterdam. Eight ethnic populations were distinguished: Dutch (n = 1608), Surinamese-Hindustani (n = 57), Surinamese-Creole (n = 123), Antillean (n = 41), Turkish (n = 171), Moroccan (n = 261), Ghanaian (n = 57), and other non-Dutch countries (n = 800). Overweight status at age 2 was defined by the International Obesity Task Force (IOTF) age and gender specific guidelines. We assessed the explanatory role of prenatal factors (maternal age, height, parity, diabetes, hypertension, pre-pregnancy-BMI, maternal education, smoking habits, alcohol use and physical activity of the mother all during pregnancy), birth outcome (birth weight, gestational age and gender) and postnatal factors (weight gain in the first 6 months and duration of exclusive breastfeeding). Multivariate logistic regression was used to analyse the data. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Prevalence of overweight was significantly higher in the Turkish (19.3%), Moroccan (16.9%) and Ghanaian (17.5%) children (reference Dutch: 7.2%). Pre-pregnancy-BMI of the mother was associated with overweight of the child and partly contributed to the ethnic differences. Nevertheless, the risk of being overweight remained increased in Turkish (OR: 2.43;

95% CI: 1.42–4.15) Moroccan (OR: 2.09; 95% CI: 1.29–3.40) and Ghanaian (OR: 2.58; 95% CI: 1.16–5.70) children. Most of the ethnic differences were accounted for by weight gain during the first 6 months of life. This factor reduced odds ratios for most ethnic minority groups, however the risk was still increased for the Turkish (OR: 1.78; 95% CI: 1.01–3.13) and Moroccan (OR: 1.70; 95% CI: 1.03–2.83) children. Of all other factors, only birth weight affected overweight but did not explain ethnic differences.

Conclusions: Turkish, Moroccan and Ghanaian children in the Netherlands have a 2 to 3 fold higher risk of being overweight, which is partly explained by maternal pre-pregnancy BMI. The most important explanatory factor was weight gain during the first 6 months of life. In contrast to ethnicity and maternal pre-pregnancy BMI, infants' weight gain might be eligible for intervention. More research is needed to the underlying factors of this early weight gain to tackle ethnic differences in overweight in children. Supported by the Netherlands Organisation for Health Research and Development (ZonMw).

P-1B-12

Growth from birth to adulthood and abdominal obesity in a Brazilian birth cohort

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Rapid weight gain in childhood may increase the risk of chronic adult diseases. Few studies have examined the effects of lifecourse weight gain on waist circumference (WC), hip circumference (HC) or waist-to-hip ratio (WHR).

Objective: To evaluate the effects of birthweight and weight gain from birth to age 23y on WC, HC and WHR in young adults.

Methods: Population-based birth cohort study. Individuals born in 1982 in Pelotas (southern Brazil) were visited on a number of occasions from birth to age 23–24y. Assessment of the three dependent variables (WC, HC and WHR) was carried out in a sample 856 subjects in 2006 (442 males, 414 females). Weight gain from birth to early adulthood was evaluated by using a conditional weight model in each period (0–2, 2–4, 4–15, 15–18/19 and 18/19–23y). Conditional growth analyses were carried out with adjustment for confounders (family income at birth, maternal variables – education, skin colour, height, BMI before the pregnancy, smoking in pregnancy – and gestational age). WC and HC were also mutually adjusted. Multiple linear regressions were used in adjusted analyses, all stratified by sex. Stata 9.0 was used for analysis. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Weight gains during all age ranges studied were positively associated with WC and HC in both sexes. These effects were strongest from the 4–15y range ($\beta = 5.0$ cm for both circumferences; $P < 0.001$ in all the cases). Proxies for visceral adipose tissue (WHR, and WC adjusted for HC) were associated with weight gain after 2y in females ($\beta = \sim 1.0$ cm and $P < 0.01$) and after 4y in males ($\beta = \sim 0.7$ cm and $P < 0.05$). Subcutaneous adipose and muscular tissues, assessed by HC adjusted for WC, were associated with birthweight and weight gain from 0–2y in both sexes ($\beta = 0.5$ cm for males and 1.0 cm for females; $P < 0.05$), and again with weight gains from 4–18y in males ($\beta = 0.5$ cm; $P < 0.05$) and 4–15y in females ($\beta = 1.7$ cm; $P < 0.001$). Interactions between weight gain and baseline nutritional status were found for girls only. Rapid weight gain from 2–4y had a stronger effect on WC adjusted for HC, as well as on WHR, for girls born with intrauterine growth restriction (IUGR) than for those without IUGR (P for interaction = 0.007). For the 0–2y period, there was no such interaction. Likewise, rapid weight gain from 2–4y had a stronger effect on these outcomes among girls who were stunted at 2y than for those who were not stunted (P for interacion = 0.09).

Conclusions: Weight gains in utero and in the first two years had long term effects on HC, but weight gain after age four years was strongly associated with WC. Weight gains up to age 2 years may reduce cardiovascular risk associated with adult fat patterns in a middle-income setting. This study was partially funded by The Wellcome Trust. The initial phases of the cohort study were supported for the PRONEX, the Brazilian Ministry of Health, International Development Research Centre of Canada, and the United Nations Development Fund for Women (UK).

P-1B-13

Socioeconomic position and trajectories of growth and adiposity across childhood: the Avon Longitudinal Study of Parents and Children

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Objective: Growth is frequently used as an indicator of overall child health, given its responsiveness to health, social and environmental conditions. Additionally, it is associated with longer-term health; shorter adults have increased risk for cardiovascular disease and type 2 diabetes. Adiposity in childhood has health and psychological consequences and is associated with both adiposity and adverse health in adulthood. Socioeconomic differentials in height and adiposity have been reported in children. There is, however, limited understanding of the pattern of these differentials across childhood: at what age do they emerge, do they change over childhood, do they differ between boys and girls?

Methods: Using data from 7269 boys and 6769 girls from the Avon Longitudinal Study of Parents and Children, we examined

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trajectories of height and adiposity in children from birth to ten years (median and IQR numbers of repeat measures per child 11, 5–19 for height; 9, 4–16 for weight). Adiposity was measured as ponderal index (PI, kg/m^3) for 0–2 year olds and body mass index (BMI, kg/m^2) for 2–10 year olds. Trajectories were modelled using random effects multi-level models. We explored how trajectories differed between socioeconomic groups using several socioeconomic indicators.

Results: Four growth periods were estimated for height, separately for boys and girls: boys 0–3, 3–10, 10–29 and 29–120 months, girls 0–2, 2–11, 11–32, and 32–120 months. There was some indication of each overall growth pattern differing between socioeconomic groups, but socioeconomic differences in growth for each period were small. Most of the socioeconomic differentials in height appear to be driven by birth length, with higher socioeconomic mothers having longer babies. Socioeconomic differentials in PI at birth were not observed. There was some socioeconomic gradient in PI across the first two years, with children from lower socioeconomic groups being more adipose, but the differences at this age were very small. Important socioeconomic differentials in adiposity emerged later in childhood, with the difference being more pronounced and occurring earlier in girls compared with boys. For girls, BMI started to increase more rapidly among lower socioeconomic groups after approximately 6 years old, leading to earlier and more pronounced socioeconomic inequalities in BMI than in the boys. At seven years old, the mean BMI in girls whose mothers were educated to less than O-level was 16.6 kg/m^2 (SD 2.4), compared with 16.1 kg/m^2 (SD 1.8) for girls of mothers educated to degree level. The corresponding BMIs for boys were 16.1 kg/m^2 (SD 1.9) and 15.9 kg/m^2 (SD 1.5). Observed inequalities tended to be wider using maternal education as the socioeconomic indicator, compared with paternal education or household social class.

Conclusions: Since socioeconomic differentials in linear growth (indicated by length or height) are established at birth, interventions to reduce these should look at risk factors in parental or earlier generations. The emergence of socioeconomic differentials in adiposity in later childhood suggests interventions in infancy and early childhood may also be relevant here, especially for girls.

P-1B-14

Maternal smoking during pregnancy and offspring overweight: the role of familial factors

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Objectives: An association between maternal smoking during pregnancy and childhood obesity has been found in several recent studies, although the mechanism by which prenatal smoking exposure influences offspring's risk of overweight remains unclear. We aim to investigate whether familial factors confound the association between maternal smoking during pregnancy and overweight in early adulthood in Swedish males.

Methods: In a population-based cohort of 124 203 singleton males born to Nordic mothers in Sweden between 1983 and 1988, we examined the association between maternal smoking during pregnancy and the risk of overweight in the offspring at around age 18 years. Data on pregnancy and birth characteristics was obtained from the Medical Birth Register, parental social characteristics from Census and Education Register, and offspring outcome variables from conscript examinations. Maternal smoking was measured and recorded during the first antenatal visit, usually at 8 to 12 gestational weeks. We also investigated the association between maternal smoking in pregnancy and offspring body mass index within 8250 full siblings and 182 half siblings. By examining effects of change in maternal smoking behaviour between pregnancies, we aimed to partially control for unmeasured familial confounding such as common genes and shared environment. Fully adjusted analyses include adjustments for offspring birth weight, head circumference, gestational age, birth order, urban living and age at conscription, maternal age, height, maternal body mass index and pregnancy weight gain, parental education and socioeconomic status. Analyses were performed using generalised estimating equation models and linear mixed models in SAS taking into account the correlated structure of the data. The study was approved by the research ethics committee of the Karolinska Institutet.

Results: The risk of overweight was increased in sons of smoking mothers compared to sons of non-smokers (adjusted OR 1.42, 95% CI 1.35–1.49 and 1.56, 95% CI 1.46–1.66, for 1–9 cigarettes per day, and more than 10 cigarettes per day, respectively). Stratifying for maternal smoking habits across two subsequent pregnancies, there was a significantly increased risk of overweight in the second born son (adjusted OR 1.71, 95% CI 1.40–2.09) if the mother smoked in both pregnancies. On the other hand, neither first born sons of mothers who quitted smoking between pregnancies nor second born sons of mothers who started to smoke in the second pregnancy were at significantly increased risk of overweight. The effect of smoking during pregnancy on the offspring's body mass index was not present when the association was evaluated within full and half sibling pairs.

Conclusions: Our results support the importance of familial factors, more specifically shared environmental factors, in the association between maternal smoking during pregnancy and

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overweight in young adult males. Funding: Swedish Research Council and Swedish Council for Working Life and Social Research.

P-1B-15

Mother's smoking in pregnancy modifies the effects of size at birth and concurrent body mass index on blood pressure in adolescents

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Objective: To investigate a modifying effect of maternal smoking on associations of size at birth and length of pregnancy with blood pressure at age 18 years.
Methods: We studied associations of length of pregnancy, birth weight for gestational age, and body mass index at age 18 with systolic blood pressure, pulse rate and hypertension at age 18 in a register-based cohort of 1657 Swedish men born 1982–1985 (third generation UBCoS Multigen) in multivariable regression analyses, adjusted for age at measurement, place of residence and maternal education. We used a path analysis approach to examine the relationships of mother's pre-pregnancy body mass index with birth weight for gestational age, body mass index and systolic blood pressure in a sub-sample of 731 men with additional information on maternal height and pre-pregnancy weight. Analyses were conducted in STATA version 10 and Mplus version 5.1. Ethics committee clearance was obtained.
Results: Among sons of smoking mothers, standardised birth weight was negatively associated with systolic blood pressure at age 18, both before and after adjustment for concurrent body mass index. Such an association was not present among sons of mothers who did not smoke in pregnancy (p-value interaction 0.037). On the other hand, preterm birth increased the risk of hypertension among sons of non-smoking mothers (p-value interaction 0.027), both before and after adjustment for concurrent body mass index. Among sons of non-smoking mothers, standardised birth weight was positively associated with body mass index at age 18, and body mass index was the strongest predictor of high systolic blood pressure (p-value interaction 0.036), pulse rate and hypertension. Our path analysis models fit the data very well, both for smokers and non-smokers separately and overall (assessed using a multiple group analysis with no constraints). Some of the estimated effects are clearly similar between the two models (mbmi → bmi), whilst others differ markedly (e.g. stdbwt → bmi, bmi → sbp), indicating a modifying effect of maternal smoking on the pathways.
Conclusions: Mother's smoking in pregnancy is a strong modifier of the associations between foetal growth indicators and later overweight and hypertension. Funding: VR and FAS.

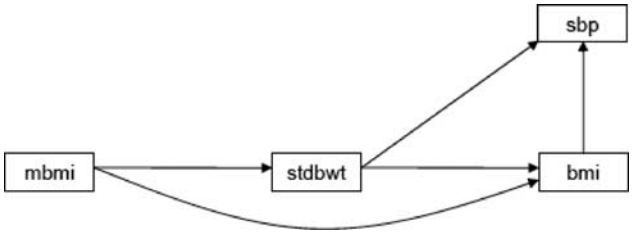

Figure 1: A priori path model fitted independently for smokers and non-smokers (mbmi: mother's pre-pregnancy body mass index; stdbwt: birth weight for gestational age; bmi: body mass index at age 18; sbp: systolic blood pressure at age 18).

P-1B-16

Patterns of food intake and risk of diabetes and cardiovascular disease in 12 y children in Pune Maternal Nutrition Study

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Objective: The developing countries are undergoing a rapid transition which has contributed to the escalating epidemic of chronic non-communicable diseases. The key component of this transition is the nutritional transition which involves change in dietary pattern and physical activity associated with urbanization. We examined the association between dietary patterns and body composition and cardiovascular risk factors at 12y in rural Indian children.
Methods: Pune Maternal Nutrition Study (PMNS) is an ongoing, prospective study of maternal nutrition, fetal growth and cardiovascular risk. At 12y follow-up the children (n = 690) were examined for body composition (DXA) and cardiovascular risk factors (glucose, insulin, lipids, and blood pressure). A customized food frequency questionnaire (FFQ) was used to assess intake over the preceding one year for 16 food categories (114 food items). Principal component analysis (PCA) was used to summarize the food intake. The components summarise the intake information and are independent of each other, hence can be used in multivariate analysis. Appropriate institutional ethics committee clearance and patients consent were obtained.
Results: These children were 12 y old with a BMI of 14.5 Kg/m^2 and fat% of 15.5. Their median (IQR) total plasma cholesterol, HDL cholesterol and triglyceride concentrations were 130 (116,145), 41 (37,47) and 55 (45,68) mg% respectively. Median (IQR) systolic blood pressure was 106

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(99,113) mmHg. None of the children were vegans. 20% were lacto vegetarian and 67% non vegetarian. In the PCA, 6 components (patterns) emerged, explaining more than 60% variation. These were: increased frequency of consumption of micronutrient rich foods like green leafy vegetables, fruits (P-1), energy dense and bakery products (P-2), lentils-rice (P-3), saturated fats (P-4), rotis (bread) (P-5) and more frequent consumption of non-vegetarian and less of fermented foods (P-6). We performed multivariate analysis for associations between food intake and risk factors, results are shown as sd change in risk factor per 1 sd change in frequency of food intake. P-1 was associated with higher adiposity (fat %) ($\beta = 0.11$, $p < 0.01$). P-2 with plasma higher triglyceride concentrations and adiposity (both, $\beta = 0.1$, $p < 0.01$), P-4 with plasma cholesterol concentrations ($\beta = 0.09$, $p < 0.05$), and P-6 with higher systolic blood pressure ($\beta = 0.09$, $p < 0.05$).

Conclusion: Our results demonstrate the dietary patterns that may contribute to the NCD risk in a rapidly transiting society like Indians. On the background of intrauterine programming by maternal nutrition, childhood exposure to urbanized and globalised patterns of nutrition could predispose these children to increased risk of type 2 diabetes and cardiovascular diseases. Nutritional education of parents and children may have a significant role in the prevention of NCDs.

Funding source: The Wellcome trust, UK.

P-1B-17

'SYM-KEM': School based lifestyle intervention project – improvement in physical fitness with exercise program

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Objectives: (i) To evaluate the effect of an exercise program on physical fitness parameters in school children. (ii) To study the association of these parameters with birthweight, current BMI, waist, skinfolds and parental BMI.

Methods: *Participants:* We studied 411 children (average age 11.6 yrs, 53% boys) from Grades VI & VII of Symbiosis School, Pune, who were enrolled, in an intervention program for prevention of childhood obesity since 2005 ('SYM-KEM'). *Anthropometry:* The height, weight & waist measurements (children & parents), birth weights & sexual maturity ratings (SMR) of children were obtained from the annual health records. ***Fitness tests:*** Fitness tests were conducted before & after a 6 months intervention program with informed parental consent. Four health & three sports related fitness tests were conducted under the supervision of a sports consultant. The test readings were compared with local standard norms[1]. *Intervention (Exercise) program:* A 6-month basic fitness program covering flexibility, endurance & muscular strength was implemented 4 times a week.

Results: All fitness parameters except push-ups improved significantly after intervention. In push-ups, children were already above 75th centile at baseline (Table 1). The improvement was independent of age, sex, BMI & SMR.

Table (1) Fitness parameters before & after intervention (n = 411).

Fitness Test	Before Intervention	Centile	After Intervention	Centile	% Improvement Median (range)
Health Related					
9 min run walk (mtrs)	1241 (199)	35–40	1291 (244)	45–50	2.4 (−5.8 to 11.6)***
Sit ups (counts/min)	22.0 (10.1)	40–55	25.9 (10.2)	60–85	13 (−6.9 to 50)***
Push ups (counts/min)	20.2 (10.9)	75–95	20.5 (10.6)	75–95	4.8 (−34 to 48)NS
Sit & Reach (cm)	18.0 (5.6)	<5	19.0 (5.9)	5–10	3.3 (−10 to 23)***
Sports Related					
Skipping (counts/min)	68.5 (40.0)	55–75	87.5 (34.1)	85–95	28.9 (2.1 to 73.4)***
Shuttle Run (seconds)	12.8 (1.2)	35–40	12.4 (0.9)	55–60	2.3 (−2.2 to 7.1)***
Standing Broad Jump (mtrs)	1.28 (0.22)	50–55	1.35 (0.24)	70–75	6.0 (−2.9 to 14.7)***

Values are Mean (SD). P-value by MLRA adjusted for age, sex, BMI and SMR grading.
***$p < 0.001$.
NS: Non-significant.

All baseline fitness parameters showed significant correlation with current BMI ($r \sim = -0.35$, $p < 0.001$ for all fitness parameters), waist (~ -0.28, $p < 0.001$) and triceps skinfold (~ -0.25, $p < 0.001$) after adjusting for age, sex & SMR. Five of the seven fitness parameters correlated significantly with maternal BMI (~ -0.18, $p < 0.05$) (none with paternal BMI). Birthweight correlated significantly with 9 min walk (0.12, $p < 0.05$) and shuttle run (0.11, $p < 0.05$) but not with other parameters.

Conclusions: The exercise programme implemented showed significant effect in improving the health and sports related fitness within a short time of 6-months. Child's adiposity was associated with baseline fitness parameters. Birthweight and maternal BMI also showed correlations with some fitness parameters.

1. Nimkar, ND. Doctoral thesis, University of Mumbai, 2008.

P-1B-18

Obese status and metabolic syndrome among school-age children in Beijing, China

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Obesity, with an increased risk for developing chronic disease, is increasing dramatically in China. Few studies estimate the obesity related metabolic syndrome (MetS) in Chinese children and adolescents.

Objective: To determine the prevalence and clinical phenotype of MetS among overweight and obese school-children in Beijing.

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Methods: Based on Beijing Child and Adolescent Metabolic Syndrome (BCAMS) study, 1 885 children with overweight and obese were screened and recruited in a clinical examination including measurements of waist circumference, fasting plasma glucose and insulin, serum lipid profile. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The prevalence rates of MetS, based on the modified NCEP definition for children, were 0.9%, 7.6% and 29.8% in the normal weight, overweight and obese children, respectively. Abnormal obesity (81.6%), elevated BPs (47.7%) and high TG (35.6%) were the leading three metabolic abnormities among obese children. More than one quarter of children suffered from elevated BPs (29.8%), abnormal obesity (27.4%) and high TG (26.0%). With the increase of body mass index, the clustering of MetS components and insulin resistance (HOMA-IR) were remarkably increased ($P<0.001$).
Conclusions: MetS has been in an epidemic status among the obese children and adolescents in Beijing. Acknowledgments: The study was supported by Grants (H030930030031, D08050700320801) from Beijing Municipal Science & Technology Commission.

P-1B-19

Maternal dietary type in pregnancy and child health outcomes

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Objective: Maternal diet during pregnancy has been related, particularly in extreme circumstances, to early outcomes such as birth weight. The objective of this analysis was to determine whether the effects of maternal diet may have long term consequences. As such we analysed whether maternal dietary type during pregnancy has an effect on childhood health outcomes in terms of BMI, body fat and cardiovascular risk factors.
Methods: The Auckland Birthweight Collaborative Study is a longitudinal case-control study. All infants were born at term and approximately half of the sample were born small for gestational age (SGA) and the other half born appropriate for gestational age (AGA). Data was collected from mothers shortly after the birth of their infants in relation to socio-demography, ante-natal and obstetric related factors. Mothers also filled in semi-quantitative food frequency questionnaires (FFQ) relating to dietary intake during the first and last months of their pregnancies. From these FFQ's we have previously defined dietary types during pregnancy (junk, traditional and healthy) using principle components analysis. The children have been followed up at approximately 1, 3.5 and 7 years of age. At each time point weight, height and percentage body fat (PBF) (via bio-electrical impedance) were measured. The assessment at 3.5 and 7 years of age also included measurement of systolic and diastolic blood pressure. Analysis was carried out using standard regression techniques that allowed for the disproportionate sampling of the SGA infants. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: We were unable to detect any significant effect of maternal dietary type during pregnancy on the children's health outcomes. For example a change of 1 s.d. in the junk diet score during pregnancy was associated with an increase in PBF at 3.5 (0.17%, $p = 0.70$) and at 7 the effect was similar (0.13%, $p = 0.81$). The effects of the traditional and healthy diet scores were in the opposite direction but again not significant. Similarly the effects on blood pressure were not of note, and relatively small in magnitude.
Conclusions: We have been unable to show any effect of maternal dietary type on measures of obesity and cardiovascular disease in childhood. This suggests that the effects of maternal diet on child health may be relatively short term. Alternatively, other factors such as child diet and environmental exposures could be correlated with maternal diet or simply over ride the effects of maternal diet due to the relative closeness of these factors to the time of measurement of the outcomes. On behalf of the Auckland Birthweight Collaborative Study.

P-1C-20

Maternal obesity and a high maternal nutritional plane result in initial fetal overgrowth followed by slowing in the fetal growth trajectory in late gestation

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Objective: Maternal obesity (MO) is paradoxically associated with both macrosomia and IUGR reflecting competing mechanistic processes dependent on multiple placental and maternal and fetal metabolic, cardiovascular and endocrine systems which clearly differ at different stages of gestation. We used our established ovine model of MO and high maternal nutritional plane (HNP) to determine their impact on fetal growth rate, placental vascularity and circulating hormone and metabolite concentrations in maternal and fetal blood in the first and second halves of gestation.
Methods: Study design*:* We assigned mature ewes to control (C, 100% NRC recommendation) or obesogenic (OB, 150% NRC) diets from 60 days before conception to necropsy at 0.5 gestation (G, Term = 150days; n = 7/dietary group) or 0.9G (n = 7/dietary group). Only singleton pregnancies were studied. Analyzed by GLM procedures of SAS.

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Results: MO ewes body weight increased by ~30% from diet initiation to mating ($P < 0.05$) and 43% and 52% from diet initiation to 0.5 and 0.9G respectively. In contrast, C ewes exhibited only modest nonsignificant body weight increases - diet initiation to conception (2.9%), and 5.7% and 7.0% to 0.5 and 0.9G respectively. At 0.5G MO fetuses were ~30% heavier and had greater crown rump length (CRL) than C fetuses ($P < 0.05$). At 0.9G, fetal weight and CRL of C and MO ewes did not differ suggesting slowed late gestational fetal growth in MO versus C fetuses. Placental arteriole diameters were markedly greater (~37%; $P < 0.01$) in OB versus C ewes at 0.5G, but were similar at 0.9G.This corresponded to a decrease in angiogenic factor mRNA and protein expression of VEGF, PLGF, FGF-2, ANG-1 and ANG-2 in the placental vasculature in OB ewes from 0.5 to 0.9G. At 0.5G, maternal blood concentrations of glucose, insulin, and IGF-1 were greater ($P < 0.05$) in OB than C ewes. At 0.9G, while glucose and insulin remained elevated ($P < 0.05$) in the blood of MO versus C ewes, IGF-1 concentrations had returned to levels observed in the blood of C ewes. While blood glucose, insulin, and IGF-1 levels were also elevated in fetal blood of MO versus C ewes at 0.5G ($P < 0.05$), at 0.9G, only insulin remained elevated in fetal blood of MO versus C ewes, while glucose concentrations were reduced to levels found in C fetuses. Also at 0.9G, fetal IGF-1 concentrations were markedly lower ($P < 0.02$) in the blood of MO versus C fetuses.

Conclusions: Our findings clearly implicate altered placental angiogenesis as a contributory factor to the slowing of fetal growth and the paradoxical occurrence of both macrosomia and IUGR in the presence of MO and HNP. NIH INBRE 1P20RR16474.

P-1C-21

Long term effects on infant capuchin monkey adrenal function after maternal exposure to constant light during late gestation

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During pregnancy the fetus may be exposed to some detrimental conditions. However it copes with them and programs for adaptation to the new environment to come, as a newborn, infant and adult. It is clear that some detrimental conditions experienced in utero have negative effects that become apparent late in adult life, suggesting the presence of compensatory mechanisms that allow normal function between these life stages. The adrenal gland may be important in this compensation. A condition that inevitably impacts the fetus is the photoperiod to which the mother is exposed. In fact, in capuchin monkey, maternal exposure to constant light during the last third of gestation induced precocious maturation of the fetal adrenal and resulted in increased plasma cortisol concentrations in the newborn soon after birth[1,2].

Objectives: explore effects of this treatment in the infant monkey adrenal function.

Methods: Four pregnant capuchin monkeys were maintained in constant light (LL) from 63% gestation to term and other four remained in light:dark cycle (14:10; Control). After delivery the newborns and their mothers returned to 14:10 photoperiod. We measured: i) plasma DHAS and cortisol (RIA) response to exogenous ACTH at one and ten month in infants pretreated with dexamethasone to suppress endogenous ACTH and ii) we assessed directly adrenal function at 10 months of age by measuring in vitro the cortisol and DHAS response to ACTH and mRNA and protein levels (RT-PCR and immunoblot) of the key factors in steroid synthesis StAR and 3β-HSD and adrenal weight.

Results: At one month of age plasma levels of cortisol and the cortisol response to ACTH were doubled compared to control infants whereas, plasma levels of DHAS and the DHAS response to ACTH were markedly reduced. At 10 month of age, DHAS was still lower but closer that those observed in control animals whereas cortisol response to ACTH was similar to that of the control group. A compensatory response was detected at the adrenal level, consisting in a 30% increase in adrenal weight and about 50% reduction of protein and mRNA levels of StAR and 3β-HSD and of the magnitude of cortisol and DHAS response to ACTH in vitro.

Conclusion: Our results support that chronic maternal exposure to constant light during the last third of gestation induced different effects on newborn adrenal function in DHAS and cortisol production and in the response of these steroids to ACTH, at birth and at the 10 month of postnatal life. We observed that the adrenal hyperfunction present at one month of age subsided by 10 months of age, providing a normal plasma cortisol response to ACTH. The mechanisms involved at the adrenal level, were a decrease in steroidogenesis accompanied by an increase in adrenal size. We speculate that these compensatory mechanisms overcome the adrenal function alterations induced during pregnancy allowing the infant to grow with a normal plasma cortisol concentration.

1. C. Torres-Farfan *et al.*, *J Pineal Res.*, 41:58–66, 2006.
2. C. Torres-Farfan *et al.*, *J Physiol.*, 554:841–856, 2004.

P-1C-22

The method of cross-fostering *per se* induces obesity and hypertension in male offspring of control and obese mice

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Objective: Cross-fostering is often used in studies of developmental programming to investigate the relative contribution of exposure to a specific maternal environment during the critical *in utero* and post natal periods. Our objective was to determine the potential effects of the cross-fostering procedure *per se* on the development of obesity and blood pressure in offspring of control and obese mice.
Methods: Female C57BL/6J mice were fed either a control diet (3% fat, 7% sugar) or a highly palatable, hypercalorific diet (16% fat, 33% sugar) for six weeks and throughout pregnancy and lactation[1]. On postnatal day 2, half of the litters were cross-fostered to dams on the same diet as their biological mother. The remainder of the litters were suckled by their biological mother. All offspring were weaned onto a control diet. Offspring body weights and food intake were measured weekly. At three months of age offspring blood pressure was measured by radio-telemetry in conscious, unrestrained animals, following surgical implantation of a probe into the left carotid artery. Offspring inguinal fat pad mass was determined following sacrifice.
Results: Both male and female cross-fostered offspring were significantly heavier than the respective non-cross-fostered 'controls' over the time period studied ($p < 0.01$ or 0.001, RM ANOVA). Calorific intake was increased in male and female cross-fostered offspring of control but not obese dams compared to the respective 'controls' ($p < 0.001$, RM ANOVA). Inguinal white adipose tissue (WAT) mass was significantly heavier in male, but not female cross-fostered offspring compared to respective 'controls'. Systolic blood pressure was significantly increased in male cross-fostered offspring compared to the respective non-cross-fostered 'controls' during both the day and night (male SBP [mmHg, mean ± SEM] Day: OC/C, 114.3 ± 0.7, $n = 6$ *versus* OC, 100.4 ± 1.7, $n = 5$, $p < 0.001$; OO/O, 120.3 ± 1.8, $n = 6$ *versus* OO, 105.3 ± 2.4, $n = 6$, $p < 0.001$; Night: OC/C, 123.2 ± 1.1, $n = 6$ *versus* OC, 108.8 ± 2.2, $n = 5$, $p < 0.001$; OO/O, 134.3 ± 2.1, $n = 6$ versus OO, 121.4 ± 1.9, $n = 6$, $p < 0.001$; unpaired t-test). In addition, diastolic blood pressure (DBP) was significantly increased during both day and night in male cross-fostered offspring of obese dams compared to the respective 'controls' ($p < 0.05$ and $p < 0.01$ respectively). In female offspring, no effect of cross-fostering on SBP was observed, whilst DPB was significantly raised only in cross-fostered offspring of obese dams during the night-time period ($p < 0.05$).
Conclusions: The method of cross-fostering itself can programme significant obesity and systolic hypertension in male, but not in female offspring. An interaction of cross-fostering with the maternal environment leads to diverse effects on offspring diastolic blood pressure and appetite, with hyperphagia only observed in cross-fostered offspring of dams fed a control diet. These findings have important implications for the use of this technique for investigation of critical periods relevant to developmental programming.

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1. A.M. Samuelsson *et al.*, *Hypertension*, 51:383–392, 2008.

P-1C-23

Perinatal programming of appetite control – influence of birth weight and early postnatal growth on leptin

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Objectives: To determine the influence of maternal caloric intake during the period of maximal fetal growth on long term programming of appetite control through modulation of leptin action. Additionally, we aimed to establish the influence of early postnatal growth on later metabolic outcomes.
Methods: Pregnant twin-bearing sheep were fed either a control diet (C; $n = 20$) or a 60% nutrient restriction diet (NR; $n = 20$) from 110 days up to term. (147 days). Mothers gave birth naturally at term to twins. Ten offspring in each group were then reared by their mother as singletons in order to promote postnatal growth (high weight gain – HG). Another group of 10 offspring from each group were reared by their mother together as twins in order to restrict postnatal growth (low weight gain – LG). The male:female ratio of each group was 1. After weaning, all offspring were kept in a control indoor environment up to 17 months of age. Overnight fasted plasma samples were taken at weaning (3 months of age), sexual maturity (7 months of age) and adulthood (17 months of age) to measure fasting plasma leptin. At 17 months of age, animals were kept for 2 weeks in individual pens to measure their daily food intake. Appropriate institutional animal ethics committee approval was obtained.
Results: A 60% reduction in maternal food intake during late gestation significantly reduced their weight gain during this period. Over this time whilst NR sheep gained 10% body weight, C gained 40% in body weight ($p < 0.001$). Offspring born to NR mothers weighed less (C: 4.8 ± 0.21 kg, NR: 3.9 ± 0.15 kg, $p < 0.01$). As expected HG offspring gained more weight during the postnatal period (C-HG: 0.31 ± 0.001, C-LG: 0.23 ± 0.01, NR-HG: 0.30 ± 0.01 and NR-LG 0.24 ± 0.01 kg/day). The NR-HG group exhibited the highest fractional growth rate up to weaning when they increased their body weight ~6 times compared with the NR-LG group that only gained 4.5 times their body weight ($p < 0.01$). At weaning, all animals had similar plasma leptin concentrations. However,

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by 17 months of age, NR-HG had significantly higher plasma leptin compared to both the C-HG group and NR-LG groups ($p < 0.05$). However, this alteration in plasma leptin was not related to any significant changes in energy intake.

Conclusions: We have demonstrated that caloric restriction during the period of maximal fetal growth is related to long term changes in plasma leptin that are dependent on postnatal growth. These changes in plasma leptin are, however, unable to modify energy intake, implying central leptin resistance. We are now undertaking further analysis of adipose tissue and hypothalamic samples from these offspring to determine potential changes in leptin production and central leptin signalling.

P-1D-24

Paternal age and offspring survival

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Objectives: Advanced paternal age (APA) has been linked to a number of adverse health outcomes in the offspring such as intrauterine death, schizophrenia, and some cancers[1–3]. The mechanisms behind such associations are debated, but point mutations in the male germ cells[4] and epigenetic changes[5] have been suggested. It has been demonstrated that daughters of old fathers in European aristocratic families had an increased mortality. The authors suggested an x-linked susceptibility to be the responsible mechanism[6]. The aim of this study was to examine the association between paternal age and long-term survival of the offspring as well as cause specific mortality beyond their childhood years.

Methods: From the population covering Danish Twin Registry we used the cohort of subjects born in 1870–1930 who survived to an age of 6 years. For 5,834 subjects the age of their father at birth was registered, and these subjects constituted the study population. We used survival analysis to estimate the mortality hazards in four paternal age groups (< 25 y, 25–34 y, 35–44 y, 45+ y). Finally we estimated the paternal age-related hazard ratios of death from cardiovascular diseases, cancer, and other causes, respectively, to examine whether there was any association between fathers age and cause specific mortality. The Danish Twin Registry and the analyses are approved by the Danish Data Protection Board.

Results: The overall mortality from age 6 years and onwards did not differ between the paternal age groups. The Kaplan-Meier survival curves for the four paternal age groups were identical. We found no interaction between paternal age and sex on mortality risk. In offspring of fathers aged 45+y, we found a hazard ratio of 1.26 (95% CI: 1.00–1.60) of dying from cardiovascular diseases by, but no such increase for deaths from cancer or other causes.

Conclusions: Overall, there was no evidence of an increased mortality by offspring of older fathers, neither in men nor in women. However, an increased mortality from cardiovascular diseases with advanced paternal age was suggested.

1. Andersen *et al. Am J Epidemiol.,* 160:1214–1222, 2004.
2. Yip *et al. Int J Epidemiol.*, 35:1495–1503, 2006.
3. Malaspina *et al. Arch Gen Psychiatry,* 58:361–367, 2001.
4. J.F. Crow. *Science*, 301:606–607, 2003.
5. Perrin *et al. Schizophr Bull.*, 33:1270–1273, 2007.
6. L.A. Gavrilov, N.S. Gavrilova. Springer-Verlag, 7–31, 2000.

P-2A-25

Maternal 1-C metabolism and cognitive function in the offspring: The Pune Maternal Nutrition Study

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Objective: Folate and vitamin B_{12} (B_{12}) are required in the remethylation of homocysteine to methionine and then to S-adenosylmethionine. S-adenosylmethionine is involved in numerous one carbon transfer reactions involving proteins, phospholipids, DNA, and neurotransmitter metabolism. B_{12} and folate are important in neuronal development and their deficiency may lead to neuropsychiatric dysfunction. In a subset of the Pune Maternal Nutrition Study (PMNS) cohort, maternal B_{12} concentrations during pregnancy were positively related to cognitive performance in the offspring at 9y of age. We now report the relationship between maternal and offspring B_{12} and folate nutrition with offspring cognitive function at 12y of age in the whole PMNS cohort.

Method: We studied 690 children (331 girls). Maternal plasma B_{12}, total homocysteine (tHcy) and methymalonic acid MMA, and red cell folate concentrations, were measured at 18 wks and 28 wks gestation. Tests of cognitive function in the children at 12y included: Colour Progressive Matrices (CPM-fluid intelligence), Digit span (working memory), Colour trail (focused attention), UCLA version of Rey's auditory verbal learning (AVLT-verbal learning & memory), Picture completion (visuo-conceptual ability), and Block design (visuo-spatial skills). Associations between maternal plasma B_{12} and folate status and offspring cognitive function were analysed using linear regression, adjusting for appropriate confounding variables. KEM Hospital, Research Centre ethics committee clearance and participants' informed consent were obtained.

Results: The children were 12y old, 99% school going and studying in the 5th standard. When compared with Indian reference norms (NIMHANS), their cognitive performance was

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between 50th–75th percentile for CPM, picture completion and colour trail A tests, and between 25th–50th percentile for colour trail B, auditory verbal learning and block design tests. Age, gender, head size, weight, socio-economic status and maternal education were significantly positively associated with the child's cognitive function. We performed multivariate analysis for associations between indicators of maternal 1-C metabolism and cognitive function in the child, results are shown as sd change in cognitive function per 1 sd change in exposure. After adjusting for confounders, indicators of maternal 1- C metabolism at 28 wks gestation were significant predictors of cognitive functions except memory. Maternal tHcy (visuo-conceptual ability, $\beta = 0.09$), MMA (intelligence $\beta = 0.11$, sustained attention $\beta = 0.11$) and folate concentrations (visuo-spatial skills $\beta = 0.16$) favorably predicted cognitive function ($p < 0.05$ for all) while maternal B_{12} unfavorably predicted visuo-spatial skills ($\beta = 0.11$, $p < 0.05$). The child's own B_{12} status at the time of testing favorably predicted sustained attention, verbal learning and memory ($\beta = 0.11$, $p < 0.05$ for all), however folate was not associated with any cognitive function. Indicators of maternal 1-C metabolism at 18 wks gestation were not associated with child's cognitive performance.

Conclusion: Our results indicate that 1-C metabolism during intrauterine life may influence brain development and cognitive function. The second half of pregnancy may provide a window of opportunity for nutritional intervention (vitamin B_{12} & folate) to improve neuro-cognitive function of the fetus. Support: Wellcome Trust UK and Department of Biotechnology of India.

P-2A-26

Life-course sociodemographic conditions and quality of life among adolescents in a Brazilian birth cohort

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Objective: To evaluate the associations of gender, skin colon, maternal education at birth and socioeconomic position (SEP) change from birth to 11–12y on familiar and social aspects of Quality of Life (QoL) in early adolescence.

Methods: Population based birth cohort study. All the 5249 individuals born in Pelotas (southern Brazil) in 1993 were repeatedly visited from birth to age 11–12y (follow-up rate 87.5%). Socioeconomic variables were collected in 1993 and 2004–05. This cohort is one of the few available in low and middle income countries with data on sociodemographic and health aspects of the children and their family. Information of QoL was obtained in 2004–05 and seven variables were selected for analysis, including familiar (bad relationship with father, bad relationship with mother, frequent family conflicts and frequent physical punishment by parents) and social aspects (sensation of discrimination, fear of the neighbourhood and academic failure). All the analyses were developed in STATA 9.2, using Poisson regression in order to estimate the relative risks. A hierarchical model was considered for adjustment. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Skin colour was associated with near all the familiar outcomes (30–40% higher risk among non-whites compared to whites), with the exception of family conflicts. Sex was only associated with physical punishment by parents (30% less frequent among girls). Maternal education was inversely associated with bad relationship with father and mother, physical punishment and family dysfunction. The association with family conflicts was U-shaped and the intermediate level of maternal education showed a protective effect. Current poverty (always poor and non-poor/poor) was adversely associated with all family aspects of QoL with the exception of family conflicts, which was more frequent only among those always poor. In relation to social aspects of QoL, fear of the neighbourhood of residence and discrimination were 20–30% more frequent among women and non-whites individuals when compared to their respective reference groups. Non-whites adolescents also showed higher risk of academic failure, but conversely female sex provided a protective effect. Maternal education was inversely associated with discrimination, but not with fear of the neighbourhood. Academic failure was 20 times more frequent among adolescents with lower maternal education at birth than the better off, even after controlling for potential confounders. Likewise, SEP change was not associated with fear of neighbourhood, but was associated with discrimination, which was more frequent among those currently poor (always poor and non-poor/poor). Academic failure was also 2.5 times more frequent among subjects who were always poor when compared to those who were never poor.

Conclusions: Lower SEP experienced across the life had an accumulative effect on social and familiar aspects of QoL, principally for academic failure. Skin colour and gender also showed important long-term effects on different outcomes. These results suggest than politics should be directed to improve access to education of quality, especially in the lower SEP groups, involving both parents and children. This would help to minimize iniquities across the life and in next generations.

P-2A-27

Maternal vitamin C administration prevents memory impairment in adulthood following prenatal hypoxia

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Objective: Previous studies have shown that prenatal hypoxia can impair memory consolidation in the chick[1], and if

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combined with preterm delivery, can delay learning ability in lambs[2]. To date, it is unclear whether adverse conditions in pregnancy such as prenatal hypoxia can programme neurological disease in adulthood. Furthermore, the mechanisms underlying such impairments remain unknown. This study tested the hypothesis that the developmental programming of neurological disease by prenatal hypoxia is secondary to oxidative stress. We investigated in rats the effects of prenatal hypoxia on behaviour and cognitive function in adulthood, and determined whether vitamin C had any neuroprotective effects.

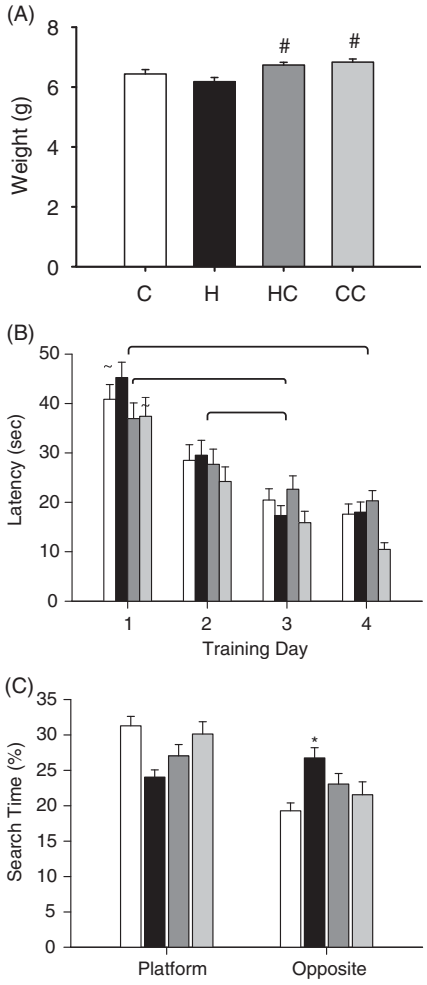

Figure 1. Values are mean ± SEM for (A) birth weight (B) latency to find the submerged platform in the water maze over 4 days of training, and (C) search time in the target quadrant during the probe trial, in offspring of control (C, white), hypoxic (H, black), hypoxic + vitamin C (HC, dark grey) or control + vitamin C (CC, light grey) pregnancies. *$P < 0.05$ *vs.* control, #$P < 0.05$ *vs.* hypoxic, ~$P < 0.05$ *vs.* day 1 of training (One-way ANOVA or Two-way ANOVA + Tukey's test).

Methods: From days 6–21 of pregnancy, 44 female Wistar rats (n = 5–6 per group) were divided into control (C: 21% O_2) and hypoxic (H: 14% O_2) pregnancies, with and without vitamin C (0.5 g.100 ml^{-1} in drinking water). At birth, litters were culled to 8 pups, and weighed weekly until the completion of the study. At 3.5 months, open field and Morris water maze testing was performed to assess exploratory behaviour and cognitive function, respectively. To control for sex and within litter variation, only up to 2 male offspring from any one litter were studied (n = 10–12 per group).

Results: Relative to controls, hypoxic pregnancies tended to reduce birth weight (Fig. 1A), and significantly increased catch-up growth from postnatal days 7–14 (fractional growth rate: H: +7±2%, $P < 0.05$). Maternal treatment with vitamin C significantly improved birth weight in hypoxic pregnancies ($P < 0.05$); fractional growth rates were unaltered (−0.4±2%). All rats learnt the position of the submerged platform in the water maze as the experiment progressed (decrease in latency, $P < 0.001$, Fig. 1B). After four days of training, the platform was removed, and a probe test was performed. Relative to controls, hypoxic animals spent less time searching in the quadrant that had previously contained the submerged platform, and more time in the opposite quadrant ($P < 0.05$, Fig. 1C), suggesting impaired memory retention. Maternal treatment with vitamin C significantly reducedthigmotactic(wall-hugging) behaviour ($P < 0.05$, data not shown), and improved performance in the probe trial ($P < 0.05$). There was no treatment effect on open field performance, as measured by path length, speed, and the percentage of time spent in the centre and periphery of the arena.

Conclusions: The data show that prenatal hypoxia can impair memory retention in adulthood. Maternal treatment with vitamin C improved performance in the Morris water maze following prenatal hypoxia, suggestingthat oxidative stress-could be a key link in the developmental programming of neurodegenerative disease. Support: The British Heart Foundation, The Royal Society and the BBSRC.

1. E.J. Camm *et al.*, *Reprod Fertil Dev.,* 12:165–172, 2000.
2. E.J. Camm *et al.*, *Dev Brain Res.,* 132:141–150, 2001.

P-2A-28

Developmental programming of dysfunctional hypothalamic neural stem cells in leptin deficient, low birth weight newborns

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Objective: Appetite regulatory circuits in the hypothalamus develop in utero to assure neonatal food intake, and the set-points for appetite regulation are programmed during the gestational and lactational newborn periods. Low birth weight (LBW) offspring have a programmed dysfunction in hypothalamic neuronal development with reduced anorexigenic neural pathways and dysregulation of central signaling

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of orexigenic and anorexigenic neuropeptides. Programmed hyperphagia and obesity in LBW offspring results, in part, from impaired anorexigenic mechanisms. Although leptin and insulin serve as hypothalamic modulators of appetite/satiety in the adult, they have critical neurotrophic properties during fetal life, including development of hypothalamic appetite pathways. As LBW offspring have decreased cord blood leptin and insulin levels, we hypothesized that reduced neurotrophic stimulation during critical periods may alter the development of appetite pathways. We utilized neural stem cells (NSC) to investigate the growth and differentiation of hypothalamic neuronal cells, in response to leptin and insulin. We hypothesized that LBW-associated leptin-deficiency (LD) causes impaired neuronal NSC proliferation and differentiation.

Methods: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to 21 to produce LBW-LD newborns. At day 1 of age, hypothalamus was dissected and cultured in complete medium (CM) containing growth factors and heparin. At day 8–9 of culture, NSC were digested and seeded in CM or differentiating medium (DM; without growth factors and heparin) for basal studies. For studies of neurotrophic proliferation responses, NSC cultured in CM were treated with leptin (10, 20, 40 ng/ml) or insulin (10, 20, 40 μg/ml) every 48 h for 8 days and proliferation rate measured by MTT assay. For differentiation responses, NSC cultured in DM were treated with leptin or insulin (as above) and cell differentiation quantified by expression (Western Blot) of neuronal (NeuN, Tuj1) or astrocyte (GFAP) markers.

Results: The *basal proliferation index* of NSC was significantly reduced in LD newborns (15%). Although LD and Control NSC responded to leptin and insulin with dose-dependent increments in proliferation, LD NSC displayed reduced *proliferation* at all doses as compared to Controls (50–60%). Further, LD had reduced *basal differentiation* to both neuronal (Tuj1, 22%) and astrocyte (GFAP, 42%) cell lines, as compared to Controls. In response to leptin, both LD and Controls showed dose-dependent increments in differentiation though at all times, the LD exhibited reduced neuronal (~ 34%) and astrocyte (~ 29%) differentiation as compared to Controls. In response to insulin, both LD and Controls showed dose-dependent increment only in neuronal differentiation. Once again, LD exhibited reduced neuronal (~ 32%) and astrocyte (~ 40%) differentiation as compared to Controls.

Conclusions: Low birth weight, leptin-deficient newborns have programmed dysfunctional hypothalamic neuronal stem cells, evident by reduced basal and stimulated proliferation and neuronal/astrocyte differentiation. These results indicate that impaired NSC proliferation and differentiation is the likely etiology for reduced anorexigenic neural pathways in LBW offspring, and contribute to the resulting hyperphagia and obesity.

P-2A-29

Rat embryonic hypothalamic neural stem cells response to trophic factors: Selective differentiation responses to leptin and insulin

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Objective: Although leptin and insulin serve as hypothalamic modulators of appetite/satiety in the adult, they have critical neurotrophic properties during fetal life, including development of hypothalamic appetite pathways. Low birth weight (LBW) offspring have a programmed dysfunction in hypothalamic neuronal development with dysregulation of central signaling of orexigenic and anorexigenic (satiety) neuropeptides. As LBW offspring have decreased cord blood leptin and insulin levels, we hypothesized that reduced neurotrophic stimulation during critical periods may alter the development of appetite pathways. We utilized neural stem cells (NSC) to investigate the growth and differentiation of hypothalamic neuronal cells in response to leptin and insulin. We determined the putative signaling pathways essential for proliferation versus differentiation.

Methods: Hypothalamus from E20 control rat embryo and cultured in complete medium (CM) containing growth factors and heparin. At day 8–9 of culture, NSC were seeded in CM or differentiating medium (DM; without growth factors and heparin) for basal studies. For studies of neurotrophic proliferation responses, NSC cultured in CM were treated with leptin (10, 20, 40 ng/ml) or insulin (10, 20, 40 μg/ml) every 48 h for 8 days and proliferation rate measured by MTT assay. For differentiation responses, NSC cultured in DM were treated with leptin or insulin (as above) and cell differentiation quantified by expression (Western Blot) of neuronal (NeuN, Tuj1) or astrocyte (GFAP) markers. Signaling pathways were examined by measure of select molecules (Notch 1, Hes1, pERK1/2 and pSTAT3) and NSC responses in the presence of selective pathway antagonists.

Results: Both leptin and insulin enhanced NSC *proliferation*. In CM cultures, there was a dose-dependent effect of leptin (35%, 39%, 72%) and insulin (23%, 28%, 43%) on NSC proliferation. However, there were selective NSC *differentiation* and signaling responses to leptin and insulin. Leptin treatment of NSC in DM cultures resulted in marked increase in expression of neuronal markers (NeuN: 185%; Tuj1: 46%) with a non-significant trend towards increased astrocyte marker (GFAP). In contrast, insulin treatment caused significant increase in GFAP (78%) with non-significant increment in NeuN and Tuj1. In studies of signaling responses, leptin and insulin induced NSC proliferation in association with increased Notch1 and Hes1, as well as increased phosphorylation of ERK1/2 and STAT3. In contrast, NSC differentiation was associated with inhibition

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of Notch1 and activation of ERK1/2 and STAT3 pathway. Inhibition of ERK activation by PD98059 or STAT3 by AG490 completely blocked leptin/insulin induced NSC proliferation and differentiation.

Conclusions: Leptin and insulin have potent effects on NSC proliferation, with selective effects on NSC differentiation, enhancing neuronal or glial cells, respectively. Both NSC proliferation and differentiation include ERK1/2 and STAT3 pathways, though only proliferation includes Notch1 and Hes1 signaling. These results indicate a critical role of in utero neural trophic factors and suggest that excess or deficient leptin/insulin associated with IUGR or macrosomic fetuses, may permanently alter neural pathway development and adult behavior.

P-2A-30

A comparison of intra-uterine and early postnatal head growth and subsequent cognitive development in term low birthweight infants in Brazil and the UK – evidence for a critical period?

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Infants born at term with low birthweight (LBW) are recognized to show impairments in their mental and motor development, but debate continues as to the relative importance of intra-uterine and post-natal insults on subsequent development, and on the contribution of poverty and continuing undernutrition on outcome. We have used birth cohorts from very different social settings to investigate the relationship between head growth and subsequent IQ in term LBW, and to assess whether there is evidence for a critical period.

Objective: to investigate the relation between head growth during different time periods and IQ at 8 years, and to identify factors associated with more rapid head growth.

Methods: a) Brazil: Two parallel cohorts of term LBW and appropriate birth weight (ABW) infants were enrolled at birth in Northeast Brazil. Anthropometric measurements were made at birth, 2 m, 6 m, 12 m, 24 m and at 8 years. Cognition was assessed at 8 years (n = 164) with the Weschler Intelligence Scale for Children (WISC III). b)UK: A population-based birth cohort from South West England- the Avon Longitudinal Study of Parents and Children (ALSPAC)- was used. Anthropometric measurements at birth, 2 m, and 9 m and were available from 7035 term infants, of whom 158 (2.2%) were term LBW. Cognition was assessed at 8 years (n = 3901) using the WISC III. In both datasets, the head circumference measurements were converted to SD scores using the updated British 1990 Growth Reference. Multivariable analysis with a two-stage residual model was used to relate head growth between successive time points with IQ. Appropriate institutional ethics committee clearance and participants' informed consent were obtained in both the Brazil and UK studies.

Results: Mean birth weight in the term LBW group was 2.35 kg in Brazil vs 2.28 kg in UK, and in the ABW group 3.21 kg in Brazil vs 3.49 kg in UK. At 8 years, mean IQs were lower in the LBW vs the ABW groups in both settings: 75.2 vs 79.4 in Brazil and 97.8 vs 104.8 in UK. In the LBW group from Brazil, head growth from birth-2 m and from 2–6 m, conditional on previous size, were significant independent predictors of IQ at 8 years. Head growth from 6m-8 years and head size at birth were unrelated to IQ. In the ABW group there was no significant relationship between conditional head growth and IQ for any time period. Determinants of more rapid head growth from birth-6m in LBW infants were maternal height and rate of weight gain. In the LBW group from UK, head growth between birth and 2m was associated with IQ at 8 years. Head size at birth and head growth from 2–9m were unrelated to IQ. In the ABW group from the UK there was also an association between head growth between birth-2m and 2m–9m and IQ, although the relationship between increasing head gain and IQ was more striking amongst the LBW group.

Conclusions: In term LBW infants, head growth from birth-6m is more important than prenatal or later postnatal head growth in predicting IQ at 8 years. Funding support: Wellcome Trust, UK and CNPq, Brazil.

P-2A-31

Antioxidant defenses in hippocampus of adult rats submitted to neonatal handling

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Objective: Neonatal handling is an experimental paradigm of an early experience which permanently alters hypothalamic-pituitary-adrenal axis function resulting in increased ability to cope with stress and decreased emotionality and sexual behavior. It also produces morphological changes in critical neuroendocrine areas that are involved in social bonding in adult rats (1–3). As early as the prenatal or neonatal period, stress can alter the rate of cognitive decline and neurodegenerative changes in the brain, with prenatal restraint and maternal separation usually causing damage to the brain, whereas brief neonatal handling confers resilience to stressors in adulthood and to age-related memory decline (4–6). The occurrence of negative outcomes of early

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stress can be reversed by subsequent events known to be beneficial to the ageing process. After the early developmental period, it is currently unknown how stress will impact on the ageing process, due to a lack of studies. On the other hand, the brain is specially susceptible to oxidative stress because it has high oxygen consumption and low antioxidant defenses system. In the present work we investigated the effect of neonatal handling (10 minutes/day during 10 days) on antioxidant defenses and on DNA damage in adult rat hippocampus, a structure of brain involved with memory and particularly susceptible to influences of stress hormones.

Methods: Litters were divided into intact and handled. In the handled group, pups were placed in an incubator (32° C) 10 min/day, from days 1 to 10 after birth. Litters were weaned and separated by sex on postnatal day 21. On postnatal day 60, the animals were sacrificed and the hippocampus was dissected and part of it was immediately used in single-cell gel electrophoresis (Comet) assay in order to detect DNA damage. The other part was kept at −70°C until analysis of the antioxidant enzymes activities SOD (superoxide dismutase), GPx (glutathione peroxidase) and CAT (catalase).

Results: Results were analyzed by Student's t test. No differences between handling and intact groups were found on SOD, GPx and CAT activities in hippocampus neither in Comet assay.

Conclusions: We concluded that handling does not confer antioxidant protection, nor induces oxidative stress on the rat hippocampus. Therefore, the antioxidant defenses probably do not contribute to long-term alterations observed in the animals handled in early life.

1. A.S. Todeschin *et al., Horm Behav.,* 56:93–100, 2009.
2. R.L. Huot *et al., Psychopharmacol.,* 158:366–373, 2001.
3. D.D. Francis *et al., Neurosci J.,* 22:7840–7843, 2002.
4. M.C. Pardon, I. Rattray. *Neurosci Biobehav Rev.,* 32:1103–20, 2008.
5. M.J. Meaney *et al., Neurobiol Aging*, 12:31–38, 1991.
6. A.L. Rodrigues *et al., Brain Res.,* 1002:94–9, 2004.

P-2A-32

Effects of neonatal malnutrition caused by milk production inhibition at distinct periods of lactation on the behavior of adult offspring

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Objective: Maternal hypoprolactinemia induced by bromocriptine (BRO) treatment at the end of lactation programs for obesity[1] and hypothyroidism[2] in adult offspring and these changes may affect behavior, but if the prolactin inhibition occurs in mid-lactation the obesity does not happens, but hypothyroidism will still be present. In the present study, we analyzed the long-term effects of maternal hypoprolactinemia at mid- and late lactation associated with learning/memory, novelty-seeking and anxiety levels in adult male rat offspring.

Methods: 1) Mid of lactation: lactating rats were divided into 2 groups: injected twice a day with 1 mg of BRO (MIDBRO = 17) or saline (MIDC = 22) at 7th, 8th and 9th days; 2) End of lactation: injected using the same protocol at 18th, 19th and 21th days (ENDBRO = 44; ENDC = 34). Offspring were tested at adulthood. *Test 1:* Anxiety levels were assessed in elevated plus-maze (EPM). Time spent in the open arms, total number of arm entries (open+closed), and decision making (time in the central area) were recorded over a period of 10 min (subdivided into four 2.5 min segments: S1 to S4). *Test 2:* Rats were tested in the hole board arena (HB) in order to assess novelty-seeking behaviour. Rats were allowed 10 min to explore. The number of explored holes (head-dips) was noted.

Results: *EPM:* Mid lactation period – An interaction (ANOVA: $F = 2.9$, $df = 3$, $P = 0.038$) between TREATMENT (MIDBRO X MIDC) and SEGMENT (S1 to S4) was observed regarding the duration of open arms visits: the decrease in time spent in the open arms throughout the 4 segments was significantly more accentuated in MIDC (from 10.0 ± 3.1 s in S1 to 1.0 ± 0.9 s in S4) than in MIDBRO (from 3.2 ± 1.8 s in S1 to 1.6 ± 1.1 s in S4). End of Lactation – ENDBRO rats (8.8 ± 3.3 s) spent less time (Mann-Whitney: $Z = 2.4$, $P = 0.018$) in the open arms than C (27.0 ± 8.2 s). ENDBRO rats (15.7 ± 2.1 entries) had smaller number (ANOVA: $F = 15.9$, $df = 1$, $P < 0.001$) of total arm entries than ENDC (27.6 ± 2.1 entries). ENDBRO rats (7.4 ± 1.0 s) spent less time in the central area (ANOVA: $F = 16.4$, $df = 1$, $P < 0.001$) than ENDC (13.5 ± 1.0). *HB:* There were no significant differences regarding the number of head dips between groups neither during mid- or late lactation.

Conclusions: Irrespective of the milk inhibition period, anxiety levels were more susceptible to the effects of malnutrition than novelty-seeking behavior. Support: FAPERJ, CNPq, CAPES, SR2-UERJ.

1. I.T. Bonomo *et al., J Endocrinol.*, 192:339–344, 2007.
2. I.T. Bonomo *et al., J Endocrinol.*, 198:331–337, 2008.

P-2A-33

Effects of maternal hyperleptinaemia during lactation on learning/memory, anxiety-like and novelty-seeking behavioral traits in adult rat offspring

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Objective: Previously, we have shown that hyperleptinaemia caused by neonatal injections of leptin in the rat pups results in increased anxiety levels and novelty-seeking behavior at adulthood, without affecting memory/learning[1]. Here, we studied whether maternal hyperleptinaemia during lactation could affect these behaviors in the adult offspring.
Methods: During the first 10 days of lactation (from PN1 to PN10), lactating Wistar rats were s.c. injected once per day with either 50 μl of saline (SAL, n = 11) or murine leptin (LEP - 8 μg/100 g of body mass, n = 11). *Experiment 1:* Learning/memory was assessed at PN140 in the radial arm water maze (RAWM). Each animal was tested 4 times (trial) a day for 5 consecutive days. They were allowed 2 min to explore and find the hidden escape platform. The latency to find the platform was noted. *Experiment 2:* At PN148, anxiety levels were assessed in the elevated plus-maze (EPM). Entries into the open arms (Entries OA) and total number of arm entries (Entries TT) were recorded over a period of 10 min.*Experiment 3:* At PN 149, animals were tested in the hole board arena (HB) in order to assess novelty-seeking behavior. Animals were allowed 10 min to explore. The number of explored holes (head-dips) was recorded.
Results: *Exp. 1*: LEP rats (567 ± 140 s) displayed a significantly (ANOVA: $F = 5.5$, df=1, $P = 0.03$) shorter time than C ones (728 ± 212 s) to find the hidden platform. *Exp. 2:* LEP rats (1.8 ± 0.6) presented significantly higher number of Entries OA (ANOVA: $F = 5.9$, df = 1, $P = 0.026$) than C ones (0.4 ± 0.2). No differences between groups were observed regarding Entries TT (ANOVA: $F = 0.002$, df = 1, $P > 0.05$). *Exp. 3:* There were no significant differences (ANOVA: $P > 0.05$) between groups regarding the number of head dips.
Conclusions: Maternal hyperleptinaemia reduces anxiety levels and improves learning and memory performance at adulthood without interfering with novelty-seeking behavior. Thus, the programming effect of neonatal hyperleptinemia on behavior depends on the via of administration and maternal hyperleptinemia may induce milk or gastrointestinal imprinting factors on the offspring. Support: FAPERJ, CNPq, CAPES, SR2-UERJ.

1. M.C. Fraga-Marques *et al., Horm. Behav.*, 55:272–279, 2009.

P-2A-34

Gene expression profiling in laser-microdissected hippocampal subregions of prenatally undernourished adult female rat offspring

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Objective: Prenatal nutrition has the potential to program brain function and change the development of metabolic and cognitive outcome during postnatal life. (1–3) To elucidate the molecular mechanisms by which early life undernutrition may impact on the development of the hippocampus, a brain structure important in establishing learning and memory, and hence for cognitive performance, we compared the transcriptional profiles of hippocampal subregions cornu ammonis (CA)1, CA2/3 and dentate gyrus (DG) from adult female rat offspring whose mothers were undernourished or fed *ad-libitum* during pregnancy.
Methods: Pregnant Wistar rats were fed either *ad libitum* (AD) or 30% of AD intake of a standard chow diet throughout gestation (UN). At weaning (3 weeks), female offspring from AD or UN mothers were fed a chow diet for the remainder of the study. Brains were collected at 20 weeks and hippocampal subregions CA1, CA2/3, and DG were isolated from coronal rat brain slices by laser microdissection and RNA was isolated, amplified, and hybridized to a rat oligonucleotide microarray containing approximately 10,000 transcripts (~50 mer oligonucleotides). Gene expression analysis included Genespring, GeneGO Metacore, and Ingenuity Pathway analysis. To gain insight into the biological relevance of observed gene expression changes by perinatal undernutrition, genes with at least a 1.5-fold change and p-value ⩽0.05 (Fisher's exact test) were selected and categorised using gene ontology-based algorithms into biological functions, canonical pathways, and gene networks.
Results: Numerous signalling, biosynthesis and metabolic pathways, including oxidative phosphorylation, amyloid processing, LXR/RXR activation, aminoacyl-tRNA, phenylalanine, tyrosine and tryptophan biosynthesis, cell cycle and cell death regulation, mitochondrial dysfunction, β-adrenergic and IL-4 signalling pathways were significantly influenced by prenatal undernutrition.
Conclusions: The results of this study provide insight into molecular pathways by which prenatal undernutrition may influence the development of cognitive function in adult life and provide a basis for generating hypotheses about the impact of nutrition before birth on long-term cognitive function and learning outcome.

1. B.A. Ikenasio-Thorpe *et al.*, *J. Endocrinol.*, 193:31–37, 2007.
2. J. Landon *et al.*, *Learning and Behavior.*, 35:79–86, 2007.
3. J. Landon *et al.*, In: *Developmental Origins of Health and Disease*. 370–378, 2006.

P-2A-35

Early childhood obesity is associated with poor cognitive development

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Objective: To determine the relationship between obesity and intellectual development in 5y-old children attending public nursery schools in Santiago, Chile.

Methods: Cross-sectional evaluation nested in a cohort study, anthropometric data was collected in 104 non-obese (N-Ob) (BMI ≥ -1 Z to ≤ 1) and 109 obese children (Ob) (BMI $>$ 2 Z) (WHO 2006). We applied the Intelligence Wechsler Scale WPPSI-R (IQ) and assessed socioeconomic status (SES: medium, low-medium, and low) from household resources and educational level of the main provider. Outcomes were analysed as continuous variables; normality was tested prior to using parametric statistics, uni and multivariate analyses were used to meet the objective. The study was approved by INTA's Ethics Committee; participants provided informed consent.

Results: 49% were girls, average IQ was 92 ± 9, differences by age (4y: 94 ± 9; 5y: 91 ± 9 $p < 0.01$) were noted, no differences found by obesity status (N-Ob vs Ob) and sex. Mean IQ in N-Ob preschoolers was higher than in Ob of medium SES ($p < 0.001$). A trend for lower IQ was observed in obese children by SES ($p < 0.001$) (Figure 1). The interactions between obesity and socioeconomic status were significant ($p < 0.01$); thus, multivariate analysis (MA) was conducted for each SES. Table 1 summarizes results showing a negative effect of BMI on IQ for children of medium SES ($p = 0.038$). In older children the IQ were lower. The multivariate model explained 20.5% of the variability in IQ. The effect of BMI on IQ was not significant for other SES levels. Children of lower socioeconomic levels had lower IQ scores independent of Ob status. Our results strengthen the hypothesis that obese children have a lower performance on cognitive tests. This might be due to sedentary lifestyles and psychosocial-emotional problems[1–3].

Conclusions: this study provides evidence that childhood obesity is associated with poorer cognitive development in pre-school children of low income families, especially those slightly better off. Young children's cognitive performance is determined by socioeconomic conditions prevailing at home and by nutritional status. Countries with high prevalence of childhood obesity should consider interventions aimed at healthy eating and active living in order to prevent chronic diseases as well as enhancing cognitive development. This study was funded by Fondecyt # 1090252 and Junta Nacional de Auxilio Escolar y Becas.

Table 1. Multivariate Analysis for IQ by Medium SES.

Variables	R^2	β	95% CI	p
	20.5			
BMIZ score		−1.73	−3.35;−0.10	0.038
Age (months)		−0.60	−1.09;−0.12	0.016
Sex (girls = 0, boys = 1)		4.03	−0.08;8.15	0.055
Height for Age Z		−1.57	−3.84;2.69	0.725

R^2: Determinant coefficient adjusted (%); CI: Confidence interval.

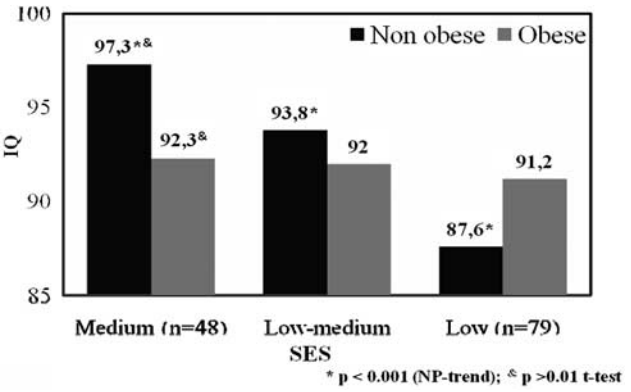

Fig. 1. Mean of intellectual coeficient (IQ) in preschoolers non-obese and obese according to socioeconomic status (SES).

1. J.L. Mille *et al.*, *Dev Neuropsychol*; 34:272–283, 2009.
2. F. Wang, P. Veugelers. *Obesity Rev;* 9:615–22, 2008.
3. J.M. Mond *et al.*, *Int J Obes (Lond).* 31:1068–1073, 2007.

P-2A-36

Maternal vitamin B_{12} and folate status during pregnancy and excessive infant crying: results from a large prospective cohort study

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Objective: The origins of excessive infant crying are largely unknown. Excessive crying may be a symptom of a dysregulated hypothalamic-pituitary-adrenocortical axis and associated dysregulation in the sleep-wake circadian rhythm.[1] Besides the effect of maternal psychosocial problems during pregnancy on crying behavior, maternal nutritional status may be involved. Maternal vitamin B_{12} and folate deficiencies

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during pregnancy affect fetal brain development; infants born to deficient mothers show symptoms like irritability and failure-to-thrive.[2] Furthermore, vitamin B_{12} has been reported to be successful in the treatment of sleep-wake rhythm disorders.[3] This explorative study is the first to examine whether 1) maternal vitamin B_{12} and folate status during pregnancy are associated with excessive infant crying, and 2) whether this association is modified by the presence of psychosocial problems during pregnancy.

Methods: From January 2003 till March 2004, all pregnant women in Amsterdam were approached during their first prenatal visit (±13 weeks of gestation); 8266 women (response rate 67%) filled out a questionnaire covering sociodemographic data, lifestyle and (psychosocial) health; 4389 women also provided a blood sample for biomarker analyses. A few months (±3) after delivery, 5132 women filled out a second questionnaire about the health of mother and infant. Vitamin B_{12} and folate concentrations were determined in serum, standardized for gestational age at blood sampling and then categorized into quintiles (Q1–Q5). Infant crying was measured by the question: 'How many hours per day (24 hours) on average did your baby cry in the past week?' Excessive crying was defined as crying ⩾ 3 hours per day on average in the past week. The following maternal psychosocial problems were measured by self-report: depressive symptoms, anxiety, pregnancy-related anxiety, jobstrain, and parenting stress. For this study, multiple births were excluded. Finally, complete data were available for respectively 2944 (vitamin B_{12} analyses) and 2644 (folate analyses) women. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The prevalence of excessive infant crying in our sample was 3.4%. Logistic regression analyses showed that vitamin B_{12} concentration was significantly associated with excessive crying, univariate as well as multivariate (adjusted for ethnicity, education, maternal age, parity and maternal smoking) (Q1:OR = 3.59[1.58–8.16]; Q2:OR = 2.63[1.12–6.17]; Q3:OR = 2.73[1.17–6.41]; Q4:OR = 2.75[1.18–6.42]; Q5:reference group). Stratified analyses showed a much stronger association between vitamin B_{12} concentration and excessive crying among women who experienced one or more psychosocial problems during pregnancy compared to women who did not. Folate concentration was not significantly associated with excessive crying.

Conclusions: This explorative study showed that maternal vitamin B_{12} status during early pregnancy is associated with excessive infant crying in the first months after birth. This association is modified by the presence of maternal psychosocial problems during pregnancy. Maternal folate status is not associated with excessive infant crying.

1. M.F. Van der Wal *et al., J. Dev. Behav. Pediatr.,* 28:431–437, 2007.
2. M.M. Black. *Food Nutr. Bull.,* 29:S126–S131, 2008.
3. M. Okawa *et al., Sleep*, 13:5–23, 1990.

P-2A-37

Life long oestrogen exposure and later adulthood cognitive function in naturally post menopausal women from Southern China: The Guangzhou Biobank Cohort Study

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Objective: In animal and in vitro studies oestrogen has both neurotrophic and neuroprotective properties. Epidemiological evidence is inconclusive, but suggests a positive association between reproductive period and later life cognitive function. We examined the interrelationships of several reproductive milestones, all proxies of endogenous oestrogen exposure, with cognitive function in a large cohort of older Southern Chinese women.

Methods: Structural equation modeling (SEM) was used in a cross-sectional study of naturally post menopausal older (⩾50 years) Chinese women from the Guangzhou Biobank Cohort Study (phases 2 and 3) to examine the interrelationship of age of menarche, age of menopause, age of first pregnancy, parity and duration of breast feeding with cognitive function assessed by the immediate and the delayed 10-word recall task in 10,875 women (phases 2 and 3) and the mini-mental state examination (MMSE) in 5,235 women (phase 3).

Results: Earlier age of menarche and later age of menopause were associated with better cognitive function on all three outcomes, adjusted for age, education, childhood and adulthood socio-economic position and current physical activity: one year earlier age of menarche was associated with 0.02 (95% confidence interval 0.008 to 0.03) more words on the 10-word delayed recall task and one year later menopause with 0.02 (0.004 to 0.03) more words. Lower parity, younger age at first pregnancy and shorter duration of breast feeding were also associated with better cognitive function and did not affect the associations of age of menarche or menopause with cognitive function. Further adjusting for adiposity (waist hip ratio and body mass index), skeletal growth (leg length and sitting height) and cardiovascular risk factors (blood pressure, lipid profile and fasting plasma glucose) did not change the pattern of results.

Conclusion: In a large cohort of naturally postmenopausal women from a population with very different socio-cultural and reproductive histories to those usually studied, we found most proxies of higher endogenous oestrogen exposure associated with better cognitive function. Greater endogenous oestrogen exposure throughout the life course may have long

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lasting benefits for cognitive development and maintenance, with corresponding implications for healthy aging. These findings support the biological evidence for a cognitively protective role of endogenous oestrogen and may add insight to the ongoing debate regarding the cognitive effects of Hormone Replacement Therapy. However, any attempts to modify these factors will need to consider any potential detrimental effects, such as increased risk of cardiovascular disease and breast cancer with younger age of menarche and loss of the beneficial effects of breast feeding for the mother and baby. Acknowledgements: The University of Hong Kong (HKSAR), Guangzhou Public Health Bureau (China), Guangzhou Science and Technology Bureau (China), The University of Birmingham (UK).

P-2A-38

Is better nutrition in childhood in a developing population associated with better cognitive function in later adulthood?: The Guangzhou Biobank Cohort Study

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Objective: There is growing evidence that early life exposures, such as childhood socioeconomic status, are related to later adulthood cognition. However, the specific aspect of early conditions underlying this association is not clear. Animal protein intake is positively associated with earlier walking in infants. Dietary supplementation with meat in infants and children in developing countries results in better cognitive function, independent of iron status. Protein energy supplementation with vegetables, milk and sugar (not meat) given from birth to 24 months in developing populations is associated with better cognitive function in early adulthood (mean age 32 years), especially amongst women. Inadequate childhood nutrition is associated with poor short term academic and cognitive outcomes. However, it is not known whether childhood nutrition has life long effects on cognitive function. We examined the association of childhood meat eating with adulthood cognitive function in southern China where the older population lived through significant hardship during their early years.

Methods: Multivariable linear regression was used in a cross-sectional study of 20,086 Chinese men and women aged ⩾50 years from the Guangzhou Biobank Cohort Study (phases 2 and 3) 2005–8. We assessed the association of childhood meat eating with amnesic-MCI and delayed 10-word recall score. The 10-word recall is a test of new learning ability from the CERAD (Consortium to Establish a Registry for Alzheimer's Disease) test battery which has been validated as a culturally and educationally sensitive tool for identifying dementia in population based research in developing countries. Amnesic-MCI was defined as a delayed recall score of 3 or less out of 10, corresponding to 1 standard deviation below the mean.

Results: Adjusted for age, sex and education, childhood meat eating 1–6 days per week and daily childhood meat eating were associated with a higher 10-word recall score (number of words recalled = 0.08 [95% confidence interval = 0.02 to 0.13] and 0.24 [0.16 to 0.33] respectively) and with lower odds of amnesic-MCI (odds ratio = 0.80 [95% confidence interval = 0.72 to 0.89] and 0.79 [0.67 to 0.94] respectively). Additional adjustment for childhood and adulthood socio economic position and current physical activity attenuated these findings, however daily childhood meat eating remained associated with a higher 10-word recall score (0.17 [0.08 to 0.26].

Conclusions: A diet that includes a small amount of daily meat in childhood (after infancy) may have long-term positive effects on cognitive function. If confirmed, these results highlight the importance of adequate childhood nutrition. Alternatively childhood meat eating may reflect a generally more cognitively protective childhood environment and nutrition. Irrespective, these findings also emphasise the childhood and adolescent antecedents of adult disease, with corresponding public health implications for healthy aging. Future research should examine the role of childhood exposures in long term cognitive development and if a role for childhood meat eating is verified, should elucidate the type and quantity of macro and micro nutrients that may be cognitively protective and the biological mechanisms behind these effects, so that preventive strategies can be implemented. Acknowledgements: The University of Hong Kong (HKSAR), Guangzhou Public Health Bureau (China), Guangzhou Science and Technology Bureau (China), The University of Birmingham (UK).

P-2A-39

Transgenerational effects of prenatal synthetic glucocorticoid exposure on molecular regulation of the pituitary gland

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Objective: Approximately 10% of pregnant women are at risk of preterm labour. Almost 50% of infants born preterm develop respiratory distress syndrome (RDS). Pregnant women at risk of premature delivery are administered

synthetic glucocorticoids (sGCs) to promote fetal lung maturation and greatly reduce the prevalence of RDS. Until recently, many centres were treating with multiple courses of sGCs. However, we and others have shown in animal models that in *utero* exposure to sGCs permanently modifies hypothalamic-pituitary-adrenal (HPA) function. Recently, we have shown that these modifications extend into the second generation (F_2). Specifically, the F_2 generation offspring of grandmaternal guinea pigs that had been exposed to 3 repeated courses of sGCs exhibit both a reduction in basal HPA function as well as reduced responsiveness to various stressors, despite no manipulation of the F_1 generation mothers during pregnancy. In the present study, we hypothesized that the reduction in basal HPA activity and reduced responsiveness to a challenge in F_2 offspring results from transgenerational influences of sGCs on pituitary expression of HPA-related genes.

Methods: Pituitaries were collected from adult male and female offspring (F_2) of mothers (F_1) that had themselves been prenatally exposed to betamethasone (1 mg/kg; BETA), dexamethasone (1 mg/kg; DEX) or saline (VEH). Anterior pituitary expression of proopiomelanocortin (POMC), adrenocorticotrophic hormone (ACTH), corticotrophin releasing hormone receptor 1 (CRH-R), arginine vasopressin receptor 1b (AVP-R) and glucocorticoid receptor (GR) mRNA and protein were analyzed using *in situ* hybridization and western blot analysis.

Results: Grandmaternal exposure to BETA resulted in a significant decrease in the expression of POMC mRNA ($p < 0.01$) in the anterior pituitaries of F_2 male and female offspring. There was also a corresponding reduction in ACTH protein ($p < 0.05$). Compared to controls, the F_2 BETA females also displayed a significant decrease in GR mRNA ($p < 0.05$) and protein ($P < 0.01$), as well as a significant reduction in CRH-R mRNA ($P < 0.05$). F_2 BETA males displayed a trend towards increased GR mRNA. F_2 DEX animals exhibited intermediate expression profiles – consistent with the degree of modification of the HPA axis.

Conclusions: There are transgenerational influences of prenatal exposure to sGCs on HPA function in adult F_2 offspring, and the present data indicate that these effects are mediated, in part, by altered molecular regulation within the anterior pituitary. The reduction in POMC, ACTH and CRH-R expression is consistent with the inability of these animals to mount a normal HPA response to stress. BETA, as compared to DEX, has more pronounced transgenerational effects on HPA-related gene expression, and this mirrors the magnitude of the effects of BETA and DEX on F_2 HPA phenotypes. The difference in sGC efficacy may be due to differences in formulation and metabolism of these compounds. We are currently investigating whether these transgenerational effects extend to the hypothalamus and higher brain centres. These findings clearly have implications for the clinical use of sGCs, and underscore the importance of avoiding overuse of these compounds in pregnancy. Funded by: Canadian Institutes for Health Research.

P-2A-40

Birth weight is associated with hyperactivity among primary school children in rural Malaysia

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Objective: Low birth weight continued to be one of the major problems among children and mothers, especially those living in rural area. It is one of the factors that affect brain development and cognitive functions[1]. However, the implication of low birth weight on children behaviour needs special attention. Hence, the main objective of this study was to determine the association between birth weight with behavioural problems among primary school children.

Methods: A cross-sectional study was conducted in randomly selected five rural primary schools in Kelantan, which is a state located at north-east Malaysia. This study involved 210 children aged between 6–10 years old comprising of 106 boys and 102 girls. Socioeconomic data were obtained using a standard questionnaire, relevant anthropometry data such as weight and height were obtained using standard methods. Body fat was also measured using Innerscan® Body Composition monitor (Model BC545, Tanita, Japan). Birth weight data was obtained from the birth certificate. Behavioural problems were assessed using a Malay Language translated and validated Strength and Difficulty Questionnaire (SDQ). There were 25 items of psychological attributes that were divided into 5 scales, namely emotional symptoms, conduct problems, hyperactivity, peer relationship problems and prosocial behavior. Appropriate institutional ethics committee clearance and participants' informed consent were obtained. Parents' informed consent was also obtained.

Results: The results showed that 15% children were born with low birth weight and the rest (85%) were born with normal birth weight. Overall, birth weight ranged from 1.5 kg to 4.8 kg.Low birth weight children had consistently higher mean score for all of the SDQ scales compared to normal birth weight children but the difference was not statistically significant. However, among the SDQ scale, birth weight showed significant and inverse correlation with hyperactivity scale ($r = -0.2$, $P < 0.05$).

Conclusions: Low birth weight is a public health concern and it is important to overcome this problem as it affects the behaviour of the children. More intensive research is required to highlight the importance of developmental origins on children behaviour especially among rural community.

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Table 1. The association between birth weight and SDQ scales.

	Strength and Difficulty Questionnaire Scales					
Birth weight	Emotional symptom	Conduct problems	Hyperactivity	Peer relationship problems	Pro-social behavior	TOTAL
r value	0.1	−0.1	−0.2	0.1	0.0	−0.1
P value	0.47	0.96	0.04	0.27	0.97	0.69

1. S.M. Grantham-McGregor *et al.*, *Proc. Nutr Soc.*, 59: 47–54, 2000.

P-2A-41

Apgar scores at birth predict autonomic nervous system activation later in childhood

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Objective: The neonatal Apgar score, assessed at one and five minutes after birth, consists of a standardized evaluation of heart rate, respiratory effort, muscle tone, facial response to stimulus, and peripheral circulation – all measures reflective of, or influenced by, regulatory processes in the autonomic nervous system (ANS). We hypothesized that Apgar scores might index early autonomic reactivity to the stress of birth and could thus be associated with later standardized measures of ANS reactivity.
Methods: In a cohort study of 270 children of low-income, Latino farm workers near Salinas, California, demographic and health measures were collected at birth and ANS reactivity was evaluated at ages 6 and 60 months using standardized, developmentally appropriate protocols for rest and responses to challenges. ANS measures included cardiac pre-ejection period (PEP) and respiratory sinus arrhythmia (RSA), representing the sympathetic and parasympathetic branches of the ANS, respectively. Mean PEP and RSA resting, challenge (mean across challenges) and difference (challenge minus rest) scores were calculated. Profile scores summarized patterns of activation (+) or inhibition (−) of sympathetic (S) and parasympathetic (P) responses (i.e., S+/P+, S−/P−, S+/P–, S−/P+).[1] ANOVA and logistic regression were used to assess relations among Apgar scores and 6- and 60-month profiles of ANS regulation. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The cohort comprised children of predominantly low-income (97%), Spanish-speaking (96%), first-generation immigrant (86%), married (82%) mothers (mean age 26.1, SD 5.2 years). Of the study sample (mean gestational age 38.8 weeks, SD 1.9), 147 (54.4%) were evaluated at 6 months, 214 (79.3%) at 60 months, and 91 (33.7%) at both. Children at both time points were statistically representative of the cohort as a whole. Using ANOVA, Apgar scores were not associated with mean sympathetic or parasympathetic resting, challenge or difference scores at 6 or 60 months. However, at both ages Apgar scores were associated with specific ANS profiles. At 6 months, low 5-minute scores predicted a "reactive" ANS profile (S+/P−) with odds ratio 3.24 (95% CI 0.98–10.7, $p = .05$) adjusted for sex, birthweight, gestational age, maternal age, parity and cesarean delivery. At 5 years, both low 1- and 5-minute Apgars exclusively predicted "co-activation" (S+/P+) profiles with adjusted ORs 3.60 (95% CI, 1.62–8.03, $p < 0.01$) and 3.68 (95% CI, 1.44–9.38, $p < 0.01$), respectively.
Conclusion: Low neonatal Apgar scores, after adjustment for important confounders, were strongly and significantly associated with profiles of autonomic regulation later in childhood. Though no link was found to sympathetic or parasympathetic activation alone, low Apgar scores were associated with a "reactive" ANS profile at 6 months and a "co-activation" profile at 60 months. These findings suggest that abnormal responses to birth stress, as marked by low Apgar scores, may reflect an early predisposition to ANS reactivity followed by parasympathetic compensation over time to a more adaptive sympathetic/parasympathetic balance by five years of age. Associations between Apgar score at birth and later physiological measures have not been demonstrated previously and suggest possible longer-term health and developmental implications.

1. A. Alkon *et al., Dev Psychobiol.*, 42:64–78, 2003.

P-2A-42

Low protein diet *in uteri* affects the cerebral renin-angiotensin system expression and impairs centrally angiotensin II dipsogenic responses in rats

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Studies in maternal protein restricted diet animals confirmed the link between alterated hypothalamic-pituitary-adrenocortical (HHA) system and adult cardiovascular, metabolic and behavioral disease. In rats, angiotensinogen is synthesized in many areas of the brain. Although no renin expression appears to occur in the brain, recently discovered tonins can produce the active peptides angiotensin II (AngII) and III. However, to our knowledge, there is little information in the literature concerning the effects of maternal protein-restricted diet on the expression of components of the intracellular

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renin-angiotensin system (RAS) in the brain and, the contribution of this system to dipsogenic behavior in rats.
Objective: Investigate the effects of maternal low protein diet exposure *in uteri* on centrally RAS signaling compounds and intracerebroventricular (*i.c.v.*) AngII thirst-induced response.
Methods: Virgin female Wistar rats were fed during pregnancy a normal-protein diet (NP17% casein, n = 6) or protein-restricted diet (LP6% casein, n = 6). The male pups were followed and maintained with normal chow until adulthood. Cerebral tissues were obtained from male offspring of time-mated rats at 16 week-old. Protein expression of AngII receptors (AT1R and AT2R) and intracellular RAS pathways was measured (n = 6) by western blot. To examine the thirst-induced effect after i.c.v. AngII administration, 16-week-old rats were randomly assigned to one of the following protocol groups: (a) *i.c.v.* single-dose of 3 μl, 400pmol or 4 nmol AngII (Experimental) compared to (b) *i.c.v.* 3 μl 0.15 M NaCl (Control) and, the volume of water drunk was measured during the next 30 min.Data are presented as mean ± SEM and n refers to animals sourced from separate litters. Data obtained over time were analyzed using appropriate ANOVA. *Post hoc* comparisons between selected means were made by Bonferroni's contrast test.
Results: The arterial blood pressure increased significantly more in LP than in NP rats at 16 weeks of age; LP pressure increased from 116.2 ± 6.5 mmHg to 137.9 ± 6.9 mmHg ($P < 0.01$), as compared with a smaller and non-significant rise from 114 ± 7.4 mmHg to 128.8±8.7 mmHg in NP. In LP, this significant rise appeared after 12 weeks of age. Western blot analysis in male offspring of NP and LP rat cerebral tissues yielded a single band at the expected weight of corresponding proteins. The expressions of AT_1R (71.33%) and SOCS-3 (43.8%) protein studied in the brain of 16-week-old LP rats were significantly lower, when compared to NP rats ($P < 0.001$). Conversely, the AT2R expression increased in LP (141.56%) when compared to NP offspring ($P < 0.05$). The *i.c.v.* AngII injection on dipsogenic response in LP offspring showed significant decreased water consumption over 30 minutes in rats to 4 nmol dose compared to response in NP rats (LP = 1.35 ± 0.76 ml *vs.* NP = 3.27 ± 1.35 ml, $P < 0.05$).
Conclusion: The present study might indicate that, also in the brain areas, protein underfeeding exerts a modulator effect on AngII receptor expression. Although the precise mechanism responsible for the subsequently fall in water AngII-induced consumption in LP offspring rats is still unclear, at first time, the current study supports the association of decreasing *i.c.v.* angiotensin-induced dipsogenic responses with also decreased central AT1R/AT2R receptor rate when compared with age-matched NP rats. Support: FAPESP.

P-2A-43

Association between antenatal maternal anxiety and neurocognitive functioning in five year old children

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Objective: Symptoms of anxiety and stress are reported to occur frequently during pregnancy. These antenatal maternal factors are known to have a negative influence on the behavioural and emotional development of the offspring. Increasing empirical evidence is found for an association between prenatal exposure to maternal anxiety and altered cognitive functioning. So far, little attention has been paid to the effects of antenatal maternal anxiety on the cognitive functioning of children in the preschool age. The current study aims to assess the relation between maternal anxiety during pregnancy and several aspects of cognitive functioning at the age of five.
Methods: The present study is part of a longitudinal community based multi-ethnic cohort (ABCD study). Antenatal maternal anxiety was measured using the State-Trait Anxiety Inventory around the 17th (SD = 4) week of pregnancy. Children's neurocognitive profile is currently examined using four tests from the Amsterdam Neuropsychological Tasks. These tasks are estimates of sensory-motor speed, response speed stability, visuo-motor skills, response selection and response inhibition. Preliminary analyses using a subsample ($N = 556$) are based on a single neurocognitive task that estimates sensory-motor speed and response speed stability. We expect to assess and evaluate the complete neurocognitive profiles (including visuo-motor skills, response selection and response inhibition) of approximately 2000 children in the coming year. Analyses were conducted using the regression function in SPSS 17.0 (SPSS Inc.). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Antenatal maternal anxiety (Mean = 35.83, SD = 9.48) was associated with a delay in children's sensory-motor speed ($F(17, 462) = 2.34$, $p < 0.01$) and less stability in children's response speed ($F(17, 462) = 1.69$, $p < 0.05$), even when corrected for possible confounding variables: birth weight, gestational age, parity, maternal ethnicity, SES, postnatal anxiety and depression, antenatal smoking and alcohol use. No relation was found between antenatal maternal anxiety and response inhibition or errors made by the children.
Conclusions: Promising preliminary results supported our hypothesis that five year olds, who were exposed to high levels of maternal anxiety and stress in utero, have a compromised neurocognitive profile compared to children prenatally exposed to low levels of maternal anxiety.

P-2A-44

Association of perinatal factors and school performance in fourth graders: a nationwide cohort of Chilean children

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Objective: To analyze the association between school performance and perinatal variables with national information of primary school children and to determine whether this possible association is influenced by either the socio-economic characteristics of the family or the educational environment.

Methods: School performance was measured using national data from the SIMCE test for the measurement of educational quality, Ministry of Education, Chile, which is taken annually and nationwide to evaluate educational achievements.[1] A historical cohort of fourth graders who took the SIMCE test in year 2006 and had full perinatal data was studied.[2] Perinatal information was obtained from the National Registry of live births.[3] Both databases were anonymously linked using the official individual identification number assigned at birth to each child. Outcomes were achievement levels in the SIMCE language and mathematics tests according to sex, birth weight (BW), birth length (BL), gestational age, maternal years of education (MYE) and type of school ownership (TOA). SIMCE scores are classified in three achievement levels: poor, intermediate and advanced, according to specific cut-off points determined using a standardized procedure by the Ministry of Education.[2] SPSS version 15.0 was used for statistical analysis which included contingency tables with chi square and logistic regression to calculate odds ratios (OR) for poor achievement. Univariate and multivariate analyses were performed. Appropriate institutional ethics committee clearance was obtained.

Results: 247.648 subjects entered the study; 96.7% of the children who took the SIMCE tests that year. Males represented 50.8% of the study population. LBW (<2,500 g) and preterm (⩽37 weeks) incidences were 4.6% and 10.9%, respectively. 48% of children attended public schools and only 6.3% attended private schools. Females had better results in language, and males in math. The distribution of language scores resulted in 40%, 27.2% and 34.7% for poor, intermediate and advanced achievement levels, respectively. The distribution of mathematics scores resulted in 38.9%, 34.7% and 26.4% for poor, intermediate and advanced levels of achievement, respectively. Univariate analyses of each unadjusted OR for poor achievement showed an inverse association of BW and BL in both tests, stronger for BW and for mathematics. Level of achievement improves with higher BW, up to 3501–4000 g for language and in all BW categories for mathematics. These results were similar when using multivariate analysis adjusting for MYE and TOA.

Conclusions: This study showed an inverse and graduated association between selected perinatal variables and the proportion of poor achievement in both SIMCE tests, which is independent of the socio-economic characteristics of the family and the educational environment. Our results are similar to previously published data from developed countries.[4,5] This is one of the first studies to show this association in a developing country.

1. SIMCE web page: http://www.simce.cl/ Accessed March 2008.
2. SIMCE database [2006]. Santiago, Chile: SIMCE, Ministry of Education, 2006.
3. Yearly live births databases [1994–1998]. Santiago, Chile: D. of Statistics, Ministry of Health.
4. M. Richards *et al.*, *BMJ.,* 322:199–203, 2001.
5. T.D. Matte *et al.*, *BMJ.,* 323:310–314, 2001.

P-2A-45

Maternal seafood consumption and neurodevelopment at 14 months in a prospective Spanish cohort: The INMA study

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Objectives: Maternal seafood consumption during pregnancy may be beneficial for child neurodevelopment, perhaps via mechanisms involving fatty acids and other nutrients concentrated in certain types of seafood. However, at high intakes, seafood consumption may increase fetal exposure to potentially neurotoxic contaminants, some of which are lipophilic and may be most abundant in fatty fish. Relatively few population-based studies to date have sufficient data on women at high intakes to adequately explore these effects. This study uses data from a population-based sample of women from the Mediterranean coast of Spain, a setting with high seafood intakes, to further examine these relationships.

Methods: Data come from the INMA (Environment and Childhood) birth cohort in Sabadell, Barcelona, which recruited 657 women in the 1st trimester of pregnancy. Neurodevelopment was assessed in 561 children at 14 months using the Bayley Scales of Infant Development. Maternal seafood intakes were assessed using a food frequency questionnaire (FFQ) administered in both the 1st and 3rd trimesters. Maternal serum organochlorine levels and cord blood mercury were measured. Multivariable linear regression was used to assess associations between different types of seafood and test scores in full-term infants, adjusting for covariates including maternal education, parity and breast-feeding duration. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

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Results: Mean intakes of lean fish in the 1st and 3rd trimesters were linearly and positively associated with test scores after multivariate adjustment, but associations with fatty fish were non-linear. Compared to non-consumers, children whose mothers reported intermediate intakes of fatty fish (7–20 g/d) had significantly higher test scores, while the highest intakes (20–80 g/d) were associated with lower scores (NS). Trimester of intake appeared to influence associations particularly for fatty fish, as positive associations for moderate consumption were observed only for intakes reported in early pregnancy. Adjusting for PCB levels slightly strengthened associations; adjustment for mercury had no meaningful effect. Other seafood types were not meaningfully associated with performance.

Conclusions: These results suggest maternal intakes of both lean and fatty fish are positively associated with child neurodevelopment at 14 months, but that elevated fatty fish intakes may not be beneficial. Similarly, another Spanish cohort found beneficial effects of maternal fish intakes up to 3 times/week on neurodevelopment at 4y, but lower scores among children exposed to higher intakes. Additional studies in other high-intake settings are needed to better understand these relationships. Support: Spanish Ministry of Health, Instituto de Salud Carlos III, the Generalitat de Catalunya-CIRIT, The European Union projects EARNEST FOOD-CT-2005-007036 and NewGeneris FOOD-CT-2005-01632.

1. M. Mendez *et al., Public Health Nutr.,* 25:1–9, 2008.

P-2A-46

Neonatally-handled rats show different reactions to changing the position of the food in a sex-specific manner

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Objective: Early life experiences have profound influences on neuroendocrine systems and on behavior in the adult life. Rats subjected to repeated brief maternal separation (handling) during the first two weeks of life show reduced stress response, are less emotionally reactive, consume more palatable food, and show different performances in learning and memory tasks. The higher motivation to ingest palatable food may be related to hedonic factors, or to the formation of a habit. The purpose of the present study is to evaluate the effects of neonatal handling on the habituation to a sweet food in a Y-maze, and the effects of changing the position of the food in the maze, using male and female animals. In addition, we studied memory in a water-maze and the effects of changing the position of the platform (the animals should switch behavior from one mode of responding to another, apart from motivational changes).

Methods: Pregnant Wistar rats were randomly selected. Litters were divided into non-handled and handled. In the handled group, pups were placed in an incubator 10 min/day, days 1–10 after birth. Behavior procedures started after postnatal day 60. 38 males and 20 females were used. Sweet food was placed in one arm of the Y-maze, which was explored for 5 min, during 5–7 days. Afterwards, the food was placed in the other arm. Number of entries and consumption were measured. Performance in the water-maze test was also evaluated. This behavioral task consisted of two phases: (1) acquisition, where rats were trained for 7 days, four trials/day, to find a submerged platform; (2) reversal learning, when the platform place was changed and animals were trained for 3 days. The latency to find the platform was measured.

Results: Results were analyzed by repeated measures ANOVA. On the Y-maze handled animals increased sweet food consumption during the habituation (males, $P < 0.01$; females, $P < 0.001$). When the sweet food was placed in the other arm, handled females continued consuming more sweet food ($P = 0.05$), but handled males consumed the same as controls ($P > 0.05$). Handled rats did more entries ($P < 0.05$, both arms). We next evaluated perseverative behavior in the water-maze. No significant differences were observed between handled and control male animals.

Conclusion: There was a sex-difference in the way males and females handled rats react to a change in the position of a reward. This behavior in the males is not related to perseverative behavior in general, since these animals were able to find a new position of the platform in the water-maze. We believe that factors such as anxiety or perception of sweet flavor could modify the final answer. These possibilities however need to be tested to a better understanding. Support: CNPq and CAPES.

P-2A-47

Cognitive abilities in young adults with very low birth weight

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Objective: Very low birth weight (VLBW, ⩽1500 g) constitutes a risk for cognitive impairments and lower IQ in

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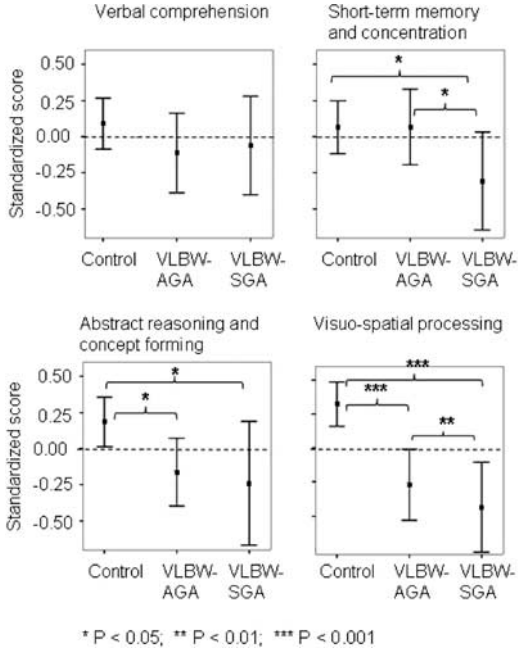
later life, even if individuals with major handicaps are excluded. Some evidence also suggests that intrauterine growth restriction defined as being small for gestational age (SGA, birth weight for gestational age $\leqslant -2$ SD) can be related to impaired cognitive abilities. However, studies concerning adulthood are scarce and effects of both VLBW and SGA status are rarely tested together. Our aim was to examine whether VLBW influences the neurocognitive performance in young adulthood and whether being born SGA or appropriate for gestational age (AGA) has an effect on these outcomes.

Methods: A total of 103 VLBW adults without neurosensory impairments and 105 term-born controls participated in the study. Of the VLBW adults, 37 (35.9%) were born SGA. Mean age of the participants at the time of the cognitive testing was 24.6 years (SD 2.1 years). Cognitive abilities were tested using four subtests of the Wechsler Adult Intelligence Scale (WAIS) III. These subtests measured verbal fluency and comprehension (Vocabulary), verbal short-term memory and concentration (Digit Span), abstract reasoning and concept forming (Similarities), and visuo-spatial perception and processing (Block design). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The figures present the mean performance ($\pm$95% confidence intervals) in standardized scores for the control, VLBW-AGA and VLBW-SGA adults in four subtests of WAIS III, adjusted for sex, age and parental education. Scores represent the difference from the mean score of the whole sample in SD units.

Conclusions: Results related to four subtests of WAIS III indicate that the neurocognitive performance is weaker among VLBW adults compared to adults born at term. This impairment pertains particularly to verbal and nonverbal tasks that require abstract reasoning and processing. Being born SGA may increase the risk of impaired cognitive performance even within the VLBW group.



P-2A-48

Low-moderate prenatal alcohol exposure and risk to child behavioural development

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Objective: A high maternal consumption of alcohol during pregnancy has been associated with developmental risks for the fetus. However, associations with low-moderate drinking in pregnancy have been inconsistent with alcohol exposure during pregnancy proving difficult to measure accurately in many studies. The aim of this study was to examine the association of fetal alcohol exposure during pregnancy with child and adolescent behavioural development.

Methods: In the Western Australian Pregnancy Cohort (Raine) Study, 2,900 women provided data at 18 and 34 weeks gestation on weekly alcohol intake: no drinking, occasional drinking ($\leqslant$ one standard drink per week), light drinking (two-six standard drinks per week), moderate drinking (seven-ten standard drinks per week) and heavy drinking ($\geqslant$ 11 standard drinks per week). Their children were followed up at ages two, five, eight, ten and 14 years. The Child Behaviour Checklist (CBCL) was used to measure child behaviour. Regression models were used to analyse the effect of prenatal alcohol exposure on CBCL scores over 14 years, assessed by continuous z-scores and clinical cut-points, after adjustment for confounders. The control variables included maternal sociodemographic information from the prenatal period as follows: maternal age, maternal education, family income, the presence of the biological father in the family home, maternal smoking and maternal experience of stressful events in pregnancy. The child's age at follow-up was also adjusted for in the models. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Light drinking and moderate drinking in the first three months of pregnancy were associated with child CBCL z-scores indicative of positive behaviour over 14 years after adjusting for maternal and sociodemographic characteristics. These changes in z-score indicated a clinically meaningful reduction in total (OR = 0.63, 95%CI = 0.46, 0.86), internalizing (OR = 0.57, 95%CI = 0.42, 0.76), and externalizing (OR = 0.69, 95%CI = 0.51, 0.93) behavioural

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problems across the 14 years of follow-up for the children of light drinkers compared with the children of non-drinkers at 18 weeks gestation. Alcohol intake at 34 weeks gestation showed no significant relationship with child behavioural problems.

Conclusions: Low levels of alcohol consumption by women in early pregnancy do not appear to be harmful to the subsequent mental health of the offspring whereas high levels of alcohol exposure during pregnancy should be discouraged during pregnancy due to the consistent finding across multiple studies that high levels of alcohol exposure during pregnancy are associated with an increase in adverse outcomes for the offspring. Our data suggest that women who conceive unexpectedly whilst consuming limited amounts of alcohol have not placed their unborn child at increased risk of behavioural problems during childhood. *Abbreviations*: OR (odds ratio), 95%CI (95% Confidence Intervals)

P-2A-49

Intrauterine growth restriction is associated with increased impulsivity using the Snack Delay Test in 3 years old girls

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Objective: Low birth weight increases the risk for cardiovascular diseases, diabetes and overweight in adulthood. Intrauterine growth restriction (IUGR) is associated with an increased preference for carbohydrates over protein in adult women (1). Because eating disorders associate with impulsive behaviors, we investigated the association between IUGR and impulsivity using a snack-delay task in 3 year-old Canadian children.

Methods: Eighty-eight children derived from a cohort based in two different cities in Canada (Montreal and Hamilton) were tested using the Snack Delay Test at 36 months of age as part of the Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN) project. During this test, the child, with hands on the table, must wait for the experimenter to ring a bell before retrieving a Smartie® from under a glass cup (four trials, delays of 10, 20, 30, and 15 sec). The ability to wait for the signal is the measure of impulsivity. IUGR was defined by a birth weight ratio lower than 0.85 (BWR = children's birth weight/mean population birth weight, gender and gestational age specific) (2). Data were examined using a repeated-measures ANOVA with IUGR category as the independent variable and the time to reward in the different trials as dependent variable. A correlational analysis between time to reward and BWR (used as a continuous variable) was also performed.

Results: IUGR girls showed a shorter waiting time in the different trials ($p < 0.05$). In addition, in IUGR girls BWR correlated with time to reward at a level that reached a borderline significance ($p = 0.06$). None of these effects were detected in boys.

Conclusions: IUGR interacted with gender to increase impulsivity in 36 month-old girls in a task using palatable food as a challenge. Considering that IUGR women also prefer to eat more carbohydrates in adulthood, it is plausible that impulsivity is an important behavioral component influencing feeding behavior in IUGR girls/women. This altered feeding preference could contribute to an increased risk for binge eating, obesity, metabolic syndrome and related disorders in adulthood in this group.

1. M.A. Barbieri *et al., Pediatr Res*., 65:215–220, 2009.
2. M.S. Kramer *et al., Pediatrics*, 103:599–602, 1999.

P-2A-50

Morningness propensity among young adults born prematurely – The Helsinki Study of Very Low Birth Weight Adults

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Objective: In a previous study concerning sleep in young adults born prematurely at very low birth weight (VLBW; <1500 g), we found that adults with VLBW go to bed and get up earlier than their term-born peers, suggesting an advanced sleep phase. Because that finding was *post hoc* and since circadian rhythm disturbances may adversely affect health, we now conducted a prospective follow-up to assess circadian rhythmicity.

Methods: During 2007–2008, 218 young adults (aged 21 to 29 years) from the *Helsinki Study of Very Low Birth Weight Adults* participated in a follow-up study. Of them, 190

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(87.2%) filled out the Morningness-Eveningness Questionnaire (MEQ). Of the participants, 97 (51.1%) were born prematurely at VLBW, and 95 (48.9%) at term gestation. The MEQ is a widely used method to measure self-reported individual differences in circadian preferences, such as preferred bed times/awakening times and perceived optimal times for physical and mental activities. A summary score is generated from the questionnaire, with higher scores indicating more tendency towards morningness. We excluded 6 individuals with neurosensory impairments (blindness, cerebral palsy) from the analyses. Information on current health status and working conditions was gathered by self-report in conjunction with the clinic visit. T-tests and ANCOVA were used to compare the two groups. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Young adults born at VLBW scored higher than term-born controls on the MEQ (47.1 versus 44.6, group difference 2.4, 95% confidence interval [CI] 0.5 to 4.4, $p = 0.016$). After adjustment for age and gender (group difference 2.4, 95% CI 0.4 to 4.4, $p = 0.019$) and additionally for depressive symptoms measured by the Beck Depression Inventory (group difference 2.2, 95% CI 0.3 to 4.2, $p = 0.026$), the results remained significant. Controlling for working conditions (night shifts or current gainful employment) did not explain the findings (p-values ranging from 0.032 to 0.040).

Conclusions: Young adults born prematurely at very low birth weight report a propensity towards morningness. The finding may reflect biological programming of the suprachiasmatic nucleus. The finding is potentially significant, given the central role of circadian rhythms in promoting health.

P-2A-51

Intrauterine social exclusion by fetal programming: an experimental paradigm to poor countries reality

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According to Food and Agriculture Organization of the United Nations, one billion people will be suffering from starviness at the end of 2009. Epidemiological studies have shown adverse environmental factors leading to intrauterine growth retardation and low birth weight, suggesting individuals predisposing to later onset of metabolic syndrome. This has led to the concept of the developmental origin of adult diseases – "fetal programming". During mammalian neural development and maturation, stimulation from environment can influence brain development and function, and such effect is at least in part due to distinct sensory systems activation. In our laboratory we have verified that angiotensin, minaralo and glucocorticoid receptors, which has a key role in stress, anxiety response, modulation of exploratory behavior and facilitator role in learning and memory acquisition, is significantly altered in adult rats whose mothers were submitted to gestational undernutrition.

Objective: Evaluate physical, sensory motor and behavioral effects in rats exposed to undernutrition *in uteri*.

Methods: Pregnant rats were divided into two groups. The daily food supply of one group (FR50) was restricted to 50% of the food consumed by the other group (NF), fed ad libitum. Ano-genital distance (AGD) was measured at birth. After birth, were assessed sensory motor functions (palmar grasp, surface righting and negative geotaxis reflex), physical parameters (opening eyes, first body hair appearance, incisive eruption and ears unfolding). In the postnatal day 23 (PND23) offspring were tested in the open-field (OF), elevated plus maze (EPM) and hole board (HB) apparatus. In the PND90 animals were tested in OF and EPM only.

Results: HB tests showed none difference between FR50 and NF groups. AGD values were significantly higher in FR50 (2.56 ± 0.11 v 1.81 ± 0.05 $p < 0.0001$, in NP). Both PND23 and PND90 FR50 offspring presented lower scores to open-field freezing and grooming, impaired to higher ambulation and rearing frequency compared to NF. Number of entries and permanency time were increased in open arms to FR50 animals with consequent decreased both values in close arms. Sensory motor values were higher in FR50 to negative geotaxis and surface righting reflex.

Conclusion: Similar results obtained to PND23 and PND90 offspring suggest that alterations caused at intrauterine environment level persist from infancy to adulthood, by imprinting. Since maternal steroids cross placenta, raised maternal androgen levels during gestation may contribute to increased AGD in male pups of food-restricted mothers. It might be possible that precocious enhanced AGD indicate that alterations of intrauterine environment can result in development of disease in later life. Increased FR50 activity in EPM observed here reflects a conflict between rodent's preference for protected areas and innate motivation to explore novel environments. Sensory motor values alterations in FR50 may indicate labyrinth alterations, since negative geotaxis and surface righting reflex are associated to postural behaviors. Freezing and grooming behaviors have been used widely as a measure of fear in the open-field, and decreased values observed suggest lower levels of social reinstatement. These data reproduce characteristics clinically classified as marasmatic undernutrition, an epidemiologic public health problem in developing countries. Support: FAPESP.

P-2A-52

Association of birthweight and head circumference at birth to cognitive performance in 9–10 year old children in South India: prospective birth cohort study

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Objective: To examine whether birthweight and head circumference at birth are associated with cognitive ability in 9–10 year old South-Indian children.
Methods: We studied 505 (239 boys and 266 girls) healthy, full-term born children from the Mysore Parthenon birth cohort, for whom weight and head circumference were measured at birth. Their cognitive function was assessed at a mean age of 9.7 years using 3 core tests from the Kaufman Assessment Battery for children and additional tests measuring long-term retrieval/storage, attention and concentration, visuo-spatial and verbal abilities. We also collected data on a variety of potential confounders like maternal age, parity, gestational age at birth, season of birth, mode of delivery, sex, children's current age and size, time of cognitive testing, parents' area of residence, educational attainment and current socio-economic status. Associations between birth measurements and cognitive function were examined by multiple linear regression analysis using stata version 10. The study was approved by the Holdsworth Memorial Hospital, Mysore, research ethics committee and informed verbal consent was obtained from parents and children.
Results: Girls scored better than boys in word order (short-term memory), pattern reasoning (planning and fluid reasoning), verbal fluency-names (broad retrieval ability, speed and flexibility of verbal thought process), and coding-Wechsler Intelligence Scale for Children-III (visual-motor processing speed and coordination, short-term memory, attention and concentration) ($p < 0.05$ for all). In multiple linear regression, adjusted for a variety of confounding variables listed above, Atlantis score (learning ability/long-term storage and retrieval) rose by 0.1 SD (95% CI: 0.01 to 0.19; $p = 0.02$) and 0.1 SD (95% CI: 0.04 to 0.22; $p = 0.006$) per 1 SD increase in newborn weight and head circumference respectively. Kohs' block design score (visuo-spatial ability) increased by 0.1 SD (95% CI: 0.02 to 0.20; $p = 0.02$) per 1 SD increase in birthweight. These associations were similar in boys and girls. The associations were reduced, and mainly non-statistically significant, after adjusting for current head circumference, suggesting that they may be mediated partly by increased post-natal brain growth.
Conclusions: Higher birthweight and larger head circumference at birth are associated with significantly better learning, long-term storage and retrieval, and visuo-spatial abilities in childhood, independent of confounding factors such as socio-economic status and parents' educational attainment. The effect of pre-natal growth on childhood cognitive function may act partly through increased post-natal brain growth.
Acknowledgements: We are grateful to the families who participated in the study and to the entire research team who made substantial contribution to the study. Support: The Parthenon Trust, Switzerland, the Wellcome Trust and Medical Research Council, UK.

P-2A-53

Effects of prenatal glucocorticoids on the functional development of the cerebral cortex in sheep

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Antenatal betamethasone (BM) therapy is clinically used to accelerate fetal lung maturation in babies threaten premature labor. In spite of the benefit of such a therapy for the survival of preterm babies there is increasing concern because antenatal glucocorticoids inhibit brain growth.[1] While postnatal glucocorticoids affect neurodevelopment,[2] the functional consequences of antenatal glucocorticoids for intrauterine brain maturation are not clear. Postnatally, late neuronal responses to an auditory stimulus generated in the auditory cortex are an established marker of maturation of complex cortical brain function. Therefore, we detected for the first time development of cortical acoustic evoked potentials (cAEP) in the sheep fetus.
Objective: To examine if antenatal (BM) treatment has persistent effects on maturation of cAEP as a measure of functional brain development.
Methods: Pregnant ewes carrying chronically instrumented fetuses received three courses of BM (2×110 μg/kg body weight 24 h apart corresponding to 2×8 mg BM administered to a 70 kg pregnant woman, $n = 9$) or an equal volume saline ($n = 6$) at 105, 112 and 119 dGA (days gestational age, term 150 days). cAEP were induced before each BM course and six days after the last course using a tone of 500 Hz, 100 dB SPL and 50 ms duration with randomly chosen stimuli intervals of 0.8 1.2 sec applied at the maternal abdominal wall for 4×5 min.cAEP were recorded from the Cz position at the fetal skull and averaged from 100ms prior to 500ms after the stimulus. Peak latencies and amplitudes of the components P1, N1, P2 and N2 were detected. Results: cAEP were detected in 7/9 BM and 5/6 control fetuses at 105 dGA and in all fetuses at 111 dGA. Latency of all components decreased continuously until 125 dGA ($p < 0.05$, Fig. 1) reflecting ongoing myelination. Betamethasone delayed the developmental decrease of peak latencies. P2 and N2 did not and P1 and N1 started to decrease at 125 dGA. Compared to controls, latency of all components was delayed at 125 dGA ($p < 0.05$, Fig. 1). Developmental increase of the amplitudes reflecting maturation of cortical neuronal circuits was not affected by BM.

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Conclusions: Maturation of cAEP is affected even by a single course of BM. The results are in agreement with the delay in myelination shown previously.[3]

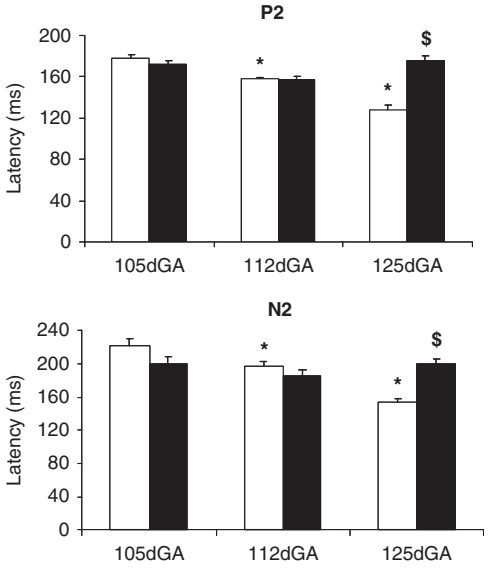

Fig. 1. Development of the latencies of the P1 and N2 in saline (white) and BM treated fetuses (black bars). Mean+SEM, *$p < 0.05$ compared to 105 dGA, $^{\$}p < 0.05$ compared to controls.

1. J.P. Newnham, T.J. Moss, *Sem Neonatol.,* 6:285–292, 2001.
2. T.F. Yeh *et al.*, *N Engl J Med.* 350:1304–1313, 2004.
3. I. Antonow-Schlorke *et al., Obstet Gynecol.,* 113:142–151, 2009.

P-2A-54

Effects of maternal smoking during pregnancy and preterm birth on physical size in late childhood: Possible Moderation by ADHD Status?

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Objective: Attention Deficit Hyperactivity Disorder (ADHD) is associated with a number of prenatal risk factors. Two of these are maternal smoking during pregnancy and preterm birth, and both are associated with low birth weight. The Developmental Origins of Health and Disease (DOHaD) concept recognizes low birth weight as a non-specific marker of adverse intra-uterine environments hypothesized to produce adaptive changes in organ structure and function with long-term consequences. We evaluated the effects of maternal smoking during pregnancy and birth weight on height, weight, and body mass index in school-aged children.

Method: Within the Multimodal Treatment study of children with ADHD (MTA) were selected two groups for evaluation: stimulant-naïve children with diagnoses of ADHD ($n = 83$) and a local normative control group (LNCG) of age and sex group-matched classmates ($n = 246$). From parent interview we obtained reports of birth weight, preterm status, and maternal smoking during pregnancy. We measured physical size and body composition during childhood at 9–11 years of age. Our primary analyses were conducted with a 0.05 significance level to evaluate in a $2 \times 2 \times 2$ design main effects of Smoking Status, Preterm Status, or Group and all of the interactions of these factors. Appropriate institutional ethics committee clearance and patients consent were obtained.

Results: The standardized birth weight for the pre-term children was lower than the non-preterm children for both the ADHD group ($z = -0.65$ for Preterm vs. $z = 0.05$ for non-Preterm) as well as the LNCG ($z = -0.66$ for Preterm vs. $z = 0.14$ for non-Preterm). The effect of Smoking Status on birth weight was evident in both groups also ($z = -0.76$ for Smokers vs. $z = 0.06$ for non-Smokers in the ADHD group and $z = -0.59$ for Smokers vs. $z = 0.07$ for Non-Smokers for the LNCG). In analyses of childhood size, the main effect of Smoking Status was not significant, but there was a significant interaction of Smoking Status x Group in the analyses of weight ($F(1,328) = 6.29$, $p = 0.013$) and body mass index ($F(1,326) = 4.89$, $p = 0.028$). Specifically, in the normative control group, children of mothers who smoked during pregnancy had higher weight and body mass index than children of mothers who did not, but this expected pattern was not observed in the ADHD group.

Conclusions: According to the DOHaD hypothesis, maternal smoking during pregnancy may result in low birth weight and produce a ''thrifty'' phenotype in offspring (e.g., increased insulin resistance) that contributes to higher weight and BMI in later life. This prediction was confirmed in the LNCG but not in the ADHD group. The ADHD children exposed to maternal smoking during pregnancy and having low birth weight were still lighter and had a lower BMI in childhood. This suggests that some prenatal influences on postnatal physical size in childhood may act differently in children with ADHD than in the general population. Support: The National Institute of Mental Health by a Cooperative Agreement for the design and implementation of the MTA.

P-2A-55

Increased corticosterone levels and decreased hypothalamic dopaminergic activity in neonatal rats submitted to cold stress

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Objective: The first two weeks of life in rodents is known as the "stress hyporesponsive period", in which the pups show decreased neuroendocrine responses to stress. However, this is a neurodevelopmental sensitive period in which the animal is susceptible to environmental events, such as stress and the quality of maternal care, which may have long-term effects upon the individual. Our study investigated plasma corticosterone and hypothalamic dopaminergic activity in neonatal rats submitted to cold stress. We also investigated the possible influences of gender and maternal care received.
Methods: In the end of 1° postpartum day (P0), the 60 Wistar dams had their litters culled to 8 pups. From P1 to P10, dams had their maternal behaviour analysed according to previous works, in which licking behaviour was taken as a measure of the quality of maternal care (population's mean licking frequency = 5,52 ± 0,1863). Dams showing the higher and lower frequencies of licking were selected as High Licking (mean > 6,991, n = 10) and Low Licking (mean < 4,1814, n = 12), respectively. At P13, a pair of pups was removed from the litter and immediately sacrificed by decapitation (control group) and another pair was removed from the litter and placed in a plastic container inside a cold chamber (0°C, 6 min), being sacrificed 15 min after the termination of the cold stress. Blood trunk was taken for plasma corticosterone radioimmunoassay. The brains were quickly removed for dissection of the hypothalami for dopamine and DOPAC High Performance Liquid Chromatography analysis. The dopaminergic activity was measured by the DOPAC/dopamine ratio (turnover).
Results: The three-way ANOVA with Duncan Post Hoc showed an effect of stress increasing plasma corticosterone ($p < 0,001$) and decreasing dopamine turnover ($p = 0,05$). There were neither gender nor maternal care differences in both measurements.
Conclusions: Stress increased plasma corticosterone in the pups. Surprisingly, we found that stress decreased the hypothalamic dopaminergic activity, as opposed to the effects usually found in adult and infant animals. We suggest that this is due to the peculiarity of cold stress, since the monoaminergic responses vary depending on the stressor used, additional to the particular neurochemical responses on neonatal period. Pups might have a differentiated hypothalamic dopaminergic activity driven by cold. Effects of gender and maternal care on corticosterone and dopaminergic activities are possibly seen only after the puberty.
Support: CAPES and CNPq.

P-2A-56

Nocturnal polysomnographic studies in a children's sample with Attention Deficit Hyperactivity Disorder, hyperactivity-type

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Children with ADHD can present a high prevalence rate of comorbidity with sleep disorders. The exact nature of these sleep problems are still to be determined.
Objectives: To describe the sleep disorders found by nocturnal polysomnographic study (PSG) in a sample of 10 children with diagnosis of ADHD with hyperactivity-impulsivity-type.
Methods: We studied 10 children (1 female and 9 males) with a mean age of 9,30 (SD: 4,498148) who met DSM-IV criteria of (ADHD) with hyperactivity-type ADHD (ADHD/H). They were evaluated by a nocturnal polysomnographic study and neurological exploration. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: All studies were abnormal, 7 (70%) of the children presented period movements legs syndrome (PMLS). The sleep architecture of 8 (80%) children with ADHD shows an increase in the percentage of phase III and consequently decreases of phase II of slow sleep. The REM latencies was increased in 7 (70%) of the studied children, whereas the REM percentage was diminished in 5 (50%). Epileptiform-type paroxysms were observed in 40% of the children who presented symptoms of ADHD/H and in 4 of them declarations of parasomnias were demonstrated, for 40%.
Conclusion: The increase in phase III may be related to the alterations in noradrenaline and dopamine transmission present in children who suffer from ADHD. Some children with ADHD can have a region of the brain with intense epileptic activity, which does not trigger epileptic seizures but gives rise to behavioural disorders.

P-2A-57

Long-lasting effects of maternal separation upon distinct memory tasks and DNA damage to the hippocampus in rats

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Objective: The first two weeks of life are a critical period for neural development in rats. Repeated long-term separation from the dam is considered to be one of the most potent stressors to which rat pups can be exposed, and permanently modifies neurobiological and behavioral parameters. Prolonged periods of maternal separation usually increase stress reactivity during adulthood, and enhance anxiety-like behavior. The purpose of the present study was to verify if repeated long-term maternal separation would affect performance in different memory tasks in adulthood; we also verified DNA damage to the hippocampus in different ages.
Methods: Male Wistar rats were subjected to repeated maternal separation (3h/day) during postnatal days 1–10. At 70 days of age, the subjects were exposed to sequential (7 days apart) different tasks to evaluate memory. The animals were sacrificed to analyse DNA damage to the dorsal and ventral hippocampus using the comet assay at 21 days of age and one month after the last behavioral evaluation (adult age).
Results: In the object recognition test, maternal separated animals showed a deficit in performance, exploring less the new object (Student's t test, $P < 0,05$). No effects were observed in the Morris water maze (repeated measures ANOVA, $P > 0.05$). In the inhibitory avoidance task, maternal separated animals showed a marginally significant difference, with higher latencies to step-down, especially when short-term memory was tested (90 min after training; Student's t test; $P = 0,08$). An increased defensiveness towards the cat odor (conditioning session) was detected in maternal separated rats (Student's t test; $P < 0,005$), whereas no significant effect was found during the context session 24h later. A higher score of DNA damage was observed in dorsal and ventral hippocampus of adult maternal separated rats (Student's t test, Adult: Dorsal, $P < 0,001$, Ventral, $P < 0,001$ and at 21 days of age: Dorsal, $P > 0,05$ and Ventral, $P > 0,05$).
Conclusion: These results suggest that an early stress experience such as maternal separation may increase damage to the hippocampus and also affect defensive behavior in adulthood, and this effect is task-specific. Performance was affected mainly in tasks involving emotional aversive contents, which underlies the process of memory consolidation. Support: CAPES and CNPq.

P-2A-58

Maternal smoking during pregnancy and chronic headache in schoolchildren

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Objective: To study the prevalence of chronic headache and its association with maternal smoking during pregnancy and other risk factors in schoolchildren aged 7–11 years.
Methods: Children from two population-based birth cohorts studied in Ribeirão Preto (RP) in 1994[1] and in São Luis (SL) in 1997/98[2], Brazil, were re-examined in 2004/05 and 2005/06, respectively, and mothers were re-interviewed[3]. Chronic headache was defined as at least two occurrences in the last 15 days before the interview, with or without aura[4]. Mothers were considered light smokers when smoked 1–9 cigarettes/day, heavy smokers when consumed ⩾10 cigarettes/day, and no smokers. The risk factors associated with chronic headache were evaluated by multiple Poisson regression analysis. The Strength and Difficulties Questionnaire-SDQ[5] was applied for all children. Responses were classified as normal, borderline and abnormal. The control variables were maternal schooling and occupation of the family head at birth, child's blood pressure, bruxism and gender. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The total prevalence of chronic headache among schoolchildren was 25.8% (29.8% for males and 24.4% for females) in RP, and 11.1% (15.5% for males and 11.2% for females) in SL. The prevalence of headache among children from light smoker mothers was 19.3% and 40.5% among heavy smokers in RP, and 9.6% and 24,4% respectively for light and heavy smokers in SL. In both cohorts, the factors associated with chronic headache were maternal smoking during pregnancy (RR for heavy smokers 1.560, 95%CI 1.128–2.159 in RP and RR = 2.540, 95% CI 1.148–5.619 in SL) and abnormal score of the SDQ (RR = 1.900, 95% CI 1.383–2.595 in RP and RR = 2.102, 95% CI 1.228–3.598 in SL). The remaining factors were female gender (RR = 0.731, 95% CI 0.565–0.949), bruxism (RR = 1.389, 95% CI 1.058–1.824), high blood pressure (RR = 1.421, 95% CI 1.049–1.925) for Ribeirão Preto and maternal schooling at birth ⩾12 years (RR = 3.388, 95% CI 1.321–8.686) for SL.
Conclusions: Maternal smoking during pregnancy and general difficulties detected in the mental health screening were found to be risk factors for chronic headache at school age in both cohorts. Biological factors – female gender, bruxism, blood pressure – were risk factors for chronic headache only in RP, whilst a social factor – maternal schooling – was a risk factor only in SL. Support: CNPq, FAPESP and FAEPA.

1. H. Bettiol *et al., Rev Saúde Públ.*, 32: 18–28, 1998.
2. A.A. Silva *et al., Cad Saúde Públ.,* 17: 1413–23, 2001.
3. V.C. Cardoso *et al., Braz J Med Biol Res,* 40:1165–76, 2007.
4. *Classificação Internacional das Cefaléias.* 2ª Ed. Sociedade Brasileira de Cefaléia. São Paulo. Alaúde Editorial Ltda., 2006.
5. R. Goodman. *J Child Psychol Psych*, 40:791–801, 1999.

P-2A-59

Unwanted pregnancy and its detrimental effect on early language development in the offspring: the HBC Study

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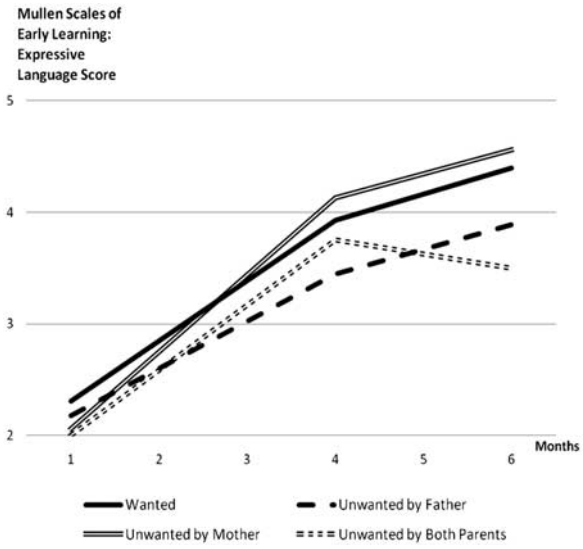
Objective: Unwanted pregnancy has been suggested to be associated with low birth weight[1–3] and delayed neurodevelopment, particularly in motor and social domains[4]. However, language development in infants in relation to unwanted pregnancy has yet to be explored. This is of a particular concern since delayed language development is a predictor for behavioural as well as cognitive problems during childhood[5]. To capture a longitudinal effect of unwanted pregnancy, we investigated the association between unwanted pregnancy and the development of expressive language during 1 to 6 months of age using a multi-purpose cohort, the Hamamatsu Birth Cohort for Mothers and Children (HBC).

Methods: The HBC study was initiated in 2007, the methodology of which is discussed elsewhere (Tsuchiya *et al.*, a poster to be presented). Among 209 dyads of mothers and their children enrolled by August of 2008, 165 children completed direct assessments at 1st, 4th, and 6th months of age of the child. Expressive and receptive language, and motor skill were assessed with the Mullen Early Scales of Learning[6]. Mothers were questioned on intention to pregnancy at approximately 20th week of gestation, and were categorised into three groups: "intended", "mistimed", and "unwanted", defined according to a previous study[1]. In the present study, "mistimed" and "intended" were conventionally merged into "wanted", since mistimed pregnancy is defined as having an intention to give birth in later life. Additionally, we asked each of mothers to provide names of two individuals who had been most pleased to know she was pregnant. If she provided the name of the husband or partner, the pregnancy was defined as "wanted by father"; otherwise, the pregnancy was categorised as "unwanted by father". As for potential confounders, socio-economic status such as annual income, educational history for both mother and fathers, and parity were also identified. Institutional ethics committee clearance and participants' consent were obtained.

Results: Although no remarkable difference was found in expressive language development by the 4th month of age, "unwanted-by-father" children showed lower scores than "wanted-by-father" children at the 6th month. Then, we compared the score across children "wanted by both parents", "unwanted by father and wanted by mother)", "unwanted by mother and wanted by father", and "unwanted by both parents", and found that it was the lowest in the group of children "unwanted by both parents" in the 6th month (figure).

Conclusions: Unwantedness of pregnancy by father appears to have a detrimental effect on expressive language development in infants. Although unwantedness of pregnancy in mothers per se exerts no similar effect, it may have an additive, detrimental effect in combination with unwantedness of pregnancy by father.



1. D.V. D'Angelo *et al., Perspect Sex Reprod Health,* 36:192–7, 2004.
2. S.A. Collier & C. J. Hogue, *Matern Child Health J.,* 11:65–71, 2007.
3. A.P. Mohllajee AP *et al., Obstet Gynecol.,* 109:678–86, 2007.
4. S.R. Crissey. *Pop Res Policy Rev.,* 24:593–615, 2005.
5. L. Rescorla *et al., J Speech Lang Hear Res.,* 50:1063–78, 2007.
6. E.M. Mullen, Pearson Assessments, Minneapolis, MN, 1995.

O-2A-60

Is better nutrition in childhood in a developing population associated with better cognitive function in later adulthood?: The Guangzhou Biobank Cohort Study

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Objective: There is growing evidence that early life exposures, such as childhood socioeconomic status, are related to later

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adulthood cognition. However, the specific aspect of early conditions underlying this association is not clear. Animal protein intake is positively associated with earlier walking in infants. Dietary supplementation with meat in infants and children in developing countries results in better cognitive function, independent of iron status. Protein energy supplementation with vegetables, milk and sugar (not meat) given from birth to 24 months in developing populations is associated with better cognitive function in early adulthood (mean age 32 years), especially amongst women. Inadequate childhood nutrition is associated with poor short term academic and cognitive outcomes. However, it is not known whether childhood nutrition has life long effects on cognitive function. We examined the association of childhood meat eating with adulthood cognitive function in southern China where the older population lived through significant hardship during their early years.

Methods: Multivariable linear regression was used in a cross-sectional study of 20,086 Chinese men and women aged ⩾50 years from the Guangzhou Biobank Cohort Study (phases 2 and 3) 2005–8. We assessed the association of childhood meat eating with amnesic-MCI and delayed 10-word recall score. The 10-word recall is a test of new learning ability from the CERAD (Consortium to Establish a Registry for Alzheimer's Disease) test battery which has been validated as a culturally and educationally sensitive tool for identifying dementia in population based research in developing countries. Amnesic-MCI was defined as a delayed recall score of 3 or less out of 10, corresponding to 1 standard deviation below the mean.

Results: Adjusted for age, sex and education, childhood meat eating 1–6 days per week and daily childhood meat eating were associated with a higher 10-word recall score (number of words recalled = 0.08 [95% confidence interval = 0.02 to 0.13] and 0.24 [0.16 to 0.33] respectively)) and with lower odds of amnesic-MCI (odds ratio = 0.80 [95% confidence interval = 0.72 to 0.89] and 0.79 [0.67 to 0.94] respectively). Additional adjustment for childhood and adulthood socio economic position and current physical activity attenuated these findings, however daily childhood meat eating remained associated with a higher 10-word recall score (0.17 [0.08 to 0.26].

Conclusions: A diet that includes a small amount of daily meat in childhood (after infancy) may have long-term positive effects on cognitive function. If confirmed, these results highlight the importance of adequate childhood nutrition. Alternatively childhood meat eating may reflect a generally more cognitively protective childhood environment and nutrition. Irrespective, these findings also emphasise the childhood and adolescent antecedents of adult disease, with corresponding public health implications for healthy aging. Future research should examine the role of childhood exposures in long term cognitive development and if a role for childhood meat eating is verified, should elucidate the type and quantity of macro and micro nutrients that may be cognitively protective and the biological mechanisms behind these effects, so that preventive strategies can be implemented.

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P-2A-61

Profiles of psychological development in children whose mothers underwent hyperthyroidism or hypothyroidism during pregnancy

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Objectives: Normal levels of production of thyroid hormones T3 and T4 in pregnant women play a critical role in the development of CNS structures of future offspring. In the case of hypothyroidism, lack, delay or inadequacy of treatment is associated to maternal and fetal morbidity including posterior poor intellectual development[1]. The objective of this study was to test if lower levels of psychological development in children are associated to previous maternal hypothyroidism during pregnancy.

Methods: Two groups of children (Group 1, N1 = 7 and Group 2, N2 = 6) whose mothers underwent hyperthyroidism or hypothyroidism and received concomitant pharmacological treatment during pregnancy, were tested using the 4 subscales of the Escala de Evaluación de Desarrollo Psicomotor (EEDP) (Asssessment Scale of Psychomotor Development)[2] and the Spanish version of the Standford-Binet Intelligence Scale. Level of psychological development (low development and non low development) of Group 1 (3,7 +/− 2,1 years old) and Group 2 (1,6 +/− 1,1 years old) and type of maternal condition during pregnancy (hyperthyroidism and hypothyroidism) were analyzed conjointly using 2×2 contingency tables and running correspondent χ^2 association tests. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: There was no significant association between level of development and type of maternal condition during pregnancy when levels of development were those measured by the Psychomotor, Sensory, or Psychosocial Subscales of EEDP while there was a significant association between level of development measured by the Phonological Subscale of EDDP and type of maternal condition ($p < ,05$) being low development associated to hypothyroidism and non low development to hyperthyroidism. Level of intelligence (low

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IQ and non low IQ) and type of maternal condition were analyzed conjointly using the same statistical procedures described above. No significant association between level of intelligence and type of maternal condition was found.

Conclusions: As the two groups under study were not equivalent in terms of age, it remains as a question to answer in prospective research using larger samples if the specific profile of phonological development found for group 2 equals profiles of phonological development of children of similar age whose mothers underwent hyperthyroidism during pregnancy or did not undergo any disorder associated to the level of production of the thyroid hormones.

1. M.L. Mitchell, M.Z. Klein. *Eur J Endocrinol.,* 151: U45–U48, 2004.
2. S. Rodríguez *et al., EEDP. Escala de Evaluación del Desarrollo Psicomotor de 0-24 meses*. Santiago: Galdoc, 1976.

P-2A-62

Maternal low protein diets during pregnancy and/or lactation impair male offspring motivation to work for reward

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Objective: Studies in several species have shown that suboptimal maternal nutrition during pregnancy and/or lactation impairs brain structure and function. The focus of these developmental programming studies has mostly been on brain reproductive and appetitive behavior. We evaluated effects of a maternal low protein (MLP) diet on motivational performance in male rat offspring (OFF) using the progressive ratio task.

Methods: Pregnant Wistar rats were assigned to a control (C - 20% casein; CC) or a restricted (R - 10% casein; RR) isocaloric diet in pregnancy and lactation. A third group received C and R in pregnancy and lactation, respectively (CR). A fourth group received R in pregnancy and C in lactation (RC). At birth litters were adjusted to 10 pups. At 90 days of age, male OFF were tested on a progressive ratio schedule of reinforcement (PRS) task. The animals were first trained to press a lever to obtain a reward in the form of a drop of 7% sucrose solution. After completing training rats were tested for 8 consecutive days on the PRS. In the PRS the animals have to systematically increase their response for each successive reinforcement. In contrast to free sucrose consumption tests using an operant schedule of conditioned reinforcement allows to distinguish between the "liking" of a reward and the motivation to obtain a reward.

Results: Fig 1 shows that compared with controls all three protein restricted male OFF groups made fewer responses and received fewer rewards.

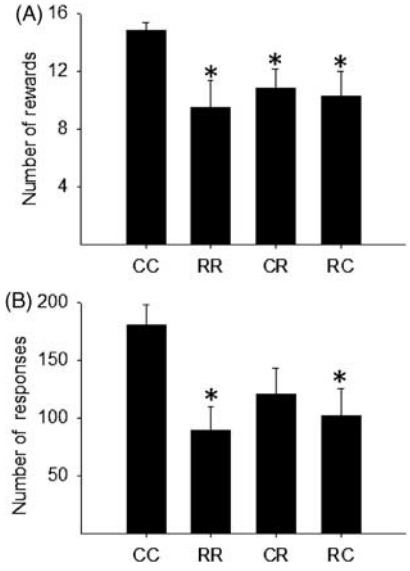

Figure 1. A) Number of rewards and B) number of responses in males at 90 days of age in the four experimental groups exposed to different diets during pregnancy and/or lactation. Data averaged over 8 consecutive days and displayed as mean ± SEM; n = 5, *vs CC, $p < 0.05$.

Conclusions: We believe this is the first demonstration of decreased motivation to obtain a reward following exposure to MLP diets in rodents. Several possible factors may influence brain development in ways that explain our findings. 1. Exposure of the developing brain to high levels of glucocorticoid. We have shown that these MLP diets raise maternal serum GC[1]. Our own studies in the baboon (unpublished) show that exposure of OFF to stress or GC at higher levels than those appropriate for the current stage of development alter OFF performance in the progressive ratio test. 2. Decrease in brain growth factors. 3. As we demonstrate at this meeting MLP diminishes the concentration of polyunsaturated fatty acids in the milk.

1. E. Zambrano *et al., J Physiol.,* 563:275–284, 2005.

P-2A-63

Limbic-hypothalamic-pituitary-adrenal angiotensina and corticosteroid receptors expression in adult rats after undernutrition in utero

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Millions of children die annually or will develop metabolic diseases, cardiovascular and cognitive deficits in adulthood, resulting from protein-calorie malnutrition both during pregnancy and in early life. The undernutrition fetal programming has medical and social relevance, especially in developing countries, like Brazil. Protein-calorie malnutrition and hypertension represent global public health problem. The central RAS play an important role in the control of fetal cardiovascular responses, body fluid balance, and neuroendocrine regulation. Recent progress have been made in demonstrating that altered fetal RAS development as a consequence of environmental insults may impact on programming of hypertension later in life. Also, altered hypothalamic-pituitary-adrenal responses to stress can occur by early environmental events**.** Aditionally, altered expression of brain mineralo and glucocorticoid receptors (MR and GR, respectively) is thought to be central to this process. In this way, we hypothesise that altered levels of expression of AT1 receptor, MR and GR in some neural pathways may influence cardiovascular control and stress response and contribute to adult cardiovascular diseases in fetal origins.

Objective: To investigate the effects of maternal undernutrition exposure *in utero* on adult AT1, MR and GR expression in hypothalamic regions, which are known to have roles in the regulation of the cardiovascular system and/or body fluid and electrolyte balance.

Methods: Pregnant rats were divided into two groups. The daily food supply of one group (FR50) was restricted to 50% of the food consumed by the other group (NF), fed *ad libitum*. Only male pups were used in the study. body weight (BW) was mesured in the day of birth. Arterial blood pressure (AP) was measured weekly, since the 42nd day of age. In the age of 90th days, the rats were perfused and their brain processed for immunohistochemistry analyze.

Results: Our results shown that FR50 offspring presented significant reduction in BW (5.67 ± 0.16 v 6.84 ± 0.13 in NF, $p < 0{,}001$) and AP increased from 6th to 12nd week (6th, 149.1 ± 3.4 v 125.1 ± 3.2 in NF, $p < 0{,}001$; 12nd, 164.4 ± 4.9 v 144.0 ± 3.3 in NF, $p = 0{,}02$). In the figure we have represented the immunoexpression found in FR50 brain when compared to NF (PVN, paraventricular nucleus; SFO, subfornical organ).

Conclusion: Impaired angiotensinergic neural pathway within the brain may have important consequences in homeostatic functions, particularly related to the control of arterial pressure. Our results suggest that HPA response to stress in FR50 rats is enhanced. We hypothesize that reduced hippocampal response to stress is probably a compensative response to systemic glucocorticoid rate enhanced. Support: FAPESP.

P-2B-64

Is flat pelvis associated with low birth weight?

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The relation of pelvis size to fetal growth restriction has not been adequately studied and has not been mentioned in intrauterine growth restriction reviews[1,2]. However, some studies have suggested that stroke is more common among the offspring of mothers who had a "flat" bony pelvis[3,4].

Objective: To verify the correlation between the mother's pelvic size and newborn birth weight.

Methods: A cross sectional study with 226 mothers with singleton pregnancy but without preterm delivery was undertaken between August 2008 and April 2009 at Instituto de Medicina Integral Professor Fernando Figueira (IMIP), Recife, Brazil. *Methods:* We measured the conjugate, intercristal and interspinous diameters of the mothers with a Collins pelvimeter. We used multiple linear and logistic regression analyses to examine the effects of each pelvic diameter on birth weight. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Low birth weight was observed in 19 (8.4%) of the newborns, and 29 (12.8%) of the mothers had flat pelvis. The mothers with a flat pelvis have a higher proportion of low birth weight offspring; 6/29 (20.6%) versus 13/197 (6.5%); Fisher exact test = 0.021. Pearson correlation for weight at birth with pelvis intercristal, interspinous and conjugate diameters, were 0.265, 0.305 and 0.289, respectively (all $p < 0.001$). After controlling a range of covariates the relation of birth weight with interspinous and conjugate diameters remained.

Conclusions: Our findings agree with the idea that intra-uterus volume restriction occasioned by flat pelvis lead to fetal growth restriction. These also support the hypothesis that malnutrition during the fetal life could lead to an inadequately development of the pelvis (flat pelvis) which is associated with transmission of fetal growth restriction to the next generation. In conclusion, the size of the pelvis assessed using clinic pelvimetry has a positive correlation with birth weight and can predict fetal growth restriction before pregnancy.

1. T.Y. Tan, G.S. Yeo. *Curr Opin Obstet Gynecol.,* 17: 135–142, 2005.
2. K. Marsál. *Curr Opin Obstet Gynecol.*, 14:127–135, 2002.
3. C. Osmond *et al., Stroke,* 38:264–270, 2007.
4. C. Berkley. Pictorial Midwifery, London, UK: Bailliere, Trindall and Cox; 1941.

P-2B-65

Correlation of mesenteric fat thickness with waist abdominal at birth

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Objective: To determine the correlation between mesenteric adiposity in neonates with the waist abdominal.
Methods: We measured the mesenteric fat thickness in 118 newborns with gestational age ⩾37 weeks. We used an ultrasound, ATL HDI 5000 (CA, Bothell) with CL 4–7 MHz or CL 2–5 MHz curvilinear transducer. Waist circumference (WC) was measured at the narrowest circumference between xiphisternum and umbilicus. The interoperator and intraoperator reliability was assessed. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The sonographic measurements showed good interoperator and intraoperator reliability, with intraclass correlation coefficient of 0.94 (95% CI; 0.88 to 0.98). There is a negative correlation between mesenteric fat and waist abdominal ($r = -0.321$; $p = 0.001$).
Conclusions: Mesenteric fat is more sensitive to the lipolytic effects of catecholamines to release more free fatty acid (FFA) which leads to an increase of gluconeogenesis, impairment of metabolism and action of insulin and increase lipoprotein synthesis [1]. Mesenteric fat is also a cardiovascular risk factor [2,3]. We showed that mesenteric fat can be recognized in newborns by ultrasound scan. Our findings suggest that intrauterine growth restriction may be associated with mesenteric adiposity. This could be one more explanation to the effect of low birth weight on accumulating visceral fat in childhood and adulthood.

1. P. Mathieu *et al.*, *Int J Biochem Cell Biol.*, 40:821–836, 2008.
2. K.H. Liu *et al.*, *Int J Obes Relat Metab Disord.*, 27:1267–7123, 2001.
3. K.H. Liu *et al.*, *Diabetes Care*, 29:379–384, 2006.

P-2B-66

Collection of muscle tissue in an epidemiological study to investigate life course influences on sarcopenia

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Objective: Sarcopenia, the loss of muscle mass and strength with age, is associated adversely with disability, morbidity and mortality. Epidemiological study findings suggest influences across the life course are important in determining muscle mass and strength as associations have been seen between birth weight and both adult muscle mass and strength. Our objective was to ascertain the feasibility and acceptability of obtaining muscle tissue from healthy older men, with historical records of growth in early life, to identify cellular and molecular mechanisms underlying the life course influences on sarcopenia.
Methods: 105 men with documented birth weight participating in the Hertfordshire Sarcopenia Study consented for detailed physiological quantification of muscle mass, strength and a biopsy of the vastus lateralis using the Weil-Blakesley conchotome. Muscle tissue was processed for immunohistochemical, and molecular studies. Acceptability was ascertained by questionnaire and a pain Visual Analogue Scale (VAS). 100 mm on the scale indicated severe pain. Appropriate institutional ethics committee clearance and participants' informed consent was obtained.
Results: Muscle biopsy was successfully performed in 102 out of 105 participants, with mean yield 107 mg (range 20–290 mg). The three participants who did not have a biopsy were on treatment that may have influenced wound healing (long term aspirin $n = 1$, methotrexate $n = 1$, long term steroids $n = 1$). 93 participants provided feedback. The median pain VAS score during the procedure was 7 mm (interquartile range [IQR] 1–34), 4 mm (IQR 0–16) one day after the procedure and 1 mm (IQR 0–4) 7 days after the procedure. 60 (64%) participants were back to their normal levels of activity one day after the procedure. 85 (91%) found this procedure acceptable and would have the procedure again for research purposes. There were no serious wound complications.
Conclusions: Muscle biopsy using the Weil-Blakesley conchotome is both feasible and acceptable in community dwelling older men participating in epidemiological research. The excellent yield of biopsy tissue will allow morphological and molecular studies of muscle to be integrated into an epidemiological study facilitating investigation of mechanisms underlying life course influences on sarcopenia. This work was supported by the Medical Research Council, UK and the University of Southampton.

P-2B-67

Protein restriction during pregnancy may alter the oxidative capacity of the skeletal muscle

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Objective: Stimulus in the pregnancy stage, such as protein restriction, can affect various morphophysiological parameters of offspring with consequences in adulthood. This phenomenon,

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known as induced phenotypic traits (fetal programming), can cause changes in skeletal muscle phenotype in the short and long term. Since stimulus during pregnancy affects the morphophysiological patterns of specific muscles, our hypotesis is that, under this condition, the oxidative metabolism of muscle fibers can be changed. The aim of this study was to evaluate the effect of the maternal low protein diet exposure, during gestation, on the oxidative metabolism of the Extensor Digital Longus muscle (EDL) of the offspring.

Methods: Wistar rats, aged 16 weeks, from mothers groups that were fed with hipoproteic diet, with 6% of the protein (GH, $n = 7$), and normoproteic diet, with 17% of the protein (GN, $n = 7$) were sacrificed and the EDL muscle were removed, weighed, and fragments frozen in liquid nitrogen. Histological sections (8 μm), obtained in a cryostat at −20°C were submitted to the histochemical reaction NADH_TR. This reaction allowed us to identify the oxidative, intermediate (oxidative/glicolytic) and glicolytic muscle fibers. Muscle fiber frequencies (%) and the cross-section area (μm^2) were calculated using a computerized imaging analysis system QWin V3 for Windows (Leica, Wetzlar, Germany). The statistic analyzis used was the test t-student, with $p < 0,05$.

Results: There was no change in muscle weight between groups ($p > 0.05$). However, fetal protein restriction reduced the frequency (GN = 30,3 ± 3,1; GH = 25,4 ± 3,5) of the intermediate fibers and increase the area of glycolytic fibers (GN = 3064,18 ± 1157,4; GH = 3199,12 ± 217,0), with $p < 0,05$. There was a considerable increase of the percentage of glycolytic fibers (GN = 36,0 ± 4,9; GH = 41,28 ± 9), but this was not significant.

There was no change in area of muscle fibers between groups ($p > 0,05$).

Conclusions: Protein restriction during pregnancy changed muscle fiber types composition in EDL muscle of the offspring. These changes may be contributing to the increase in the insulin resistance of this muscle[1,2], once the intermediate fibers are more sensitive and glycolytic fibers are less sensitive to insulin[3]. Support: FAPESP (Proc. 2007/59970-8).

1. S.E. Ozanne *et al.*, *Am J Physiol.*, 271:E1128–E1134, 1996.
2. A.E. Toscano *et al., Nutrition,* 24:270–278, 2008.
3. J. He *et al.*, *Diabetes,* 50:817–823, 2001.

P-2C-68

Detection of biofilms on endotracheal tubes in ventilated newborn and intervention in biofilms and respiratory infections of *P.seudomonas aeruginosa* in rat model

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Objective: To investigate the phenomenon of microbial colonization and associated biofilm accumulation on the surface of endotracheal tubes (ETTs) removed from neonates with intubated ventilation, to research effect of inhalation of Erythromycin and Ambroxol combining Ciprofloxacin respectively against respiratory infection with ETT-associated biofilm of *P.aeruginosa*.

Methods: Scanning electron microscopy (SEM) was used for examination of presence of biofilm on ETTs' surface, bacteria harvested from the surface of ETTs and the secretions of lower respiratory, and assessed on antimicrobial susceptibility, respectively. Rat model of *P.aeruginosa* biofilm infection was established. The biofilms were treated by follow 5 groups respectively, which include group 1 (control), group 2 (ambroxol), group 3 (erythromycin), group 4 (erythromycin+ ciprofloxacin), group 5 (ambroxol+ ciprofloxacin).

Results: SEM showed that incidence of microbial colonization increased significantly after 2 days duration of tube use (12/20), biofilm formation was observed about 3 days after intubation, and its architecture became more mature and complex over 3 days. Of 14 positive cultures from ETTs (4 grew normal flora), 7 pathogens were isolated. Of 13 positive cultures from secretions of lower respiratory (1 grew normal flora), 10 pathogens were isolated. There were 7 samples had the same pathogen both on surface of ETTs and in secretions of lower respiratory, accounted for 50%. The gram-negative bacteria isolated from the surface and the secretions presented multi-resistance to antibiotics. Rat model was intervened after 7 days inhalation. The bacterial colony counts (BCC) in lung tissues ($\times 10^4$ CFU/ml) from every group were (139.250 ± 42.0162), (101.625 ± 40.4190), (109.625 ± 33.4747), (57.750 ± 37.8295) and (22.25 ± 17.31840), respectively. It were significantly different that the group1, group2 and group3 were compared with the group4 and group5 respectively ($P < 0.05$). it also was significantly different that the group4 was compared with the group5($P < 0.05$), but not significantly different between the group 1, group 2 and group 3, respectively ($P > 0.05$). BCC from ETT biofilm were as follows ($\times 10^4$ CFU/ml): (170.000 ± 48.3263), (127.625 ± 39.0163), (133.500 ± 33.6876), (70.375 ± 35.7768) and (38.125 ± 19.1045), respectively. The biofilm of the ETT surface was thickest in the group1. It were less in the group2 and group3, least in the group4 and group 5. The group 5 was less a little.

Conclusions: The ETT-biofilm develops into mature and complex with the duration of tube use. There is correlation between microbial colonization, biofilm formation on the surface of ETTs and the lower respiratory infection in prolonged intubated ventilated neonates. The combination Ambroxol with Ciprofloxac was significantly effective on ETT-associated biofilm of *P. aeruginosa* and relevant respiratory infection, which is better than the combination Erythromycin with Ciprofloxacin.

P-2C-69

A way of managing cheaply food poisoning due to enterotoxigenic. E. Coli and shigella in community in low – resource countries – kenya

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Health King Baudouin Foundation Kenya

Objective: Several drug-resistant community acquired infections are causing global concern e.g. Enterrotoxigenic toxic *E.coli* and Shigella organisms transmitted by untreated water and unhygienically prepared foods. The study for 14 years in Kenya shows the breakdown of public health facilities, untreated, water, poverty resulting in hygienic housing and misuse of antibiotics in trying to treat enteric pathogens has increased the mortality and morbidity. This shows that although the current Enterrotoxigenic *E.coli* are sensitive to new introduced quinolones and cephalosporins; these drugs kill the bacteria byt they do not effective on already produced toxins in patients gut. A new approach to control this problem must be introduced to control the spread and management of toxin-produced in the gut. Antibiotics combined with neutralizing agents of toxins might be effective in controlling these problems.

Methods: From 1983, food poisoning agents were investigated during the outbreak in Kenya. Sensitivity was done, toxin produced was investigated, and model of transmission was studied. Health providers used antibiotics with toxin neutralizing agents and those without.

Results: Result showed 33% of food poisoning was due to heat toxins producing E-coli. These organisms continued, developing resistance to new drugs. Food poisoning due to toxigenic (*E.coli*) responded better with antibiotics combined with activated magnesium aluminium silicate. This is a chemical that has a high absorbent power to neutralize toxins produced. Those treated with antibiotics only continued to have stomach ache and constipation.

Conclusion: Our study shows that antibiotics with magnesium aluminium silicate are effective in treating food poisoning due to toxigenic E-Coli. Treating water as ''Point of use'' with Chlorine should be promoted controlling food poisoning diseases.

P-2C-70

Fish meal supplementation during gestation protects against endotoxin-induced fetal programming of the dermal immune response in sheep

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Over the past few years there has been a switch in the westernized society from a balanced omega-3:omega-6 polyunsaturated fatty acid (PUFA) ratio to a diet highly supplemented with omega-6 (n-6) PUFAs. This switch in PUFA balance combined with bacterial infection during pregnancy may have an impact on programming of the fetal immune system and may increase the risk of the offspring's susceptibility to inflammatory diseases.

Objective: To investigate differences in the antigen-specific dermal immune response of offspring born to mothers that had been supplemented with either fish meal (FM) rich in omega-3 docosahexaenoic acid or soyabean meal (SM) rich in omega-6 polyunsaturated fatty acid (n-6 PUFA), and challenged with endotoxin on day 135 of gestation.

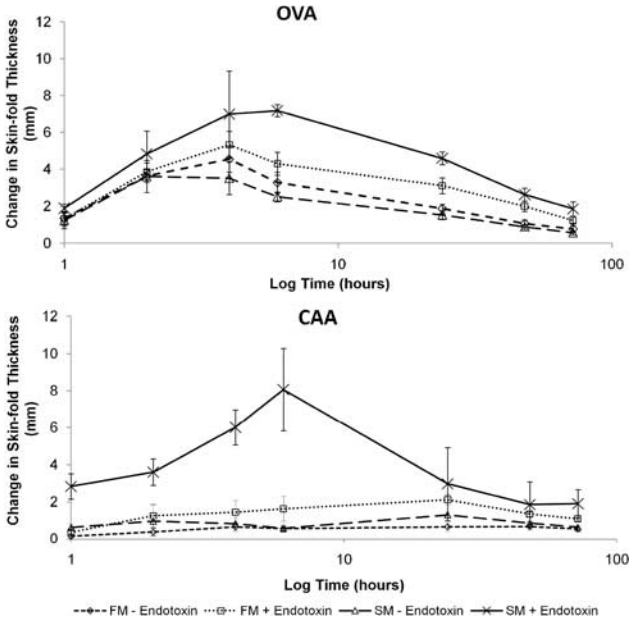

Figure 1: Dermal hypersensitivity response to OVA and CAA in offspring born to mothers supplemented with FM or SM during gestation and treated with endotoxin or saline during late gestation.

Methods: Twenty-one ewes were allocated to either a diet supplemented with FM (n = 11) or SM (n = 10). On day 135 of gestation (of an approximate 145 days gestation period) half of the ewes from each treatment were either challenged with 1.2 μg/kg *Escherichia coli* endotoxin administered *i.v.,* or saline as control. At 4 months of age, all offspring received the antigens ovalbumin (OVA) and *Candida Albicans* cellular extract (CAA) administered *i.m.* A secondary booster of OVA and CAA was repeated on all offspring 10 days later. On day 21 of the trial offspring were subjected to a dermal hypersensitivity response test using both OVA and CAA administered *i.d.* Skin-fold measurements were performed at 0, 1, 2, 4, 6, 24, 48 and 72 hours post injection to assess the dermal immune response to these specific antigens. The University of Guelph's Animal Care Committee approved the experimental protocol in accordance with the Canadian Council of Animal Care.

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Results: All offspring responded to both the CAA and OVA antigen over time ($p < 0.0001$). Amongst the offspring born to mothers fed a SM supplemented diet, there was a significant difference in the hypersensitivity response between offspring born to mothers receiving endotoxin, as compared to offspring born to mothers receiving saline (CAA, $p < 0.0010$; OVA, $p < 0.0023$). However, the offspring born to mothers fed a FM supplemented diet showed no significant difference between endotoxin treatments for either antigen (CAA, $p < 0.0982$; OVA, 0.3331). When comparisons were made across dietary treatments with respect to endotoxin challenge, it was demonstrated that offspring born to mothers fed a FM supplemented diet had an attenuated hypersensitivity response compared to offspring born to mothers fed a SM supplemented diet (CAA, $p < 0.0038$; OVA, $p < 0.0225$) (see Figure).
Conclusions: Offspring born to mothers fed a diet supplemented with FM + endotoxin in late gestation had an attenuated dermal hypersensitivity response to both CAA and OVA compared to offspring born to SM + endotoxin treated mothers. This suggests that FM may protect against endotoxin-induced fetal programming of the dermal immune response.

P-2C-71

Role of infection agent in cardiovascular disease in Slovak population.

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Objective: The participation of the infectious agents in the pathogenesis of atherosclerosis still remains a controversial issue. We tested buffy coats of the cardiovascular (CV) patients, i.e. with coronary heart disease (CHD), hypertension and those who underwent reconstructive vascular surgery (RVS) by PCR using the specific primers directed to *Chlamydophila pneumoniae*, *C. psittaci* and cytomegalovirus (CMV).
Methods: Of 228 patients CMV was detected in 77, but *C. pneumoniae* only in 11 subjects and *C. psittaci* was not detected at all. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: At the same time, high proportion of patient's sera contained specific IgG antibodies to CMV (68.7%) and IgA antibodies to *C. pneumoniae* (66.6%). Serological results correlated well with the findings of interleukin-6, C-reactive protein, hypercholestrolemia, and diabetes. Some correlation was observed also with PCR detection of CMV, but not *C. pneumoniae.* In one PCR-negative sample for *C. pneumoniae*, we detected parachlamydiae.
Conclusions: The search for their possible involvement in buffy coats and arterial specimens will be the purpose of our further study. Support: 2006/07-SZU-02 of Ministry of Health, Slovak Republic and by 2006/10-SZU-05.

P-3B-72

Study of hypothalamic leptin receptor expression in low-birth-weight piglets and effects of leptin supplementation on neonatal growth and development

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Objective: Low birth weight resulting from intrauterine growth retardation (IUGR) is a risk factor for further development of metabolic diseases. The pig appears to reproduce nearly all the phenotypic pathological consequences of human IUGR and is likely to be more relevant than rodents in studies of neonatal development.
Methods: In the present work, we characterized the model of low-birth-weight piglets with particular attention to the hypothalamic leptin-sensitive system, and we tested whether postnatal leptin supplementation could reverse the precocious signs of adverse metabolic programming.
Results: Our results demonstrated that 1) Newborn IUGR piglets present altered adipose tissue cellularity and exhibit abnormal hypothalamic distribution of leptin receptors 2) IUGR piglets present an altered postnatal growth characterised by a catch-up growth and development of increased adiposity since pre-pubertal age; and 3) postnatal leptin administration can partially reverse the IUGR phenotype by correcting growth rate, body composition, and accelerated the development and maturation of organs involved in metabolic regulation.
Conclusions: We conclude that IUGR may be characterized by altered leptin receptor distribution within the hypothalamic structures involved in metabolic regulation and that leptin supplementation can partially reverse the IUGR phenotype. These results open interesting therapeutic perspectives in physiopathology for the correction of defects observed in IUGR.

P-3B-73

S100B protein concentrations in serum and abdominal fat of neonatally handled rats chronically exposed to a highly palatable diet in adulthood

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Objectives: According to our previous findings, neonatally-handled rats demonstrate an increased preference for palatable food in comparison to controls[1], but seem less vulnerable to the damaging metabolic effects of a chronic exposure to a highly palatable diet, with attenuated abdominal fat accumulation after this challenge[2]. Serum S100B is influenced by diet and by body weight gain[3]. In humans and animals studies, myocardial ischemia increases serum S100B[4,5]. Therefore, the aim of our study was to verify if neonatal handling and further chronic exposure to chocolate would alter S100B protein concentration in serum and abdominal fat in adulthood.

Methods: Nests of Wistar rats were (a) handled for 10 minutes in the first 10 days of life or (b) left undisturbed until weaning. At 3 months of age, the females (n = 48) were assigned to receive or not chocolate + standard lab chow for 30 days and then sacrificed to dissect the abdominal fat and to collect the serum. A group of animals receiving chocolate was deprived for 30 days, receiving only rat chow before the sacrifice. S100B protein was determined in both serum and abdominal fat[6]. Repeated measures ANOVA or Two-Way ANOVA were used for the analysis.

Results: Handling did not affect the consumption of chocolate or chow nor body weight, although rats receiving chocolate had increased body weight and abdominal fat deposition. Chronic exposure to chocolate was associated with an increase in serum S100B levels in non-handled rats (NH chow = 3.65 ± 0.46, NH chocolate = 6.14 ± 1.19), while handled animals had no change in S100B levels with the exposure to the diet (H chow = 4.19 ± 0.39, H chocolate = 3.4 ± 0.62), in which we found an interaction between group × diet, $P = 0.039$. S100B in abdominal fat did not differ between groups during chocolate exposure. After 30-days-period of chocolate deprivation, the difference between groups in S100B levels was not detected anymore.

Conclusions: These results indicate that neonatal handling is associated with a differential response to a chronic ingestion of a highly palatable diet at a biochemical level, protecting against the chocolate-induced increase of serum S100B levels in females, and possibly against the development of abdominal obesity and cardiovascular risk. Supported by CAPES, CNPq.

1. P.P. Silveira *et al.*, *Physiol Behav.*, 80:739–745, 2004.
2. C.S. Benetti *et al.*, *Pediatr Res.*, 62:405–411, 2007.
3. K. Holtkamp *et al.*, *J Neural Transm.*, 115:937–940, 2008.
4. R. Pfeifer *et al.*, *Resuscitation*, 79: 46–53, 2008.
5. G.S.L. Mazzini *et al.*, *Life Sci.*, 77:882–889, 2005.
6. M.C. Leite *et al.*, *J Neurosci Methods*, 169:93–99, 2008.

P-3B-74

Introduction of a high omega-3 fatty acid diet reverses the programmed increase in adiposity, but not hypertension, in offspring of mothers treated with dexamethasone

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Objective: Maternal dexamethasone (Dex) treatment in the rat programs several phenotypic outcomes including adult hypertension[1], hyperleptinemia[1] and increased proinflammatory cytokines[2]. These adverse outcomes are prevented, however, if offspring are raised on a postnatal diet enriched with omega-3 (n3) fatty acids from birth[1,2]. The objectives of this study were to determine the effects of maternal Dex on offspring adiposity and blood pressure (BP), and to examine whether programmed changes are reversed by introduction of a high n-3 fatty acid diet in adulthood.

Methods: Offspring of control (Con) and Dex-treated mothers (0.6μg/ml drinking water, from day 13) were cross-fostered to untreated mothers at birth and males raised on normal chow for 40 weeks. A semi-pure diet (5% fat) containing either standard (Std) or high (Hn3) omega-3 fatty acid levels was then introduced. Body weights were recorded weekly throughout the study and BP was measured by tail-cuff plethysmography in trained animals before and 10 weeks after the switch to semi-pure diets. Body fat (% abdominal and % whole body) was measured by DEXA analysis at 50 weeks of age.

Results: Maternal Dex reduced birthweight ($P < 0.001$) by 18% and thereafter bodyweights remained slightly, but consistently, lower in offspring of Dex-treated mothers (overall treatment effect $P < 0.001$, two-way ANOVA). There was no significant interaction between treatment and age in this analysis of body weight, indicating an absence of catch-up growth. BP was elevated (7%; $P = 0.02$) in offspring of Dex-treated mothers at 40 weeks of age, and this difference remained evident after 10 weeks on the Hn3 diet (7% higher in Dex on Std diet; 6.5% higher in Dex on Hn3 diet; post-switch treatment effect $P = 0.05$). There was a significant interaction ($P = 0.02$) between treatment and diet for abdominal body fat (%), and so treatment effects were analysed separately for each diet. This analysis showed that offspring of Dex-treated mothers had higher abdominal fat on the Std diet (Dex/Std: $55 \pm 2\%$ vs Con/Std: $49 \pm 2\%$; $P = 0.04$), but not on the Hn3 diet (Dex/Hn3: $49 \pm 1\%$ vs Con/Hn3: $52 \pm 3\%$; NS). Total body fat (%) showed a similar overall pattern (treatment-diet interaction $P = 0.03$), although the group comparison between Con/Std and Dex/Std did not reach statistical significance ($P = 0.06$).

Conclusions: This study shows that maternal Dex treatment programs increased adiposity in adult offspring, and confirms that adult hypertension is a feature of this programming

model. Introduction of a high n3 diet in adulthood reversed the increase in adiposity but not that in BP. The latter contrasts with our previous demonstration that programmed hypertension is prevented when offspring are raised on a high n3 diet from birth[1,2].

1. C.S. Wyrwoll *et al., Endocrinology,* 147:599–606, 2008.
2. C.S. Wyrwoll *et al., J Endocrinol.,* 198:571–579, 2008.

P-3B-75

Life-course socioeconomic factors, skin colour and abdominal obesity in adulthood in a Brazilian birth cohort

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Obesity is an increasingly prevalent nutritional disorder throughout the world. In particular, abdominal obesity is associated with cardiovascular and metabolic risk.

Objective: To evaluate the effects of skin colour and life-course socioeconomic indicators on waist circumference (WC), hip circumference (HC) and waist-hip ratio (WHR) in young adults.

Methods: Population-based birth cohort study. Individuals born in 1982 in Pelotas (southern Brazil) were visited on a number of occasions from birth to age 23–24 y. A sample of the cohort was sought in 2006 and 972 individuals were located. Assessment of the three dependent variables (WC, HC and WHR) was carried out in 856 subjects (442 males, 414 females). The independent variables were collected at the different follow-up visits: self-reported skin colour (2004), family income at birth (1982), family income in adulthood (2004) and family income change (1982 and 2004). Possible confounding or mediating variables were also collected in 2004: current smoking, sedentary behaviour, fiber and fat intake, alcohol consumption, attained education of the individual and parity (for women). ANOVA was used in crude analyses and multiple linear regressions in adjusted analyses. The adjusted analyses took into account the different levels of determination. Tests for linear trend were used for ordinal variables. Stata 9.0 (Statacorp, College Station, Texas, USA) was used for analysis. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: In men, family income at birth and in 2004–05 were positively associated with WC and HC ($\beta = \sim 2.4$ cm and $P < 0.001$ in all the cases), but not with WHR ($\beta = \sim 0.005$ and $P = 0.1$ in both periods). Regardless of current income, men born to wealthier families had larger WC and HC as adults ($\beta = 1.7$ cm and $p < 0.01$ for both circumferences). Skin colour was not associated with any of the outcomes. In women, early poverty was associated with smaller HC ($\beta = 1.4$ cm; $p = 0.05$), and current poverty with larger WC ($\beta = -2.3$ cm; $P = 0.003$). Poverty at any age thus led to higher WHR ($\beta = -0.015$ for family income at birth and -0.011 for family income in adulthood; $P < 0.01$ in both cases). Black women had larger WC and HC than white women ($\beta = \sim 3.2$ cm and $P \leqslant 0.01$ in both cases), but there were no differences in WHR ($\beta = 0.006$; $P = 0.4$). All the associations were partially mediated by education and behavioural variables.

Conclusions: The effects of early socioeconomic position on WC and HC persist even after adjustment for adult socioeconomic position, highlighting the importance of interventions during the first years of life. Support: Partially funded by The Wellcome Trust. The initial phases of the cohort study were made possible by support from the PRONEX, the Brazilian Ministry of Health, International Development Research Centre of Canada, and the United Nations Development Fund for Women (UK).

P-3B-76

Intergenerational 'mismatch' and adiposity in a developing population: The Guangzhou Biobank Cohort Study

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Background: Intergenerational 'mismatch' between maternal and adult environments, common in developing economies, has been hypothesized as contributing to obesity. In a population with recent socio-economic development, we examined whether the association of maternal conditions, proxied by maternal literacy, with adult adiposity, proxied by body mass index (BMI) and waist-hip ratio (WHR) were modified by later life conditions, proxied by socio-economic position (SEP) at three life stages. We also examined if maternal conditions had sex-specific associations with adult adiposity.

Methods: In a cross-sectional study of 19,977 adults (⩾50 years) from the Guangzhou Biobank Cohort Study (phases 2 and 3 in 2005–8), we used multivariable linear regression to assess the association of maternal literacy with BMI and WHR, and whether the associations varied with sex, age or SEP.

Results: The adjusted association of maternal literacy with WHR varied with sex (p-value = 0.04). In men, maternal literacy was not associated with WHR or BMI. In women, maternal illiteracy was associated with higher WHR (0.004, 95% confidence interval (CI) 0.002 to 0.006) and higher BMI (0.17, 95% CI 0.04 to 0.29), after adjustment for age, lifestyle, life course SEP and paternal literacy. There was little evidence that associations

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varied with SEP at any stage, although continuity of poor conditions into early life may have exacerbated the positive association of maternal illiteracy with higher WHR in women.
Conclusions: Mismatched maternal and later life conditions do not appear to be associated with adiposity, but poor maternal conditions in developing populations may increase adiposity in women. Whether such sex-specific intergenerational effects are driven by epigenetics, maternal sex hormones or other mechanisms remains to be determined. Our findings, although preliminary, imply that a transient epidemic of obesity may occur in the first generation of women who experience economic development.

P-3B-77

Maternal distress in early life predicts the waist-hip ratio in schoolchildren

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Objective: This study was undertaken to determine the association between risk of overweight at age 10–11 years, and exposure to stress during pregnancy, the postpartum period and early school-age. Low birth weight, and maternal psychopathology in later childhood have been reported to contribute to stress and overweight in children. Our findings will determine whether maternal distress in early life also has a role to play in the development of overweight in children.
Methods: This was a longitudinal follow-up of the nested case-control study of the 1995 SAGE birth cohort in Manitoba, Canada. As part of a research objective to investigate the association between overweight in pre-adolescence and the onset of adolescent asthma, waist and hip measurements were obtained during a clinic visit at 10–11 years of age. In chronological order, the evaluated child stress exposures were: low birth weight (proxy for pregnancy stress), maternal distress in the postpartum period and recent stress hormone levels (cortisol, DHEA), which were assayed in a blood sample obtained in children at age 7–10 years. The ratio of cortisol and dihydroepiandrostrenone (DHEA), a proposed marker of the stress response, was also assessed. Multiple linear regression was conducted to determine the relationship between the waist/hip ratio and: low birth weight, postpartum maternal distress and recent stress markers in children (cortisol, DHEA, cortisol/DHEA). Maternal distress was defined from provincial database records, as at least one health care encounter or prescription medication for depression or anxiety during the postpartum period. Results are presented as regression coefficients at the 95% level of confidence. All analyses were adjusted for sex. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Hormone levels and waist-hip measurements were available for 375 children, 10–11 years old, in the SAGE nested case-control study. At this age, 50% of children had a waist-hip ratio of 0.84 and in 10%, the ratio approached risk for overweight (0.93 and higher). The waist-hip ratio was significantly higher in girls than boys. Sex-adjusted waist-hip ratios increased linearly with plasma levels of DHEA (increase in waist-hip ratio of 0.005 for each unit increase in DHEA level, $p < 0.03$). Independent of this correlation with a marker of recent stress, exposure to maternal distress in the postpartum period predicted an increase of 0.014 in the waist-hip ratio of children ($p < 0.02$). The waist-hip ratio was unrelated to low birth weight status, cortisol levels and the cortisol/DHEA ratio.
Conclusions: We found risk for overweight in a population of Canadian schoolchildren to be predicted by exposure to maternal distress in early life, independent of recent stress in the child, a recognized determinant of overweight. We were unable to find greater risk for overweight in children born low birth weight, a common outcome of stress during pregnancy. Although the pathways by which maternal distress leads to overweight are unknown, they may be related to the non-responsive feeding styles of depressed mothers during the postpartum period.

P-3B-78

Maternal nutrient reduction from 0.16 to 0.9 of gestation is accompanied by increases in Agouti-related peptide protein expression in fetal baboon hypothalamic arcuate and paraventricular nuclei

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Objective: Food intake in post-natal animals is regulated by hypothalamic peptides including the orexigenic Agouti-related peptide (AGRP), e.g., in hamsters, food deprivation increases AGRP gene expression in hypothalamic arcuate (ARC) neurons. Most studies of ARC control of appetite have been done in postnatal rodents, but development of these systems in non-rodent species, appears to occur during fetal life. However to our knowledge, only 3 fetal studies on this subject have been published to date: two for sheep and one for the rhesus monkey. In order to provide further data we determined the normal distribution and effect of moderate maternal nutrition reduction (MNR) on AGRP protein expression In near term fetal baboon ARC and paraventricular (PVN) nuclei. We hypothesized that AGRP protein

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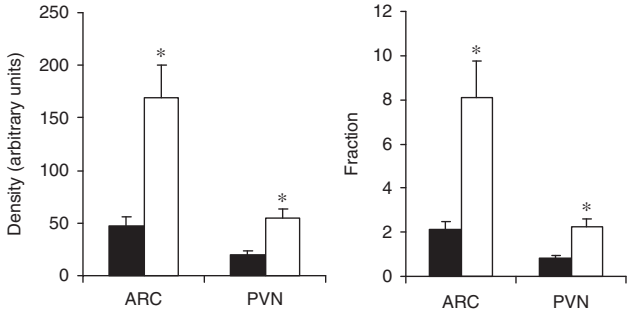
expression would be upregulated in ARC neurons and fibers and PVN fibers with MNR.

Methods: From 30 to 165 days (d) gestation (G)(0.16 to 0.9G), pregnant baboons were fed as *ad lib* controls (CTR; n = 7) or fed 70% of CTR diet (MNR; n = 7) with necropsy/fetal brain collection at c-section under general anesthesia at 165 dG. AGRP protein was quantified in the fetal ARC and PVN by immunohistochemistry and image analysis for fraction (area immunostained/area of the field × 100%) and density [arbitrary density units (DU)]. Statistical comparisons were made with the rank sum test with alpha level set at 0.05. Data are expressed as mean ± SEM, with CTR values presented first.

Results: AGRP protein expression was significantly increased ($p < 0.05$) in ARC cell bodies and fibers (fraction – 2.11 ± 0.4 vs. 8.07 ± 1.67%; density – 47.59 ± 8.68 vs. 168.77 ± 30.99 DU) while it was increased in fibers only in the PVN (fraction – 0.8 ± 0.16 vs. 2.24 ± 0.38%; density – 19.94 ± 3.97 vs. 54.72 ± 9.09, dU) in MNR vs. CTR.

Conclusions: The near term fetal baboon appears able to sense moderate reductions in nutrient availability and responds by upregulating synthesis of AGRP in the ARC and PVN. If these differences persist postnatally, they could offer an explanation for some forms of obesity. Supported by HD 21350-18.



P-3B-79

Precocious weaning programs some metabolic syndrome components in adult rat offspring

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Objective: Maternal under nutrition during lactation programs for overweight and central leptin resistance in adult offspring[1]. Also, the inhibition of the 3 last days of lactation, treating the dams with bromocriptine (a PRL inhibitor) programs for overweight, hyperleptinaemia and leptin resistance in adulthood[2] and higher triglycerides and cholesterol. Then, we evaluated how early weaning without the use of pharmacological substances can imprint the offspring and program for hormonal and metabolic dysfunctions.

Methods: After birth, excess pups were removed to kept only six male pups per dam, which were separated into the two following groups: EW (Early Weaning) – dams received anesthetic (thiopental −0.06 mg/ml/100 g) and were involved with a bandage to interrupt the breastfeeding in the last 3 days of lactation, and C (Control) – pups had free access of maternal milk during all lactation (21 days). After weaning, EW and C offspring had free access to water and standard diet until 180 days-old when they were killed to collect blood and tissues. Visceral fat mass was weighed. Fasting glycemia was determined using a glucosimeter. Body fat and protein mass were determined by carcass method. Serum hormones were determined by radioimmunoassay and lipids by colorimetric assay. Results were significantly different when $p < 0.05$.

Results: At weaning, EW pups presented lower body weight (−12%), body nose-rump length (−4%), visceral fat mass (−40%), body fat content (−30%), glycemia (−10%), serum leptin (−73%) and insulin (−20%), but higher body protein content (+40%). When adult, EW offspring showed a transient increase in body weight (+9%) with no changes in food intake. At 180 days-old, these offsprings presented higher visceral fat mass (+84%), body fat (+36%), glycemia (+15%), serum triglycerides (+96%), but lower body protein (−22%).

Conclusions: Early weaned adult rats displayed higher visceral adiposity, hyperglycemia and hypertrygliceridemia, which are components of the metabolic syndrome. Our model reinforces the idea that neonatal malnutrition caused by shortening lactation is important for metabolic programming of future diseases, even when no pharmacological treatment is used. Support: FAPERJ, CAPES, CNPq.

1. M.C.F. Passos *et al.*, *Horm Metab Res.*, 36:625, 2004.
2. I.T. Bonomo *et al.*, *J. Endocrinol.,* 192:339–344, 2007.

P-3B-80

Alkylresorcinol urinary metabolites are possible biomarkers for intake of whole grain wheat and rye

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Objective: A diet including whole grain products is considered to be part of a healthy life style and epidemiological studies have shown relationships between whole grain consumption and decreased risks of several chronic diseases, e.g. obesity, type 2 diabetes, coronary heart diseases, and some cancers. Establishment of an alternative measurement of whole grain intake through the use of a biomarker may

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overcome obstacles related to traditional intake assessment tools (e.g. food frequency questionnaires and dietary recalls). A group of phenolic lipids, alkylresorcinols (AR) are found in high amounts in the outer layers of rye and wheat grains. Among commonly consumed products, AR are only present in those containing bran or whole grain of these cereals. AR are suggested as biomarkers for whole grain rye and wheat intake. Ingested alkylresorcinols are absorbed in the small intestine and they are thought to be eliminated by hepatic metabolism, including ω – and β-oxidation, leading to formation of two major metabolites: DHBA (3,5-dihydroxybenzoic acid) and DHPPA (3-(3,5-dihydroxy)-1-propanoic acid). These can be excreted either as such or as more polar conjugates. The aim of the study was to investigate the response of urinary AR metabolites excretion after 3 different AR intakes and to evaluate the distribution of free and conjugated urinary metabolites.

Methods: The study was conducted as a randomized 3-way crossover design with seventeen participants. During each treatment period the participants were assigned to 1 of 3 doses of AR (as rye bran flake product), which should be included in their diet. At the end of each treatment the participants collected urine in two 24-h periods. Excretion of urinary AR metabolites was used to determine the recovery of ingested AR as urinary AR metabolites. Distribution of conjugates and free metabolites was estimated by analyzing urine samples (n = 9), incubated with different deconjugating enzymes. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Excretion of AR metabolites increased with increasing AR intake, from 76 ± 15 μmol/24 h at the lowest dose (85 μmol) to 189 ± 57 μmol/24 h at the highest dose (342 μmol). However, the recovery of ingested AR as urinary metabolites in 24 h collections decreased with increasing dose, from 89 ± 18% at the lowest intake level to 45 ± 15% at the highest level. Urinary DHPPA was conjugated to a greater extent (54 ± 14%) than DHBA (34 ± 10%), which is more hydrophilic. The major conjugates were glucuronides and the DHPPA/DHBA ratio in urine was 1.4 ± 0.3.

Conclusions: The large proportion of conjugated metabolites in urine suggests that suitable deconjugation methods (e.g. multipotent enzyme mixture) are essential, in order to ensure quantification of the total pool of urinary AR metabolites. The decrease in recovery could be due to a dose-dependent shift in elimination route or to the limited collection time. A wider understanding of AR pharmacokinetics is crucial for the potential use of urinary AR metabolites as biomarkers for whole grain wheat and rye intake.

P-3B-81

Effect of late gestational nutrient restriction on gene expression of markers of white and brown adipocytes in postnatal sheep

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Objectives: It has recently been shown that brown adipose tissue (BAT) is present in adult humans and that its abundance is reduced with obesity[1]. Birth results in the rapid activation of BAT that is normally accompanied by its loss and replacement with white adipose tissue[2]. Recent studies in rodents, however, have suggested that brown and white adipocytes have very different origins with bone morphogenetic protein acting through PR-domain-containing (PRDM)16[3,4] having a pivotal role in this process. The aim of our study was to establish the primary molecular changes in brown and white fat i.e. uncoupling protein (UCP)1, peroxisome proliferator-activated receptor coactivator (PGC)1α and PRDM16 in the perirenal-abdominal depot that rapidly changes its characteristics over the first month of life in sheep. This was combined with an investigation of the impact of late gestational nutrient restriction of the mother, as these offspring deposit more fat in later life[5].

Methods: Eighteen twin-bearing pregnant sheep were randomly assigned to a normal (C, 100% of total metabolisable (ME) requirements, n = 9) or nutrient restricted (NR) (60% of total ME, n = 9) diet from 110 days gestation until term (i.e. 147 days). The timing of nutrient restriction coincides with the period of maximal fat deposition in the fetal sheep. One twin was humanely euthanased on the first day of life and its sibling sampled at 30 days of age. The mRNA abundance for UCP1, PGC1α and PRDM16 in adipose tissue was measured by real-time PCR. Appropriate institutional animal ethics committee approval was obtained.

Results: Over the first month of life there was the expected decline (~100 fold) in UCP1 mRNA abundance ($P < 0.0001$) in both groups of offspring. However, gene expression for PGC1α and PRDM16 increased by ~10 fold between birth and one month of age ($P < 0.001$). The magnitude of this adaptation was greatest in male offspring born to control fed mothers, a process that was significantly down-regulated in male (e.g. PGC1α: 30 days – C 5.1 ± 0.7; NR 1.5 ± 0.5 ΔCt ($P < 0.01$)), but not, females offspring born to mothers NR through late gestation. Fat mass increased in all offspring with age but this process was much greater in females than males irrespective of prenatal diet (Females: 1.83 ± 0.18; Males 1.06 ± 0.13 g/kg body weight $P < 0.001$).

Conclusions: The rapid growth of white fat during postnatal life is accompanied by a pronounced upregulation of molecular markers previously presumed to define BAT. During early postnatal life the development of white fat also appears to be very different between males and females. This difference may be critical in determining not only the long term effects of maternal diet but also the very different fat distribution with gender.

1. W.D. van Marken Lichtenbelt *et al.*, *N Engl J Med.*, 360:1500–1508, 2009.

https://doi.org/10.1017/S2040174409990067 Published online by Cambridge University Press

2. M.E. Symonds *et al., J Endocrinol.,* 179:293–299, 2003.
3. Y.H. Tseng *et al.*, *Nature,* 454:1000–1004, 2008.
4. P. Seale *et al.*, *Nature,* 454:961–967, 2008.
5. D.S. Gardner *et al.*, *Am J Physiol.,* 289:R947–R954, 2005.

P-3B-82

Intrauterine growth restriction programs addictive/reward behavior

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Objective: Human studies during the past two decades have confirmed the developmental programming effects of low birth weight (LBW) on obesity and metabolic syndrome. In addition, animal models of LBW demonstrate the development of adult obesity and dysregulation of behavior, including central appetite/satiety pathways. Notably, LBW in humans is an independent risk factor for obesity as well as the early onset of compulsive behaviors such as binge eating. Although prior studies have focused on hypothalamic appetite and ingestive behavior pathways, food intake is also regulated by central reward pathways. One of the most important components of the central reward pathway is the mesoaccumbens dopaminergic (MADA) pathway, beginning in the ventral tegmental area (VTA) with projections to the nucleus accumbens. Our rat model of maternal food restriction results in intrauterine growth restricted (IUGR) newborns that develop hyperphagia and adult obesity. We hypothesize that hyperphagia and the predisposition to adult obesity in LBW offspring can be attributed, in part, to enhanced susceptibility to food ''addiction,'' caused by programmed changes in the central MADA reward pathway. We sought to assess the offspring dopaminergic reward pathway via expression of tyrosine hydroxylase (TH) in the VTA, as well as intermittent sucrose intake, as a measure of the propensity for food ''addiction''.
Methods: Control dams received ad libitum food, whereas study dams were 50% food restricted from pregnancy day 10 to 21. After birth, all pups were nursed by Control dams and weaned at 3 weeks to ad libitum feed. At 1 day of age, brains were collected from female offspring and stained for tyrosine hydroxylase (TH) in the ventral tegmental area (VTA). At 9 months of age, IUGR and control females were given access to 10% sucrose for 24 h followed by sucrose access for 90 minutes every other day for 2 weeks.
Results: IUGR rats at birth exhibited 50% more TH protein expression in the VTA. When provided intermittent sucrose, adult IUGR rats consumed significantly more sucrose than controls (18.4 ± 1.2 vs 12.8 ± 0.9 ml/90 min), respectively).
Conclusions: IUGR programs changes in VTA dopamine production as early as day 1 of age, suggesting an upregulation of central reward pathways. Increased intermittent sucrose ingestion is consistent with enhanced adult addictive potential. These results suggest that IUGR offspring may be predisposed to reward-mediated food intake, contributing to adult obesity, and potentially additional addictive reward behaviors.

P-3B-83

Maternal influences on offspring obesity in young adulthood: effects of BMI, parity and pregnancy weight gain

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Objective: The prevalence of obesity among women of childbearing age is increasing. Pre-gravid obesity and excessive weight gain in pregnancy are associated with adverse perinatal outcomes and increasing evidence suggests an influence on adiposity in childhood and adolescence. We examined whether maternal factors, including nutritional status and diet, have persisting effects on offspring obesity in early adulthood.
Methods: We studied 276 men and women born in Motherwell, Scotland during 1967–68, whose mothers had been advised to eat one pound (0.45 kg) of red meat daily during pregnancy and to avoid carbohydrate-rich foods. Our previous studies have demonstrated higher blood pressure and heightened cortisol responses to stress in those whose mothers reported higher meat and fish consumption in pregnancy[1,2]. At age 30 years we measured the offspring's height and weight (to derive body mass index (BMI)), waist circumference and four-site skinfold thicknesses (Harpenden callipers); sex-adjusted percentage body fat and fat mass index (FMI) were calculated. The mother's weight gain and dietary intake during pregnancy were extracted from antenatal records. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Percentage body fat was greater in offspring of mothers with a higher BMI at the first antenatal visit (rising by 0.35% per kg/m^2, $p < 0.001$) and in offspring whose mothers were primiparous (difference 1.5% in primiparous vs. multiparous, $p = 0.03$). Grouping the mother's pregnancy BMI into tertiles, offspring percentage body fat was highest (32.2%) in those whose mothers were primiparous with a BMI $> 24.15\,kg/m^2$ and lowest (26.4%) in those whose mothers were multiparous with a BMI $< 21.52\,kg/m^2$, $p < 0.001$. Higher offspring percentage body fat was also independently associated with higher pregnancy weight gain (regression coefficient 7.4% /kg/week, $p = 0.002$). There were similar significant associations of increased maternal

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BMI, greater pregnancy weight gain and parity with increased offspring waist circumference, BMI and FMI. In exploratory analyses, offspring percentage body fat was higher in those whose mothers reported a lower fish intake in early pregnancy ($p = 0.032$), but was not related to fish intake in late pregnancy. The findings were independent of current lifestyle factors influencing adiposity including smoking, gender, physical activity levels and social class. Table 1 demonstrates the combined effect size of factors predicting offspring overweight.

Conclusions: We show for the first time that risk of obesity in early adulthood is influenced by prenatal influences independently of current lifestyle factors. Maternal adiposity, greater gestational weight and parity all impact on offspring obesity. Exploratory analyses suggest a possible influence of low fish intake in early pregnancy. This study highlights the importance of maternal influences during pregnancy to prevent the intergenerational cycle of obesity. Strategies to raise public awareness of the risks of maternal obesity on offspring future health are required.

Table 1: Predictors of offspring overweight (BMI $>25\,kg/m^2$) at age 30 years.

	Odds ratio	95% CI	P value
Subject's gender (1 = male, 2 = female)	0.31	0.18–0.53	<0.001
" smoking (0 = no, 1 = yes)	0.51	0.28–0.93	0.028
Maternal age (years)	1.00	0.65–1.55	0.99
" 1st antenatal BMI (z)	1.99	1.45–2.73	<0.001
" antenatal weight gain (z)	1.40	1.06–1.85	0.018
Primiparous mother (0 = multip, 1 = primip)	1.75	1.02–3.00	0.043

1. A.W. Shiell *et al.*, *Hypertension,* 38:1282–1288, 2001.
2. R.M. Reynolds *et al.*, *JCEM.,* 92:2208–2210, 2007.

P-3B-84

Postnatal early overnutrition changes the adrenal medullary function in adults rats

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Objective: Postnatal early overnutrition (EO) is a risk factor for future obesity and metabolic disorders. Rats raised in small litters develop overweight, hyperphagia, hyperleptinemia, hyperinsulinemia and hypertension when adults. Since catecholamines have well-known effects on cardiovascular parameters, we aimed to investigate if changes in adrenal medullary function may be related to increasing blood pressure of early overfed animals at adulthood.

Methods: To induce postnatal EO, litter size was reduced to 3 pups/litter (SL: small litter) and the groups with normal litter size (10 pups/litter) were used as control. Rats had free access to standard diet and water post weaning. Body weight and food intake were monitored daily and offspring were killed at 180 days-old. We evaluated the adrenal total catecholamines (adrenaline and noradrenaline) content by the trihydroxyindole method and adrenal tyrosine hydroxylase (TH) content by western blotting. Blood pressure was measured by the tail-cuff method using a Letica LE 5000 device. The first measurement of these cardiovascular parameters was discarded and the mean the three subsequent measurements were recorded.

Results: As expected, EO induced higher body weight (+9%, $p < 0.05$) and food intake (18%, $p < 0.05$) in adult rats. These programmed rats showed higher adrenal catecholamines content (+35%, $p < 0.05$) that can suggest a higher production or a lower release. Adrenal TH expression show no significant difference compared to control group, however we cannot discard changes in enzymatic activity. Systolic blood pressure was higher in adult SL rats (+10.2%, $p < 0.05$).

Conclusions: We evidenced that postnatal EO induces long-term effects upon the adrenal medullary function. It is possible that higher total catecholamines content may due to higher biosynthesis or decrease in secretion of these hormones. However, since SBP was higher it is suggestive of a higher catecoholamine action, which can contribute to the development of adult chronic cardiovascular diseases.

Support: Capes, CNPq and FAPERJ.

P-3B-85

Birth weight and body composition in a remote Aboriginal Australian community

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Objectives: To describe the relationship between birth weight (BW) and body composition in one remote Australian Aboriginal community with high rates of chronic disease related morbidity and mortality.

Methods: 1,078 adults participated in a chronic disease health screen between 2004 and 2006. 309 males and 214 females had a recorded BW. Ages were 20–49 years. Height,

weight, fat free mass (FFM) BMI, waist circumference (WC), and percent fat (%Fat) were assessed. Data were analysed in gender specific BW quartiles. Appropriate institutional ethics committee clearance and patients consent were obtained.

Results: Mean (SD) BW was 2.80 kg (0.54) for males and 2.72 kg (0.50) for females. The tables give mean values of body composition variables by BW quartiles.

Conclusion: Males of the highest birth weight quartile had higher weight, FFM, BMI, WC, and %Fat relative to those with lower birth weights. None of the parameters were high relative to representative Australian standards (AusDiab)[1]. There were similar trends, although not significant in weight and FFM in females. Notably, however, females over the entire birth weight spectrum had average WC measurements in the obese range by nonAboriginal standards (>88 cm)[1], without a trend by birth weight. The promoters of the preferential central deposition of fat in females, with its especial conservation in those of lower birth weights, is worthy of much further study.

Table 1: Mean (SD) of body composition variables by BW quartiles in males.

BW	Height (cm)	SD	Weight (kg)	SD	FFM (kg)	SD	BMI (kg/m^2)	SD	WC (cm)	SD	%Fat	SD
1.15–2.49	171.9	5.7	63.0	14.2	51.6	5.3	21.3	4.6	82.4	13.2	16.1	9.2
2.50–2.79	170.9	5.2	62.8	11.8	51.8	5.3	21.4	3.7	81.6	11.0	16.2	7.8
2.80–3.16	171.9	4.9	63.5	11.8	52.0	5.6	21.5	4.0	82.2	10.6	15.9	7.8
3.17–4.69	172.7	7.2	71.5	17.9	56.4	5.4	23.8	5.0	86.4	13.9	19.2	8.9
P	0.4380		0.0001		0.0001		0.0001		0.0031		0.0014	

Table 2: Mean (SD) of body composition variables by BW quartiles in females.

BW	Height (cm)	SD	Weight (kg)	SD	FFM (kg)	SD	BMI (kg/m^2)	SD	WC (cm)	SD	%Fat	SD
1.07–2.40	159.2	5.3	62.9	15.5	41.6	5.0	24.8	6.0	91.2	15.3	34.1	9.0
2.41–2.72	159.9	5.3	62.1	14.3	40.8	3.9	24.3	5.5	88.5	16.0	33.0	9.4
2.74–3.03	161.1	5.0	65.8	19.3	41.7	5.1	25.5	7.7	93.1	19.2	35.6	10.2
3.04–4.31	161.5	6.0	67.4	19.2	43.6	5.7	25.8	6.7	92.3	15.4	36.1	9.0
P	0.1455		0.2081		0.0817		0.4378		0.2920		0.1876	

1. D.W. Dunstan *et al.*, *Diabetes Res Clin Pract.*, 57:119–129, 2002.

P-3B-86

Increased visceral fat mass is correlated with impaired glucose homeostasis in obese adolescents

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Objective: The prevalence of childhood obesity has increased rapidly over the past decades and is a strong risk factor for adult obesity and an important risk factor for adverse health outcomes in childhood and adulthood[1]. Increased central and visceral fat, rather than a high body mass index (BMI) is linked to higher risks of development of obesity and metabolic and cardiovascular diseases in later life[2,3]. Studies in adults have shown that increased abdominal fat mass, a measure of central and visceral fat, is associated with an increased risk of insulin resistance, dyslipidemia, hypertension and coronary heart disease and overall mortality rates[4]. In the present study we investigated the influence of body fat distribution on glucose homeostasis in obese children with impaired glucose tolerance.

Methods: A total of twenty six Caucasian children aged between ten and twelve years, with a BMI above 25 showing moderate to severe impairment of glucose tolerance, were included in the study. Plasma glucose and insulin concentrations were measured at baseline and 30, 60, 90, and 120 min after ingestion of an oral glucose load (OGTT). Whole body insulin sensitivity (WBISI) was determined by a two-step euglycemic clamp. Quantification of visceral and abdominal subcutaneous fat depots was performed using magnetic resonance imaging (MRI). In addition, intrahepatocellular lipid content (IHCL) was measured using ^{1}H-NMR spectra.

Results: The Table shows that BMI showed a significant negative correlation with WBISI ($r = -0.53$, $p = 0.005$), and a positive correlation with calculated HOMA index ($r = 0.45$, $p = 0.02$). When insulin sensitivity was clustered in three tertiles representing children that showed low (Q1), moderate (Q2) or high insulin sensitivity (Q3), we observed a distinct relation with the total amount of visceral fat mass: visceral fat mass was significantly higher in children with low insulin sensitivity (the Q1 tertile group of adolescents). Also, WBISI showed a clear correlation to IHCL ($r = 0.36$, $p = 0.00012$), independent of BMI.

Patient characteristics	Range	Mean ± SEM
Age (yrs)	10–12	10.96 ± 0.72
BMI (kg/m^2)	25.1–41.1	31.9 ± 4.7
120 min glucose (mg/dl)	53–128	96.2 ± 24.5
Fasting glucose (mg/dl)	59–125	82.1 ± 17.1
Fasting Insulin (mU/l)	6.4–43.3	22.2 ± 9.3
HOMA-IR	1.41–9.62	4.5 ± 2.2
WBISI	0.78–6.18	2.8 ± 1.5

Conclusions: These results clearly show that increased visceral fat mass is associated with a disturbance in glucose homeostasis and more predictive of insulin insensitivity than BMI. Moreover, the data suggest that insulin resistance, but

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not the obesity by itself, determines the risk for fatty liver in children. These data confirm the relevance of the abdominal fat mass as a risk factor for metabolic syndrome at the start of adolescence.

1. W.H. Dietz. *Pediatrics,* 101:518–525, 1998.
2. C.S. Fox *et al.*, *Circulation,* 116:39–48, 2007.
3. M.I. Goran *et al.*, *Diabetes,* 57:3007–3012, 2008.
4. J.P. Despres, I. Lemieux. *Nature,* 444:881–887, 2006.

P-3B-87

A study of the birth weight-obesity relationship using a longitudinal cohort and sibling and twin pairs

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Objective: Sibling and twin studies offer a unique opportunity to better understand how early life factors, such as birth weight, influence adult health outcomes. This study designs provide control for confounding factors that are typically unmeasured or poorly measured in traditional cohort studies. Further, monozygotic twins are genetically identical; thus, differences in birth weight within pairs likely reflect differences in fetal growth that are unrelated to genetic factors[1]. The objective of this study was to examine the association between birth weight and later obesity in a traditional cohort and in a subsample of matched sibling pairs.
Methods: Using nationally representative US data from the National Longitudinal Study of Adolescent Health on adolescents followed over three measurement periods across 8 years into young adulthood, we evaluated the association between birth weight and later obesity in a traditional cohort (n = 15,729) and in a subsample of matched sibling pairs (full siblings: n = 1,252; monozygotic twins: n = 287; dizygotic twins: n = 460). In the full cohort, we used random effects longitudinal poisson regression to estimate the relationship between birth weight and obesity across the three measurement periods, controlling for sex, age, race/ethnicity, parental education, and parental income. In the subsample of matched sibling pairs, we used random effects longitudinal linear regression models to regress within-pair BMI differences across the three measurement periods on within-pair birth weight differences. Appropriate institutional ethics committee clearance and participants' informed consent were obtained
Results: In the full cohort, among individuals with a non-obese mother, those born high (>4 kg, versus normal, ⩾2.5 to ⩽4 kg) birth weight were more likely to become obese later in life (IRR = 1.49, 1.30–1.72). In the sibling subsample, birth weight difference was positively associated with BMI difference later in life (β = 2.67, 0.99–4.35) for female monozygotic pairs; the twin with a heavier birth weight was more likely to have a higher BMI later in life. In contrast, we observed no association between birth weight difference and BMI difference in full sibling pairs, dizygotic twins or in monozygotic male twins.
Conclusions: Results from the cohort analysis suggest that high birth weight is positively associated with later obesity. Findings from the twin studies suggest that independent of shared maternal and genetic factors, the intrauterine environment unique to each fetus contributes to later obesity only in female monozygotic twin pairs. Support: NIH (NICHD: 1R01HD057194).

1. R. Morley, T. Dwyer. *Paediatr Perinat Epidemiol.,* Suppl 1:2–7, 2005.

P-3B-88

Offspring growth, cardiovascular and adipose phenotypes display differential sensitivity to maternal protein undernutrition at one year of age in mice

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Objectives: Numerous studies using rodent and domestic animal models have shown that altered maternal nutritional status encompassing oocyte maturation, preimplantation embryo development, and/or throughout gestation can all impact upon the long-term health and physiology of the offspring. However, most studies are terminated at young adulthood and whether identified phenotypes persist into late adulthood is less clear. Here, we extended an earlier study in mice from 6 months of age (young adult)[1,2] to 1 year (mature adults to determine the stability of our initial observations).
Methods: Offspring from mothers fed normal protein diet (NPD; 18% casein) or isocaloric low protein diet (LPD; 9% casein) during oocyte maturation (for 3.5 days prior to mating; termed Egg-NPD and Egg-LPD respectively), or LDP exclusively during preimplantation development (for 3.5 days following mating) before returning to NPD till term (termed Emb-LPD) or NPD or LPD throughout gestation (termed NPD and LPD respectively) were allowed to develop till 52 weeks of age. Measurements of body weight (weekly) and blood pressure and organ allometry (52 weeks) were taken. mRNA expression of uncoupling protein 1 (*Ucp1*) adrenergic receptor beta 3 (*Adrb3*), insulin receptor (*Insr*) and insulin-like growth factor I receptor (*Igf1r*) in interscapular and retro-kidney fat samples were analysed using quantitative

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RT-PCR. Serum insulin and glucose levels were measured using commercially available kits. All experiments were conducted using protocols approved by UK Home Office and local ethics committee.

Results: No differences were observed in body weight between Egg-LPD and Egg-NPD offspring for up to 1 year of age. Emb-LPD females displayed significantly increased body weight, whilst LPD females displayed significantly body weight profile when compared to NPD females. Egg-LPD, NPD and Emb-LPD offspring had significantly elevated systolic blood pressure when compared to respective control offspring at 52 weeks. LPD females had significantly reduced inguinal and retro-kidney:body weight ratios when compared to NPD females. Fat pad gene expression analysis revealed significantly increased mRNA levels for *Ucp1* and *Adrb3I* in female LPD interscapular fat, whilst Emb-LPD females has increased mRNA levels for *Igf1r* and *Insr* in retro-kidney fat when compared to controls.

Conclusions: These data show that maternal dietary protein undernutrition during specific windows of the reproductive cycle or development can induce long-term changes within the offspring that persist for up to 1 year into mature adulthood. Whilst elevated blood pressure was observed in all offspring, altered growth profiles, organ allometry and fat gene expression were only observed when maternal LPD was administered post fertilisation, indicating window-specific sensitivity with respect to long-term offspring health and physiology. This work was supported by the National Institutes of Health, [grant number U01 HD04435], BBSRC [BBF007450] and the Gerald Kerkut Charitable Trust.

1. A.J. Watkins *et al.*, *Biol Reprod.,* 78:299–306, 2008.
2. A.J. Watkins *et al.*, *J Physiol.,* 586:2231–2244, 2008.

P-3B-89

Insulin-like growth factor 2 regulates body weight and metabolism in adult mice on a HF diet

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Objective: Mice born to dams fed a low protein (LP) diet during gestation and lactation have similar phenotype with insulin like growth factor-1 (*Igf2*) knock out (KO) mice. A LP diet during pregnancy reduces hepatic *Igf2* expression in the fetal offspring. Given that maternal LP fed offspring fed a high fat (HF) diet from weaning have increased body weight gain[1], the aim for this study was to investigate whether *Igf2* regulated body weight in adulthood in response to HF feeding.

Methods: Both *Igf2* KO and wild type (WT) female mice were fed either a HF or chow diet for 6 months and were sacrificed.

Results: The KO female mice gained $\sim$30% ($p<0.01$) more body weight on the HF diet than mice on chow diet, whereas WT mice did not gain more weight on the HF compared to the chow diet. HF feeding significantly increased fasting plasma glucose by $\sim$30% ($p < 0.01$), triglyceride levels by $\sim$36.3% ($p < 0.05$) and total cholesterol levels by $\sim$44% ($p < 0.01$) in the KO mice. In contrast, no significant increase in plasma fasting glucose, triglyceride, HDL and LDL, except a $\sim$21% increase in total cholesterol levels in the WT mice. Consistently, HF feeding has no significant effects on adipose leptin mRNA expression, but markedly increased adipose leptin mRNA levels in the KO mice. HF feeding up-regulated hepatic mRNA levels of liver X receptor-alpha (regulator of hepatic lipid biosynthesis and secretion) in the KO mice, but not in WT mice. HF feeding increased hepatic mRNA levels of lipogenic genes including acetyl-CoA carboxylase-1 and -2 and fatty acid synthase in the WT mice, and the magnitude of these lipogenic genes appeared to be exaggerated in the KO mice. In contrast, HF diet induced significant increase in expression of fat oxidative genes such as peroxisome proliferator activated receptor-alpha, carnitine palmitoyltransferase-1a in the WT mice, but this increase was suppressed in the KO mice. HF feeding increased expression of glucokinase by $\sim$4.5-fold ($p < 0.001$) in the WT mice, but only $\sim$2.2-fold ($p < 0.05$) in the KO mice. These data are consistent with increased plasma triglyceride and glucose levels in the KO mice fed a HF diet. Furthermore, HF feeding markedly increased expression of H19 and this increase is reduced by $\sim$50% in the KO mice. H19 RNA levels are strongly correlated with mRNA levels of genes studied except pepck.

Conclusions: Igf 2 regulates body weight and metabolism in adulthood under HF feeding. H19 may also be involved in the regulation of body weight by Igf2. Acknowledgments: Igf2 KO mice were provided by University of Warwick animal unit. Molecular analysis was mainly funded by Research Develop Fund of Warwick University awarded to JZ.

1. Stocker C, *et al.*, *Int J Obes Relat Metab Disord.,* 28:129–136, 2004.

P-3B-90

Upregulated adipocyte renin-angiotensin system in intrauterine growth restricted offspring: mechanism for obesity-mediated programmed hypertension

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Objective: Intrauterine growth restricted (IUGR) newborns have an increased risk of adult obesity, hypertension and

coronary heart disease, and obesity accounts for 70% of essential hypertension. Thus, adipose tissue clearly is a critical factor in the development of obesity-mediated hypertension. Dysregulation of systemic renin-angiotensin is a primary factor in the pathogenesis of essential hypertension. All components of the renin-angiotensin system (RAS) are expressed in adipose tissue, and recent studies have shown that adipose-derived angiotensinogen (AGT) contributes significantly to plasma AGT concentrations and modulates blood pressure. Obese individuals show upregulation of adipose RAS, and adipose tissue-specific overexpression of AGT raises blood pressure and body fat in mice. The major vasopressor effects of AngII are mediated through its receptor type 1 (AT1) whereas interaction with receptor type 2 (AT2) modulates cell proliferation and renal sodium excretion. We have shown that maternal undernutrition during rat pregnancy results in IUGR pups. When allowed rapid catch-up growth, IUGR offspring exhibit hypertension and hypertrophic adipocytes prior to overt obesity. We hypothesized that adipogenic RAS may be one of the underlying mechanisms contributing to obesity-mediated hypertension.
Methods: From gestational day 10 to 21 (term), Control dams received ad libitum food, whereas study dams were 50% food restricted to produce IUGR pups. All pups were nursed by Control dams and weaned at 3 weeks to ad libitum feed. At 1 day and 9 months of age, retroperitoneal adipose tissue was analyzed for mRNA expression (real time RT-PCR) of AGT, angiotensin converting enzyme (ACE), renin and AT2 receptor in IUGR and Control male offspring. Plasma AGT levels were determined by Western Blot. Data are normalized to β-actin and presented as fold change.
Results: At 1 day of age, IUGR pups had significantly reduced adipose mRNA expression of AGT (0.5-fold, $P < 0.01$) and AT2 receptor (0.6-fold, $P < 0.05$) as compared to Controls. However, adipose ACE and renin expression, including plasma AGT levels were unchanged. In contrast, at 9 months of age IUGR adults, now obese, exhibited significant ($P < 0.001$) upregulation of adipogenic RAS: AGT (3-fold), AT2 receptor (9-fold), renin (5-fold) and ACE (2-fold). Plasma AGT levels were markedly higher in adult IUGR (7-fold, $P < 0.001$) as compared to Control offspring.
Conclusions: In IUGR offspring, adipose AGT likely contributes to elevated plasma AGT levels. Importantly, these changes occur in parallel with development of hypertrophic adipocytes and subsequent obesity. Thus, programmed hypertension in IUGR offspring may occur as a result of upregulation of adipose RAS with potential impact on RAS-induced systemic responses. These findings suggest an important role of adipose RAS in the hypertensive phenotype of IUGR offspring.

P-3B-91

Individual health risk factors and frequency of obesity in Slovakia

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Objective: Obesity results in higher morbidity, reduced quality of life, discrimination and early mortality. It represents a major threat to health systems in developed and developing countries. The prevalence of overweight and obesity is alarming and is increasing in Slovakia as well. The pathogenesis of obesity involves genetic predisposition, metabolic, hormonal and behavioral aspects. The aim of this work was to collect biological material from a large sample of population and assess the frequency of obesity and different health risk factors in Slovakia.
Methods: We have investigated 2386 forty years old volunteers from 7 towns in the country. The volunteers completed a questionneire from which we gained information about their personal and family illnesses, occurence of allergies, immunological disturbances and about the medicaments they were using. The questionnaire contained antropometrical data as well. Basic biochemical examination was performed. Obesity, dyslipoproteinemia, hypertension, smoking, diabetes, metabolic syndrom and positive family history were evaluated as individual health risk factors. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Obesity was detected in 26.1% of probands (BMI > 30), slightly more in women (26.5%) than in men (25.7%). 70.4% of voluntaires had dyslipoproteinemia (total cholesterol > = 5 *and/or* HDL-C < = 1(males); 1,2(females) *and/or* LDL-C > = 4 *and/or* triacylglycerols > = 1,7). The prevalence of hypertension (HT), defined as systolic pressure > = 140 *and/or* diastolic pressure > = 90 *and/or* treated HT, was 22.2%. 26.8% of the investigated persons were smokers. Frequency of obesity, dyslipoproteinemia, and above all, the frequency of hypertension has rised since 2003 in the population of 40 years old people when comparing with former data.
Conclusions: Early detection of individuals with health risk factors helps to prevent the development of serious metabolic and cardiovascular diseases. Support: 2006/07-SZU-02 of Ministry of Health, Slovak Republic.

P-3B-92

Growth in fetal life and infancy is associated with abdominal adiposity at the age of 2 years. The Generation R Study

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Objective: Early weight gain is associated with an increased risk of obesity. It is not known whether rapid weight gain in fetal life and infancy is also associated with increased abdominal adiposity. We examined the associations of fetal and postnatal growth characteristics with abdominal fat mass at the age of 2 years.
Methods: This study was performed in 481 children participating in a prospective cohort study from early fetal life onward. Fetal and postnatal growth characteristics in second and third trimester, at birth and at the age of 2 years were related to abdominal fat mass (subcutaneous distance and area, preperitoneal distance and area) measured by ultrasound according to the method of Suzuki at the age of 2 years. The area measures were used to optimize precision and accuracy of our technique.
Results: Fetal and birth weight showed tendencies towards positive associations with abdominal subcutaneous fat mass. Fetal weight in second trimester of pregnancy was inversely associated with preperitoneal fat area (−3.73 (95% confidence interval −7.23, −0.10) % per standard deviation score (SDS) increase in weight. Weight gain from birth to the age of 2 years was positively associated with preperitoneal fat mass measures. These associations remained significant after adjustment for age, gender, breastfeeding and body mass index. Positive associations were found between catch up growth in weight and abdominal fat mass measures.
Conclusions: Our results suggest that rapid growth rates in fetal life and infancy are associated with increased abdominal subcutaneous and preperitoneal fat mass in healthy children. Further studies need to explore whether these associations persist in later life and are related to metabolic syndrome outcomes.

P-3B-93

Maternal smoking and offspring body composition in adolescence and adulthood in a Brazilian birth cohort

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Objective: The aim of this study was to examine the association between maternal smoking and offspring body composition in adolescence and adulthood.

Methods: The 1982 Pelotas Birth Cohort Study included all children born in maternity hospitals and living in the urban area of the city of Pelotas, Southern Brazil. All males born in 1982 were legally required to enlist in the Army between January and April 2000.We were thus able to track 2,250 male participants in 2000, representing 78.9% of the original cohort. An additional follow-up of both males and females was carried out in 2004–5. Maternal smoking was collected in the perinatal study and at the 4 year postnatal follow up, where information on paternal smoking was also obtained. Offspring body composition was assessed in male participants at 18 years using bioimpedance, body mass index (BMI) and waist circumference (WC) were measured at 23 years in both males and females. In the present analysis, we used as predictors maternal smoking in pregnancy and maternal and paternal smoking collected at 4 years. Outcomes in adolescence included indices of fat and lean mass, fat to lean mass ratio and BMI. In adulthood the outcomes were BMI and WC. Analysis of variance and linear regression were used in the analyses to adjust for confounding factors (family income, maternal education, household assets, maternal skin color, pre-gestational weight, maternal height and, maternal age) and mediating factors (birth weight, own education, smoking in adolescence and adulthood, and parity at age 23 years for female). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Maternal smoking was associated with an increase in fat mass index ($\beta = 0.20$ 95%CI 0.09 to 0.32; $p < 0.001$), lean mass index ($\beta = 0.43$ 95%CI 0.26 to 0.62; $p < 0.001$) and BMI ($\beta = 0.62$ 95%CI 0.35 to 0.90; $p < 0.001$) in male offspring at age 18 years, adjusted for confounding and mediating factors. At age 23 years, maternal smoking was associated with BMI ($\beta = 0.81$ 95%CI 0.41 to 1.20; $p < 0.001$ and $\beta = 0.66$ 95%CI 0.24 to 1.08; $p = 0.002$, in males and females, respectively) and WC ($\beta = 1.83$ 95%CI 0.88 to 2.77; $p < 0.001$ and $\beta = 1.26$ 95%CI 0.25 to 2.27; $p = 0.02$, in males and females, respectively). Associations were similar for maternal smoking in pregnancy and smoking at 4 years postnatal. Paternal smoking was not associated with any indicator of offspring body composition.
Conclusions: There are many reasons why mothers should not smoke. Our data are consistent with the literature and provide further evidence that maternal smoking may increase waist circumference, BMI and indices of fat and lean mass in offspring in adolescence and adulthood.
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P-3B-94

Maternal high-fat diet induces liver and adipose tissue alterations in offspring male mice

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Objecive: This study examined the effects of maternal high-fat diet (HFD) upon liver and adipose tissue morphology in male C57BL/6 mice offspring at 3 months-old.
Methods: Virgin female C57BL/6 mice were feed during pregnancy and/or lactation with Standard Chow diet (SCD - 17% energy from fat, 19% from protein and 64% from carbohydrate) or HFD (49% energy from fat, 19% from protein and 32% from carbohydrate). Both diets, including micronutrient mineral mix, followed the American Institute of Nutrition recommendation to support growth, pregnancy and lactation phases (AIN-93G)[1]. Male pups were divided into 5 groups, according to maternal diet: SC - from SCD fed dam; HFG - from HFD fed dam during gestation period; HFL - from HFD fed dam during lactation period; HFT - from HFD fed dam during gestation and lactation periods, maintaining this diet in postnatal life; HFT-SC - from HFD fed dam during gestation and lactation periods and changing HFD to SCD at weaning. Before sacrifice, oral glucose tolerance test (25% glucose in sterile saline- 0.9% NaCl) was performed in half of males offspring after a 6 h fast[2]. At sacrifice, liver and genital fat pad were removed and blood was collected. Appropriate institutional ethics committee clearance was obtained.
Results: At birth, males HFT were heavier (+6%, $p = 0.03$) than SC. At weaning, as during all the experiment, these differences were maintained (+44%, $p = 0.01$ at 3 months old). Concerning carbohydrate metabolism, oral glucose tolerance test showed glucose intolerance in HFG in comparison with SC offspring (+36%, $p = 0.008$). HFG offspring presented hyperinsulinemia and hyperglycemia when compared to SC group (+146%, $P = 0.03$), indicating insulin resistance. These results were confirmed by HOMA-IR. Hepatomegaly was observed in HFT offspring (+ 28%, $p<0.0001$), whereas hepatic steatosis was present in HFT (+291%, $p < 0.0001$), HFT-SC (+309%, $p < 0.0001$), HFG (+270%, $p < 0.0001$) and HFL (+165%, $p < 0.0001$) offspring. Likewise, epididymal fat depot weight was biggest in HFT offspring ($p < 0.0001$). Adipocyte hypertrophy occurred in HFT (+41%, $p < 0.0001$), HFT-SC (+14%, $p < 0.0001$) and HFG (+17%, $p < 0.0001$) groups in relation to SC offspring.
Conclusion: Programming by HFD predisposes biometrical and morphological alterations in offspring adult life. HFD consumption during gestation provokes more adverse metabolic effect than HFD during lactation. HFD during gestation/lactation and during postnatal life predisposes fewer alterations, nevertheless obesity and hepatomegaly are present. Support: CNPq, Faperj, Capes.

1. P.G. Reeves *et al.*, *J.Nutr.*, 123:1939–1951, 1993.
2. C. Fernandes-Santos *et al.*, *Nutr.*, 7: 818–827.

P-3B-95

Testing the Protein Leverage Hypothesis of human obesity using dietary surveys

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Objective: The role of protein in the obesity crisis has, until recently, been largely ignored. This is for two reasons. First, protein provides the minor part of the human energy budget. Second, protein intake has remained far more constant over time and across populations than either fat or carbohydrate, both as a percentage of energy in the diet and in terms of absolute amounts eaten. Hence, while the obesity epidemic has spread, protein intake has remained relatively unchanged – giving the impression that protein cannot be responsible. Recently, we have used a geometric framework from nutritional ecology to derive a new hypothesis that identifies protein as a key nutrient in the obesity epidemic, the Protein Leverage Hypothesis (PLH). PLH postulates that food consumption in humans is adjusted to maintain a target protein intake, and consequently the consumption of foods with low protein content, as is typical of many Westernized countries, inevitably results in the ingestion of additional energy. Conversely, on protein-dense foods protein requirements are satisfied at lower energy intakes, creating the potential for weight loss. PLH has been supported by experimental studies of macronutrient regulation (1), and by meta analysis of experimental studies (2). Our objective in this study is to examine whether population-level data from dietary surveys are consistent with the PLH.
Method: We used 24-h dietary recalls from the 2005 Cebu Longitudinal Health and Nutrition Survey and the 1995 Australian National Nutrition Survey to test for a relationship between the percentage of calories from protein and total calorie intake in adult Filipino and Australian populations, respectively. PLH predicts a negative relationship between these variables.
Results: As predicted by PLH, in both populations there was a negative correlation between protein density of the diet and calorie intake. However, the relationship was asymmetrical: as predicted by PLH high energy intakes were not associated with protein-rich diets, but contrary to PLH low as well as high energy intakes were associated with low-protein diets.
Conclusion: Our analyses of population data are consistent with PLH. However, it remains to be determined whether the asymmetrical relationship found in this study but not in experimental studies is due to the sampling method, or a true

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reflection of the nutritional ecology of humans outside of the experimental setting.

1. S.J. Simpson *et al., Appetite,* 41:123–140, 2003.
2. S.J. Simpson, D. Raubenheimer. *Obesity Rev.,* 6:133–142, 2005.

P-3B-96

Effect of early-to-mid gestation maternal nutrient restriction and juvenile obesity on pericardial adipose tissue

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Objectives: Maternal nutrient restriction targeted during early fetal adipose tissue development can result in an enhanced inflammatory response within adipose tissue and is known to affect cardiovascular function1. The extent to which alterations of pericardial adipose tissue inflammatory activity induced by prenatal nutrition could increase cardiovascular risk when it accompanies obesity may be critical to the understanding of the developmental origins of cardiovascular disease.

Methods: Pregnant sheep (n = 18) were randomly assigned to a normal (7 MJ/day; C-O) or nutrient restricted diet (NR-O 3.5 MJ/day), from days 30 to 80 gestation (term = 147 days) and fed to requirements thereafter. After birth, offspring from both groups were kept with their mothers for the lactation period of 10 weeks during which all mothers were fed to metabolic requirements. To promote obesity, both groups received energy availability ad libitium and a confined space for exercise (17 animals per 50 m^2) between the 4th and 12th months after birth. Once sheep reached one year of age, they were humanely euthanased, pericardial tissue immediately frozen in liquid nitrogen and total RNA extracted for real time PCR analysis. mRNA abundance for adiponectin, interleukin (IL)s-6 and -18, monocyte chemoattractant protein (MCP)1, fat mass and obesity-associated (FTO) gene, glucose transporter (GLUT) 4 and glucose-responsive protein (GRP)78 determined by real-time PCR with all results expressed as arbitrary units (a.u.). Appropriate institutional animal ethics committee approval was obtained.

Results: Fat mass data was decreased when maternal nutrient restriction preceded postnatal obesity showed (C-O 328 ± 39; NR-O 232 ± 39 g ($p < 0.05$)). Gene expression of adiponectin, insulin receptor, IL-18, GRP78 and GLUT4, and FTO genes were unaffected. However, expression of both IL-6 and MCP-1 mRNA was upregulated in the NRO group compared to the C-O group (IL-6: C-O 0.075 ± 0.01, NR-O 2.0 ± 1.0 a.u.; MCP-1: C-O 1.1 ± 0.2, NR-O: 2.2 ± 0.6 a.u.; $p < 0.05$).

Conclusions: Maternal nutrient restriction targeted during early fetal adipose tissue development resulted in decreased pericardial adipose tissue mass. More importantly, gene expression of inflammatory markers IL-6 and MCP-1 exhibited an upregulation in response to maternal nutrient restriction. Whether the over production of these pro-inflammatory markers from pericardial adipose tissue may lead to possible adverse effects upon the heart[2] remains to be established.

1. D. Sharkey *et al.*, *FASEB J.,* 23:1314–1324, 2009.

P-3B-97

Obesity related hypertension, increased sympathetic drive and selective leptin resistance are programmed by exposure to a fat-rich diet during development

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Introduction: Maternal obesity or consumption of diets rich in saturated fatty acids during pregnancy can programme obesity related hypertension in the offspring but the aetiology is unclear. Leptin, secreted from white adipose tissue in proportion to its mass, acts at nuclei in the hypothalamus to decrease food intake and increase renal sympathetic nerve activity (RSNA), resulting in increased blood pressure. Selective leptin resistance; resistance to the anorectic but not pressor effects of leptin is thought to occur in adult obesity. It is not known, however, if selective leptin resistance or altered sympathetic drive can be programmed by maternal dietary challenge.

Objective: To determine if offspring from mothers fed fat rich diets in pregnancy and suckling become obese and hypertensive and to determine if these offspring demonstrate evidence of selective leptin resistance and increased RSNA.

Methods: Female New-Zealand white rabbits were fed either a control (3.5% fat from soy oil) or high fat diet (HFD, 13.5% fat from lard and soy oil) 3 weeks prior to mating, throughout gestation and lactation. Offspring were meal fed a control diet after weaning (6 weeks), effectively normalizing the post weaning caloric intake in all animals. At 4 months of age all rabbits were instrumented with intracerebroventricular (*icv*) guide tubes to the lateral ventricle and electrodes on the renal nerve. Blood pressure was measured by direct cannulation of the central ear artery. Basal haemodynamics and RSNA were measured then the responses to *icv* injections of leptin (5, 10, 50 and 100 μg) assessed. The anorectic response to leptin was measured by comparing 24 hour food intake after an injection of 100 μg leptin or vehicle. These studies were approved by the local animal ethics committee.

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Results: Body weight was similar between groups, however HFD offspring (n = 9) had ~40% heavier visceral white adipose deposition compared with controls (n = 8, $P < 0.05$) despite having a similar caloric intake post-weaning. HFD offspring demonstrated elevated basal blood pressure and RSNA compared with controls ($P < 0.05$). HFD offspring showed augmented pressor responses (ΔBP 4.8 ± 1.0 mmHg vs 2.6 ± 1.0 mmHg, $P < 0.05$) and renal SNA responses ($P < 0.05$) to *icv* leptin compared with controls. HFD offspring showed blunted anorectic effects to leptin compared with controls (−8.2 ± 4.8 g vs −21.3 ± 4.6 g, $P < 0.05$).

Conclusions: Exposure to a fat rich diet in development programmes increased adiposity even with a calorie controlled diet, indicating programmed changes to metabolism. These are the first data to show that offspring from mothers consuming a HFD show selective leptin resistance. This selective leptin resistance appears to result from alterations in hypothalamic integration of appetite and cardiovascular control and may contribute to the development of increased adiposity and elevated blood pressure in these offspring. Further investigation as to the location and neurochemical signature of affected neurons is now warranted. Support: National Heart Foundation of Australia Fellowship (PF 06M-2766), Monash Fellowship and Baker grants to JAA.

P-3B-98

The influence of nutrient restriction in late pregnancy and accelerated postnatal growth on glucose homeostasis following obesity in the sheep

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Objectives: Caloric restriction during pregnancy can lead to an increase in fat mass, a resetting of appetite regulation and decreased insulin sensitivity in offspring. Similar effects can also be observed if weight gain is promoted during early postnatal life. This study aimed to differentiate between the influence of these two critical periods in development in order to gain further insight into the mechanisms by which early growth can determine later control of body weight and metabolic health.

Methods: Pregnant twin-bearing sheep were either fed to requirements (R; n = 9) or nutrient restricted to 60% of this amount (N; n = 18) from 110 days up to term (~147 days). Nine offspring in each group were reared by their mother as singletons in order to promote postnatal growth (accelerated weight gain – A). Sixteen N twin offspring were reared by their mother in order to restrict postnatal growth (standard weight gain – S). After weaning, offspring were either kept in a control indoor environment in order to promote obesity or reared in an unrestricted environment and remained lean. Therefore, a total of four groups were generated: RAO (n = 9), NAO (n = 9), NSO (n = 7), and NSL (n = 9). Glucose tolerance was determined at 6 and 17 months of age from measurements of glucose and insulin plasma concentrations over a 120 minute period following 0.5 g/kg intravenous glucose. All offspring were then humanely euthanised. Appropriate institutional animal ethics committee approval was obtained.

Results: Offspring born to N mothers were smaller at birth (3.94 kg ± 0.12 vs. 4.84 kg ± 0.24, (p = 0.001)) but had similar adult weights which were enhanced in groups exposed to an obesogenic environment (RAO: 70.9 kg ± 4.6, NAO: 68.0 kg ± 3.1, NSO: 69.6 kg ± 5.0, NSL: 51.3 kg ± 1.7). This response was accompanied by increased fat mass (RAO: 13.1% body weight ± 2.2, NAO: 17.1% ± 2.8, NSO: 14.4% ± 1.0, NSL: 7.5% ± 0.9). Insulin responsiveness was highest in the NAO group and lowest in lean sheep (insulin area under the curve at 17 months, RAO: 49.2 ± 13.4, NAO: 94.4 ± 14.1, NSO: 66.4 ± 15.0, NSL: 22.0 ± 0.3 ng/ul over 120 min; RAO vs. NAO, p = 0.034; NSO vs. NSL, p = 0.030). These differences in insulin responsiveness were far more marked at 17 than 6 months of age.

Conclusions: Although maternal nutrient restriction reduced birth weight, it had no long term effect on adult body weight or composition when the offspring were exposed to an obesogenic environment. Insulin resistance occurred with the development of obesity, a process that was determined in part by maternal diet rather than postnatal growth. Future studies will establish molecular effects on adipose tissue and the extent to which appetite control and pancreatic function may be reset.

P-3C-99

Birth weight and risk of prostate cancer in Swedish men

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Objective: There is some evidence that birth size and *in utero* environment may be related to the onset of prostate cancer (PCA).[1] Similar to breast cancer, the intrauterine period and prenatal hormonal exposure have been proposed to increase PCA in adult life.[2] The earliest study[3] found a strong positive association with birth weight (BW), findings from subsequent larger studies, however, are equivocal. The present study

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examined recorded BW in relation to PCA risk controlling for other perinatal factors and adult body mass index (BMI) measured prior to diagnosis.

Methods: This case-control study was nested within the Malmö Diet and Cancer (MDC) Cohort Study[4] using available birth record data from 228 incident PCA cases diagnosed between 1991–2005 and 475 age-matched controls. The MDC cohort comprises 11,063 male participants. This analysis is restricted to PCA incidence among 3,562 men born in Malmö (1923–1945). Cases were matched with controls (1:2) by age at enrolment of the MDC-study. Cancer cases were ascertained by record linkage with regional and national cancer registries. We abstracted birth and maternal characteristics from archived hospital delivery records. We used Logistic Regression analysis to examine the effect of BW on PCA risk, with or without adjustment for gestational age (GA), maternal age, birth order, and BMI. Age at baseline (matching variable) was included in all models. BW was modelled both as a categorical (<3000 g, 3000–3499, 3500–3999, >4000) and continuous (by 100 g) variable. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Mean (±SD) BW and mean GA were 3539 ± 534 g and 39.3 ± 2.0 weeks. 19% of men had high BW (>4000 g) and 13% had BW <3000 g. Cases had a non-significantly higher mean BW than controls (3589 ± 514 g vs 3516 ± 542 g), and were more likely to have high BW than controls (23% vs 17%). BW was significantly ($p < 0.01$) and positively correlated with maternal age ($r = 0.15$), and birth order ($r = 0.25$), but not correlated with adult BMI. The risk of PCA increased by 3% per 100 g BW increase (OR 1.03, 0.97–1.06), but did not reach significance. When using categorical BW a stronger association became apparent. Men who weighed over 4000 g at birth had a greater than two-fold excess risk of PCA (OR 2.32, 1.27–4.23) compared to men with BW < 3000g (reference). The risk was also increased in the BW categories 3000–3499 g (OR 2.06, 1.16–3.61), and 3500–3999 g (OR 1.62, 0.93–2.84). The risk estimates were only marginally attenuated when adjusted for the other perinatal factors and adult BMI.

Conclusions: Despite lack of statistical significance in the linear modelling, there is a strong suggestion of increased PCA risk with higher BW in this population of Swedish men, independent of other potentially confounding factors. This preliminary analysis will be repeated with a larger cohort, including information on grade/stage of PCA.

1. A. Ekbom, *Semin Cancer Biol.*, 8:237–44, 1998.
2. RK. Ross, BE. Hendersson, *JNCI*, 86:252–4, 1994.
3. G. Tibblin *et al.*, *Epidemiology*, 6:423–4, 1995.
4. G. Berglund *et al.*, *J Intern Med.,* 233:45–51, 1993.

P-3C-100

Limited evidence of association between common genetic risk variants for breast cancer and fetal growth

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Objective: The incidence of breast cancer over the final quarter of the 20th Century increased worldwide. Higher birth weight, higher stature at 14 years of age, lower BMI at 14-years of age, peak growth at an early age and the rate of increase in height during puberty have been identified as independent risk factors for breast cancer. Between 2007 and 2008 seven common single nucleotide polymorphisms (SNPs) associated with increased risk of breast cancer were identified in genome-wide association studies (GWAS), a further six SNPs were identified from candidate gene studies; all provided independent replication. To explore the developmental programming of genes implicated in breast cancer, we investigated the association of these 13 breast cancer risk SNPs with fetal growth and birth biometry and examined both gene:smoking and gene:sex interactions.

Methods: The Western Australian Pregnancy (Raine) Study recruited 2900 pregnancies between 1989–1991. Ultrasound biometry was recorded at 18 weeks gestation for all pregnancies and at 24, 28, 34 & 38 weeks for a randomly selected half of these pregnancies. The ALSPAC Pregnancy Study recruited 14,541 pregnancies between 1991–1992. In both cohorts birth anthropometry included head circumference, birth length, birth weight and ponderal index. Skinfold thickness was also recorded in the Raine cohort. DNA was collected in both cohorts. Common variants in confirmed breast cancer risk loci, including those recently identified in genome-wide studies, were genotyped in the Raine cohort (n = 13 SNPs) and replicated in the ALSPAC cohort (n = 7 SNPs). Associations between the SNPs and fetal growth trajectories from ultrasound measurements and anthropometric measures at birth were assessed using linear mixed-effects models and multivariate linear regression, respectively. Gene:environment interactions involving sex, maternal smoking during pregnancy and each SNP were explored. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Analyses focused on 1,162 and 8,674 singleton-birth Caucasians with available genotype data in the Raine and ALSPAC cohorts, respectively. The rs13281615-GG genotype was associated with proportionally smaller birth weight (P = 0.02) and head circumference (P = 0.03) compared to the AA genotype in the Raine cohort. A similar trend was observed in the ALSPAC cohort, however, these results were only replicated in the offspring from maternal smokers during pregnancy; rs13281615:smoker interaction for birth weight (P = 0.02) and head circumference

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($P = 0.01$). No further consistent associations were detected between the SNPs and fetal growth trajectories or birth anthropometrics. No SNP:gender interactions were detected.
Conclusion: Limited evidence is available to support the hypothesis that common, known genetic risk variants for adult breast cancer act from early life in either males or females. Further investigation, based on the DOHaD hypothesis, is warranted into the role of these common adult breast cancer risk alleles in determining childhood growth and the onset of puberty.

P-3C-101

Cancer risk in women exposed to diethylstilbestrol *in utero*

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Objective: Diethylstilbestrol (DES), a potent synthetic estrogen, was administered to several million women in the United States and Europe between 1940 and the early 1970s. Initially administered in the misguided belief that it prevented adverse pregnancy outcomes, it was later promoted for use in healthy pregnancies. In 1971, a strong association was reported between DES and clear cell adenocarcinoma (CCA) of the vagina and cervix in young women.[1] The US National Cancer Institute (NCI) in 1994 combined follow-up of several cohorts of women with documented DES exposure to evaluate the incidence of neoplasia and other health outcomes. Data through 2001 demonstrated no excess risk of all cancers combined in DES daughters compared with both the general population and daughters who were unexposed to DES.[2] However; CCA continued to be strongly associated with DES. There were no significant excess risks for any of the other tumor sites with the exception of breast, which demonstrated an elevated relative risk in women 40 years of age and older.
Methods: Cancer incidence, as well as information on cancer risk factors has been ascertained by questionnaire. Preliminary analysis of new cancer cases reported through 2006 involved internal comparisons of DES exposed and unexposed women estimated with incidence rate ratios (RR) adjusted for birth year and 95% confidence intervals (CI) from Poisson regression models. During over 150,000 woman-years of follow-up, approximately 260 cases of cancer occurred in the exposed women and 100 in the unexposed women. Approvals for the study were obtained from the human investigations committees at the field centers and the NCI. Participants indicated their informed consent by completing a questionnaire or telephone interview.
Results: Overall, there was no increased risk among DES-exposed daughters compared with unexposed daughters for all cancers (excluding melanoma and cervical cancers) combined (RR 1.1; CI 0.8–1.4). No new cases of CCA of the cervix/vagina occurred in the exposed women since 2001. The RR for breast cancer comparing DES exposed with unexposed women continued to be modestly elevated (RR 1.2; CI 0.9–1.8), with a more appreciable RR among women age 40 and older (1.4; CI 1.0–2.1). With the exception of CCA, there were no significant excess risks for other tumors among exposed daughters, although continued follow-up will be necessary because of the still small case numbers.
Conclusions: Data on known biological exposures in pregnancy linked with subsequent cancer risk are rare. The DES cohorts provide some of the most relevant information bearing on the hypothesis that elevated *in utero* estrogen exposure affects subsequent cancer risk, as well as more recent concerns about the health effects of environmental endocrine disruptors. These findings suggest that high, pharmacologic doses of a synthetic estrogen do not cause an excess risk of total cancer in offspring; however, risk of breast cancer may be elevated in exposed women as they become older.

1. A.L. Herbst *et al. N Engl J Med.,* 284: 878–881, 1971.
2. R.Troisi *et al. Int J Cancer,* 121:356–360, 2007.
3. J.R. Palmer *et al. Cancer Biomark Epidemiol Prev.*, 15:1509–14, 2006.

P-3C-102

The effect of a flaxseed rich diet on breast cancer

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Objective: To verify the effect of a flaxseed rich diet on breast cancer, comparing two varieties of flaxseed.
Methods: A human breast cancer cell line (MCF-7) was injected subcutaneously into athymic mice. After seven weeks, when tumors were already established, the animals were randomly divided in three groups: control, fed a basal diet (BD); mice fed the basal diet supplemented with 10% of either brown (BFS) or gold (GFS) freshly ground flaxseed. Tumor growth was monitored weekly during the eight-week treatment with flaxseed. Appropriate institutional ethics committee clearance was obtained.
Results: The two groups of mice fed a diet supplemented with flaxseed presented a significant inhibition of the tumor

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growth as compared to the control one. There was no significant difference between tumor growth of mice fed brown and gold varieties of flaxseed (figure 1).

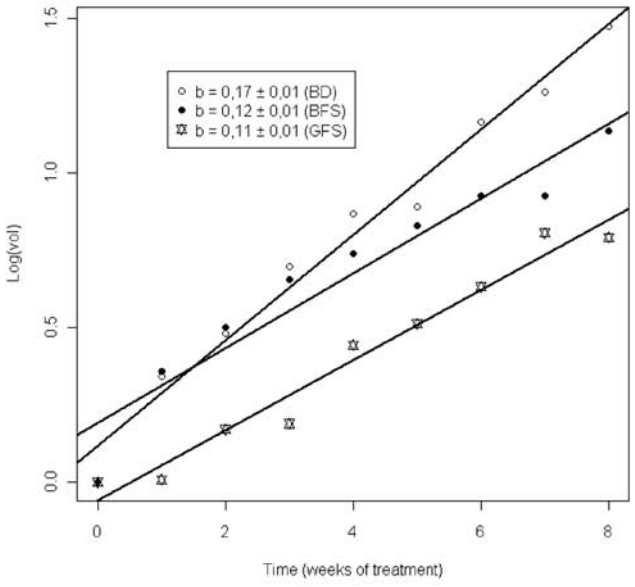

Figure 1. Logarithm of tumor volume, measured weekly, in mice fed different diets: a basal diet (BD); BD supplemented with 10% brown flaxseed (BFS); and BD supplemented with 10% gold flaxseed (GFS). Values are means; n = 9 – 10 mice; b values are the linear coefficient of the lines ± SE.

Conclusions: Our results confirm other authors' observation of the inhibition of breast cancer growth by dietary flaxseed[1,2,3] and suggest that both varieties of flaxseed – the brown and the gold – present this property. Further studies must be conducted to understand the inhibition of breast cancer by flaxseed. Support: Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP.

1. J. Chen *et al.*, *Clin Cancer Res*, 10:7703–7711, 2004.
2. M. B. Jungeström *et al.*, *Clin Cancer Res*, 13:1061–1067, 2007.
3. J. Chen *et al.*, *Exp Biol Med*, 232:1071–1080, 2007.

P-4A-103

Leptin surge inhibition at 30 days reverts programming in rats treated with leptin on the first 10 days of life

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Objective: Hyperleptinaemia in the first 10 days of lactation programmed for higher serum leptin andhigher body weight in 150 days-old rats[1]. These animals present hyperleptinemia already when they were 30 days-old[2], which may play a critical role in the establishment of this programming. Sirtuin (SIRT1) regulates glucose and lipid metabolism[3]**.** So, our aim was to assess the effects of leptin blockage, at 29 and 30 days, on the metabolic phenotype in rats programmed with leptin during lactation and the role of SIRT1.

Methods: After birth, the pups from Wistar rats were injected subcutaneously with either saline – C (control) or leptin – L (8 μg /100 g bw/day) from postnatal day 1 to day 10. At 29 and 30 days the animals from both groups received subcutaneous injections with either leptin antibody – LA and CA (3 μg/100 g/BW) or saline LS and CS. After weaning the animals received the same chow till the sacrifice at 200 days. Serum glucose and lipids were measured using commercial kits. Serum leptin and insulin were determined by specific radioimmunoassay. SIRT 1 was determined by Western Blot. All results were analyzed by two-way ANOVA followed by Newman–Keuls test, and we only reported those data with significance set at $p < 0.05$ or less.

Results: The higher visceral (+53%) and total fat mass (+33%), hyperleptinemia (+67%), hyperinsulinemia (+28%) and hypertriglyceridemia (47%) observed in the LS group that was programmed by leptin treatment on the first 10 days of life, are reverted by the treatment with leptin antibody at 29 and 30 days of life (LA). However, the control group treated with leptin antibody (CA) had most of the programmed effects of LS group, such as higher total (+33%) and visceral (+48%) fat mass, hyperleptinemia (+82%), hypertriglyceridemia (+39%) and additionally higher glycemia (+29%, $p < 0.05$), but not hyperinsulinemia. SIRT1 was higher (+41%) only in LA group.

Conclusions: Our findings suggest an important role of serum leptin concentration one week after weaning, characterizing another critical period for imprinting. In addition, the blockage of the leptin surge, which occurs in LS group at 30 days, reverts most of the programmed changes in adiposity, serum hormones, lipid and glucose metabolism. It is possible that SIRT1 stimulation may play an important role in these reversions and the glucose intolerance in CA group without hyperinsulinemia could be due to the no increment of SIRT1. So a higher SIRT1 leads to a better metabolic adaptation what could be protective against development of metabolic syndrome. Support: FAPERJ, CAPES, CNPq.

1 C. Oliveira Cravo *et al., Horm Metab Res.,* 34:400, 2002.
2 F.P. Toste *et al., Br J Nutr.,* 95:830, 2006.
3 F. Liang *et al.*, *Nat Rev Endocrinol.*, 2009.

P-4A-104

Comparison of age-related dynamics and gender differences in morbidity and mortality caused by several groups of diseases: no evidence for unique general scheme of aging potentially modifiable by perinatal programming

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Objective: Previously Barker and other researchers have shown that low birthweight was able to program higher risk of diseases like hypertension, diabetes, etc. in adult state. It could mean that there exists multiple comorbidity related to age and gender. Indeed, in our earlier studies we have shown significant coincidence in the patterns of age-related dynamics for morbidity and mortality caused by diseases of cardiometabolic group, including hypertension, diabetes mellitus and myocardium infarction, although there were clear gender differences in these epidemiologic parameters[1]. Later on, we compared cardiometabolic group of diseases with various types of cancer[2], and just recently with the group of neuropsychiatric disorders. The present work aimed at analyzing all these results together.
Methods: Primary data were extracted from national databank DataSus for the period 2001–2004 and three brazilian states of Southern region (RS, SC, PR). The data were recalculated for each age group as a percentage of the total morbidity or mortality. Thereafter, feminine fraction of morbidity and mortality was calculated in per cent for each age group. The final data were presented as arithmetic means of epidemiologic parameters for the period indicated.
Results: Only some, but not all cancer types have demonstrated age-related dynamics similar to those of cardiometabolic diseases. Besides, clear gender differences were observed for several non-reproductive cancer types. On the other hand, from all neuropsychiatric disorders, only cerebrovascular diseases have shown age-related dynamics similar to those of cardiometabolic pathologies. Alzheimer and Parkinson diseases followed pattern associated with aging, but in a mode quite different from that of cardiometabolic pathologies. Clear gender differences, as well as in age-related dynamics were observed for affective disorders, schizophrenia and epilepsy, as compared to cardiometabolic diseases. Few differences were registered between three brazilian states of Southern region.
Conclusions: In fact, there exist no evidence for unique general scheme of aging. Therefore, in spite of important unifying role of malnutrition and glucocorticoid exposure[3] in the phenomena of perinatal programming/imprinting, future studies should be directed to establish the mechanisms underlying age-related dynamics and gender differences for various diseases. It seems to us also that such peculiarities, although preliminary ones, may add some new dimensions to the complexity of DOHaD concept.

1. V.I. Goudochnikov. *Rev. Cient. JOPEF* (Curitiba), 2:97–99, 2005.
2. V.I. Goudochnikov. *FIEP Bull.* (Foz do Iguaçu), 79:235–238, 2009.
3. V.I. Goudochnikov, E.A.F. Kroth. *Diabetes Clin.* (São Paulo), 12:55–59, 2008.

P-4B-105

Prenatal exposure to undernutrition has long lasting negative effect on body mass index and waist circumference in females

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Objective: Prenatal exposure to undernutrition has long term consequences including chronic disease and obesity. We examined the association between exposure to Bangladesh famine (1974–75) during foetal life, a marker of severe nutritional deprivation, and body mass index (BMI), abdominal obesity and body fat percentage during early adulthood (27–31 y).
Methods: We randomly selected 219 individuals aged 27–31 y who were either exposed to famine (n = 68) or non-exposed, born before (n = 81) and after (n = 70) the famine with comparable sex distributions from ICDDR,B Health and Demographic Surveillance System (HDSS) database, Matlab, Bangladesh. Exposed subjects were born during or after the famine but were exposed to famine for at least 3 mo or longer during foetal life. The non-exposed were born either immediately before or after the famine and had no prenatal exposure to famine. We measured weight, height, waist circumference (WC), and skin fold thickness at multiple sites.
Results: Overall the exposed and the non-exposed groups did not differ in mean BMI, WC, or body fat percentage. When stratified by sex, exposed females, not males, had significantly lower BMI, WC and higher prevalence of chronic energy deficiency (MBI < 18.5) than non-exposed. These associations remained unaltered after adjustment for socio-economic status and educational attainment.
Conclusion: These findings suggest that prenatal exposure to undernutrition differentially affect BMI in resource poor settings with female fail to compensate for the earlier insult.

P-4B-106

Association of infant anthropometrics with left cardiac structures and blood pressure during the first 2 years of life. The Generation R study

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The human heart has its highest growth and development rate in early life[1]. Various studies showed tracking of left ventricular mass from childhood to adulthood[2,3] and therefore cardiac structure and function may be permanently affected by several factors in infancy and early childhood. The aim of this study was to investigate the influence of child anthropometrics on cardiac growth and blood pressure during the first 2 years of life.

Methods: This study was embedded in a prospective cohort study from fetal life onwards. In a subset of 1,001 children two-dimensional M-mode echocardiographic measurements of left cardiac structures (left ventricular mass, aortic root diameter and left atrial diameter) were performed at the age of 6 weeks, 6 months and 2 years. Height, weight, waist and hip circumference were obtained during the same visits. Percentage of body fat was measured with dual energy-X-ray absorptiometry at the age of 6 months and blood pressure was measured twice at the age of 2 years. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: At the age of 6 weeks, height and weight were significantly associated with left ventricular mass (increase of 1.05 (95% confidence interval (CI), 0.75, 1.34) grams and 1.20 (95% CI, 0.91, 1.50) grams per standard deviation (SD) change in weight and height, respectively), 6 months (increase of 1.32 (95% CI, 0.95, 1.69) grams and 1.22 (95% CI, 0.86, 1.58) grams per SD change in weight and height, respectively) and 2 years (1.92 (95% CI, 1.48, 2.36) grams and 2.01 (95% CI, 1.60, 2.42) grams per SD change in weight and height, respectively). Similar positive associations were found for height and weight with left atrial and aortic root diameter. The rate of increase of height and weight during the first 2 years of life was not related to left cardiac structures. Also, no associations were found of the waist to hip ratio and percentage of body fat with left cardiac structures until the age of 2. All analyses were adjusted for gender and age.

Conclusions: This study indicates that height and weight are important determinants of cardiac growth in infancy and early childhood. Small changes in body size in the first 2 years of life can have consequences for cardiac size and structure in later life. Further studies are needed to assess whether these associations persist and have influence on cardiac size in adulthood.

1. A. Ishii *et al.*, *J. Perinatal Med.*, 18: 459–463, 1990.
2. R.M. Schieken, *et al.*, *Circulation*, 97: 1901–1906, 1998.
3. J.J.M. Geelhoed *et al.*, *Am Heart J.* 158:71–77, 2009.

P-4B-107

Prevalence of chronic disease in later life among Finnish war evacuees

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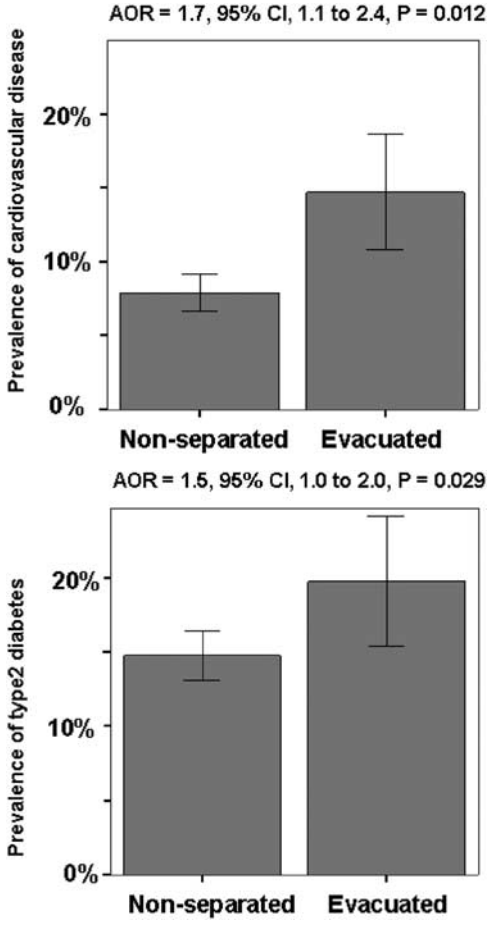
Objective: Early childhood separation from parents might have detrimental effects on health later in life. During World War II approximately 70 000 Finnish children were evacuated from their parents to foster families in other Scandinavian countries. Using the Helsinki Birth Cohort Study (HBCS) data, we have the opportunity to explore the long-term consequences of evacuation. The aim was to study prevalence of cardiovascular disease and type 2 diabetes 60 years after exposure to a traumatic separation in early childhood due to WWII.



Prevalence of cardiovascular disease and type 2 diabetes in war evacuees and non-separated controls in a clinical study. AOR refers

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to an adjusted odds ratio and 95% CI to a 95% confidence interval in an adjusting model. Error bars represent 95% confidence intervals.

Methods: This study includes 11186 subjects of whom 2003 participated in a clinical study. Of these 1505 (13.0%) and 320 (16.0%), respectively, were former war evacuees. The data on evacuations were extracted from the Finnish National Archives. The remaining participants served as controls. The average duration of the evacuation was 1.8 (SD = 1.1) years and the mean age at the time was 4.6 (SD = 2.4) years. Test for trends were based on multivariate linear regression and logistic regressions. The register data was adjusted for gender and date of birth and the clinical data for gender, age at testing, childhood and adult socioeconomic status. The prevalence of the outcomes studied was based on information obtained from a national register kept by the Social Insurance Institution. In addition, subjects in the clinical study underwent a 2-hour oral glucose tolerance test and they also reported whether they had been diagnosed with coronary heart disease by their physician. Appropriate Institutional ethics committee clearance and participants' informed consent were obtained.
Results: Based on register data prevalence of coronary heart disease was a higher among the former war evacuees (8.2% vs. 4.8%; $P = 0.010$). The figure shows that the result obtained at the clinical study confirms the former war evacuees' higher cardiovascular morbidity. Prevalence of type 2 diabetes was higher among the former war evacuees as assessed by a 2-hour 75-g oral glucose tolerance test. Based on register data there was no significant difference in diabetes prevalence (6.0 vs. 6.4; $P = 0.540$).
Conclusion: Traumatic experiences in early childhood influence later health outcomes and the prevalence of cardiovascular disease and type 2 diabetes in later life.

P-4B-108

Maternal lipid profile in early pregnancy in relation to prenatal and postnatal growth

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Objective: Growing evidence exists that maternal factors in gestation (such as total cholesterol (TC) and triglyceride (TG) levels) could act on intrauterine conditions with a subsequent effects on foetal development and newborn's health status later in life. We evaluated whether maternal TC and TG levels during early pregnancy were associated with birth weight and infant growth (defined by weight, length and body mass index (BMI)) during the first year of life.
Methods: Data were derived from a large community based cohort-study (Amsterdam Born Children and their Development study) in which pregnant woman donated venous blood during their first antenatal visit (median 13 weeks (IQR: 12–14)). In these samples, non-fasting TC and TG levels were determined. Subsequently, they completed an extensive questionnaire. Pregnancy outcome (birth weigh, gestational age, gender) and newborn's growth data at the age of 1, 3, 6, 9 and 12 months were collected from youth health care centres. Growth data was transformed into standard deviation scores (SDS). Associations of TC and TG levels (in quintiles) with birth weight and infant growth were explored by univariate and multivariate regression analyses. Only non-diabetic pregnant woman with live born singleton term deliveries were included (n = 2502). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: TG was positively associated with birth weight (estimated differences 78.1 ± 26.8 gram between highest quintile and middle quintile), which remained similar after adjustment for sociodemographic, pregnancy-related and behavioural factors). TG levels in the lowest quintile were associated with a lower birth weight (differences -28.6 ± 26.6 gram between lowest quintile and middle quintile, after adjustment). Additionally, TG was related to postnatal growth patterns. Infants in the lowest quintile displayed a lower weight (at 1 and 3 months), a shorter length (at 1 month) and a lower BMI (at 1 month) compared to the other quintiles. From 6 months onwards, growth patterns were similar for all TG quintiles. Accelerated weight gain (increase of SDS weight between 1 and 6 months >0.67) was more frequently present in infants in the lowest TG quintile (24.5%) compared to all other infants (19.6%, $p = 0.027$). TC was neither associated with birth weight nor infant growth.
Conclusion: Elevated high maternal TG levels during early pregnancy were associated with higher birth weight. On the other hand, decreased TG levels were associated with lower weight, reduced length and lower BMI during the first months of life with an accelerated weight gain during the first 6 months of life. Both a high birth weight and an accelerated weight gain are established risk factors for developing cardio-metabolic diseases later in life. New initiatives should be launched to study whether active prevention on maternal TG levels may improve newborn's growth outcome and its associated harmful cardio-metabolic profile in adulthood.
Support: The Netherlands Organisation for Health Research and Development (ZonMw), and the Dutch National Institute for Public Health and the Environment.

P-4B-109

Relationship of infant feeding patterns to cardiovascular risk factors in young adults; data from 5 cohorts in low and middle income countries

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Objective: Studies in high-income countries have shown that longer duration of breastfeeding in infancy is associated with a lower risk of later hypertension, type 2 diabetes and obesity. There is controversy as to whether this is causal or reflects confounding. Few studies have related age of introduction of complementary foods in infancy to later outcomes. Our objective was to test the hypothesis that longer duration of breastfeeding and later introduction of complementary foods are protective against adult hypertension, diabetes and overweight/obesity.

Methods: Data were pooled from 10.912 men and women aged 15–41 years, from 5 birth cohorts in low- or middle-income countries (Brazil, Guatemala, India, Philippines, South Africa) comprising the COHORTS collaboration (Consortium on Health Orientated Research in Transitional Societies). Exposure measures were 1) 'ever' versus 'never' breastfed; 2) total duration of breastfeeding (9 categories from no breastfeeding to breastfed for more than 24 months) and 3) age at starting complementary foods (6 categories from 0–3 months to >18 months). Outcomes were adult blood pressure, hypertension/pre-hypertension, plasma glucose concentration, diabetes mellitus, impaired fasting glucose, skinfolds, waist circumference, percentage body fat, and overweight/obesity. Analyses were adjusted for maternal socio-economic status, education, age, smoking, race and urban/rural residence, and infant birthweight. Each cohort study was approved by an appropriate institutional ethics committee and participants gave informed consent.

Results: There were no differences in outcomes between adults who were ever breastfed compared with those who were never breastfed. Associations between duration of breastfeeding and adult systolic blood pressure and prevalence of hypertension were U-shaped; however these were weak and inconsistent between cohorts. Duration of breastfeeding was not associated with adult diabetes or adiposity. Participants who started complementary foods later in infancy had lower adult BMI, waist circumference and subscapular skinfold thickness ($p < 0.01$ for all). BMI changed by $-0.19\,kg/m^2$ (95% CI -0.37 to -0.005) and waist circumference by $-0.45\,cm$ (95% CI -0.88 to -0.02) per 3-month increase in age at introduction of complementary foods between birth and 9 months. These associations were not significant after adjusting for 2-year weight.

Conclusions: There was no evidence that a longer duration of breastfeeding protects against adult hypertension, glucose intolerance or overweight/adiposity in these populations. Delaying the introduction of complementary foods until 6 months, as recommended by WHO, may reduce the risk of adult overweight/adiposity. This may be mediated by lower infant weight gain.

P-4B-110

Does childhood nutrition contribute to sex differences in risk factors for ischaemic heart disease in a developing population?: The Guangzhou Biobank Cohort Study

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Objective: A male epidemic of ischemic heart disease (IHD) emerges with economic development. We have previously hypothesized, based on physiological and epidemiological evidence, that this epidemic is due to nutritionally driven levels of pubertal sex-steroids, which generate a life long more atherogenic body shape and lipid profile in boys but not girls, without any sex-specific effects on glucose metabolism, which is in turn more strongly related to the somatotrophic axis. Here we tested this hypothesis by examining the association of childhood (≤18 years) meat eating with these IHD risk factors in older adults from a developing Chinese population.

Methods: Multivariable linear regression was used in a cross-sectional study of 19,418 Chinese older (≥50 years) men and women from the Guangzhou Biobank Cohort Study (phases 2 and 3) to assess the adjusted associations of childhood meat eating with waist hip ratio, HDL-cholesterol and fasting plasma glucose.

Results: Childhood meat eating had sex-specific associations with waist-hip ratio but not fasting glucose. Childhood daily meat eating compared to less than weekly meat eating was associated with higher waist hip ratio (0.007 [95% confidence interval 0.0002 to 0.01]) in men but not women, adjusted for age, life course socio-economic position and current lifestyle.

Conclusion: This study adds to a growing body of evidence suggesting that puberty may be a key developmental window when sexual dimorphism in IHD risk emerges. The male epidemic of premature IHD and sexual divergence in IHD rates which occur with economic development may be nutritionally driven in childhood or adolescence, with corresponding implications for men in the developing world currently experiencing the epidemiological and associated

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nutrition transition during early life. In elucidating the developmental origins of non-communicable chronic diseases more attention should be focused on socio-historical context and the hitherto overlooked role of puberty. Acknowledgements: The University of Hong Kong (HKSAR), Guangzhou Public Health Bureau (China), Guangzhou Science and Technology Bureau (China), The University of Birmingham (UK).

P-4B-111

The Pro12Ala polymorphism of the PPAR-γ2 gene interacts with low birth weight in increasing the risk for myocardial infarction

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Objective: The PPAR-γ2 gene regulates adipocyte differentiation and plays an important role in glucose, lipid and energy metabolism. The substitution of a proline by an alanine in codon 12 of the PPAR γ2 interacts with size at birth in its effects on insulin sensitivity, lipid metabolism, blood pressure and antihypertensive medication in adulthood. Our objective was to investigate the effects of this polymorphism and birth weight (BW) on insulin resistance, blood lipids levels and blood pressure at age 50 and on the incidence of myocardial infarction (MI) at age 50–85 in a cohort of Swedish men.

Methods: 2322 men comprise the Uppsala Longitudinal Study of Adult Men (ULSAM). Of these, we had complete data on birth weight from archives and genotyped data for 674 men from clinical investigations at age 71 with 154 cases of incident MI recorded in routine registers over 36 years of follow-up. The Pro12Ala polymorphism (rs1801282) was genotyped using a 1536-plex Golden Gate Assay and the Bead Station genotyping system from Illumina. The data was analysed by linear and Cox regression in STATA 10, with formal tests for interaction with birth weight categories. All analyses were adjusted for BMI and age. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: 158 (23%) of the 675 subjects carried either the Pro12Ala or Ala12Ala polymorphism. These subjects are compared to those carrying the wild type Pro12Pro variant in all comparative analysis. We observed statistically significant differences between the SNP variant and the wild type in mean levels of glucose measured at 0 min and 60 min after an intravenous glucose tolerance test in the lowest birth weight tertile (<3290g), but no differences in lipid levels or blood pressure. The effects of the Pro12Ala and Ala12Ala polymorphism on MI also depended on the birth weight of the subjects. Subjects with the Pro12Ala/Ala12Ala polymorphism in the lowest birth weight tertile had an increased risk for MI (hazard ratio, HR = 1.93, $p = 0.03$ adjusted for BMI and age) while no significant effect was seen in subjects in the middle and highest birth weight tertiles. The tests for interaction between birth weight and the polymorphism were not statistically significant.

Conclusions: The Pro12Ala and Ala12Ala genotype is associated with lower blood glucose levels but increases the risk for MI in subjects with a low birth weight (<3290g). These results for MI are in contradiction to previous reports of this particular genotype providing a protective effect on insulin resistance and type 2 diabetes in subjects born with low birth weight. Support source: Wallenberg Consortium North and Swedish Council for Working life and Social Research.

Table. Differences in mean glucose levels and hazard ratios (HR) for MI in men with the Pro12Ala/Ala12Ala polymorphism compared to the Pro12Pro wild type, stratified by birth weight.

Birth weight (g) Tertiles	Subjects *(n = 674)*	Glucose 0 min	*P*	Glucose 60 min	*P*	MI cases	HR	95% CI
≤3290	167	−.2178	0.03•	−1.1863	0.03†	47	1.93	1.06–3.52
3291−3959	337	−.1863	0.06	−.7201	0.08	76	1.25	0.75–2.10
≥3960	170	−.1128	0.34	0.2525	0.65	31	0.80	0.32–2.00

P-4B-112

Analysing longitudinal measurements of size early in life as predictors of later outcomes

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Objective: In the "Cohorts" collaboration a recurring analytical issue is how to measure the association between an outcome measured in adult life, for example blood pressure, and a sequence of height, weight and body mass index measurements made early in life.

Methods: We illustrate the issues using data on glucose tolerance among women in the New Delhi study. We discuss regression models, focussing especially on conditional growth models. Conditional growth models divide the age range of size measurements into a sequence of intervals in each of which growth is measured as the difference between the size observed at the end of the interval and the size predicted from

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measurements made at the start of the interval. Such differences are uncorrelated by construction and may be used as predictors of the later outcome variable. They can be thought of as the amount by which the subject's size changed in the interval beyond that which would have been expected. ***Discussion:*** Related issues on which we comment are a. pooling such results across cohorts, b. confounding, c. missing data, d. measurement error, e. selection effects, f. the optimal choice of age intervals, g. what happens when regression adjustment is made for size measured at the same time as the outcome variable (sometimes referred to the reversal paradox), h. an equivalent procedure that is retrospective not prospective, i. complementary graphical approaches.

P-4B-113

Head size at birth and mortality from coronary heart disease in adulthood

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Objective: Many studies have shown that low birth weight is associated with increased risk of heart disease in adulthood1. It is debated whether this association is caused by genetic or non-genetic factors, and whether life course exposures could influence the association2. We have studied if head circumference at birth is associated with later deaths from coronary heart disease (CHD), and assessed whether maternal height and adult body mass could modify the association.
Methods: In this population-based cohort study we have used information from birth charts of 35 846 men and women born between 1920 and 1959 who could be followed from1961 through 2005 with regards to cause specific death. Appropriate institutional ethics committee clearance was obtained.
Results: During follow up, 630 people died from CHD and there was an inverse association of head circumference with deaths from CHD (p_{trend} 0.010). The association was modified by maternal height ($p_{interaction}$ 0.01) and by adult body mass ($p_{interaction}$ 0.05). People in the lowest third of head circumference, who had a tall mother or a high BMI in adulthood, were at the highest risk of death from CHD.
Conclusions: Head circumference at birth was inversely associated with deaths from CHD, and the combination small head and tall mother, or small head and high adult body mass, was associated with the highest risk. These findings suggest that combined effects of genetic factors (growth potential and intrauterine growth) and non-genetic factors acting throughout the life course (intrauterine growth restriction and later weight gain) could mediate the effects of birth size on adult heart disease. Support: KRR was financed by the Regional Health Authority (RHA) and NTNU.

1. R Huxley *et al. Am J Clin Nutr*, 85: 1244–1250, 2007.
2. Kuh DaB-S, Y. (Ed). *A life course approach to chronic disease epidemiology*. 2 ed. 2004.

Table Hazard ratios (HR) for deaths from cardiovascular and coronary heart disease associated with one standard deviation[a] (SD) increase in birth size.

Causes of death by measure of birth size	HR[b] (95% CI)	HR[c]	*P*-value[b]
Coronary heart disease (630 deaths)			
Head circumference	0.90 (0.83–0.98)	0.90 (0.83–0.97)	0.010
Birth weight	0.92 (0.85–0.99)	0.91 (0.84–0.99)	0.020
Birth length	0.99 (0.92–1.07)	0.99 (0.91–1.07)	0.78

[a]SD-scores calculated within each sex and birth cohort (<1930, 1930–1939,1940–1949, ⩾1950).
[b]Adjusted for sex and birth cohort (<1930, 1930–1939, 1940–1949, ⩾1950).
[c]Adjusted for sex, birth cohort (<1930, 1930–1939,1940–1949, ⩾1950), maternal age (<20, 20–24, 25–29, 30–34, ⩾35 years), birth order (1, 2, > = 3), maternal marital status (married, unmarried) and paternal occupation(manual, non-manual).

P-4B-114

Long-term effects of intrauterine growth restriction on cardiac structure, function and metabolism

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Objectives: Using an animal model of hypoxia-induced intrauterine growth restriction (IUGR), our group has previously shown that adult IUGR offspring exhibit diastolic dysfunction and increased susceptibility to ischemia/reperfusion insult (I/R). A growing body of evidence suggests that alterations in cardiac metabolism could explain some of these cardiovascular characteristics of this IUGR model. However, the effects of the hypoxic prenatal insult on cardiac metabolism during adulthood are still unknown. The main objectives of this study were to determine whether this hypoxia-induced IUGR has any effect on cardiac metabolism later in life and to explore potential sex differences.
Methods: On day 15 of pregnancy (term 22 days), Sprague-Dawley rats were randomly assigned to hypoxia (12% O_2) or control (21% O_2) groups for the last 6 days of pregnancy. Pregnant dams were returned to normal oxygen conditions before birth. Cardiac susceptibility to I/R was evaluated in male and female offspring at 4 months of age using a Langendorf/working heart system. Cardiac metabolism (total ATP production and substrate use) was evaluated in basal conditions and after (I/R) using radiolabeled substrates. Appropriate institutional ethics committee clearance was obtained.

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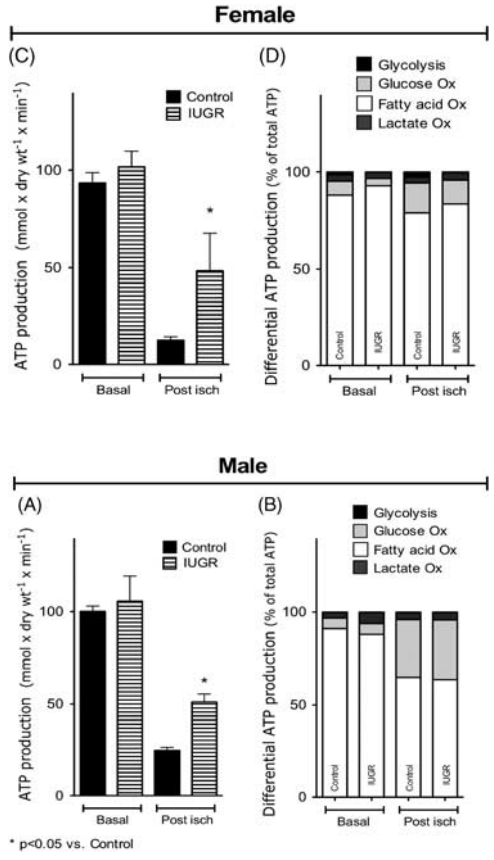
Results: Both male and female IUGR offspring exhibited an increased susceptibility to I/R and an increased post-ischemia production of ATP when compared to controls (Panels A and C of Figure). However, there were no effects of IUGR on cardiac selection of energy substrate (carbohydrates vs. fatty acids) either at baseline or following I/R (Panel B and D of Figure).



Conclusion: Our results suggest that a prenatal insult causing IUGR has long-term effects on cardiac energetic efficiency (produce more ATP but perform less work) after an ischemic insult. These changes in cardiac metabolism and efficiency are independent of the cardiac energetic substrate selection and are comparable in male and female animals. Acknowledgments: Canadian Institute for Health Research, Heart & Stroke Foundation of Canada, Alberta Heritage Foundation for Medical Research and MFN and TORCH Training Programs.

P-4B-115

Leg length and pubertal landmarks in men and women from a developing population: The Guangzhou Biobank Cohort Study

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Leg length is considered to be a reliable marker of pre-pubertal living conditions, because leg growth largely takes place before puberty. Cessation of leg growth is driven by oestrogen, and so occurs at an earlier pubertal stage in women than men.

Objective: We tested the hypothesis that components of height (leg length and sitting height) have sex-specific associations with pubertal landmarks using a large sample of older Chinese.

Methods: We used multivariable linear regression in 10,047 older (≥50 years) Chinese from the Guangzhou Biobank Cohort Study (phase 3) to examine the age adjusted associations of pubertal landmarks (age of menarche in women and mean age at voice breaking, first nocturnal emission and emergence of pubic hair in men) with leg length and seated height. We also examined whether the associations varied with sex.

Results: There were sex-specific associations of pubertal landmarks with leg length (p-value for interaction <0.001) and perhaps sitting height (p-value for interaction 0.18). In women, leg length was shorter by 0.20 centimetres (cm) (95% confidence interval (CI) 0.16 to 0.24) and seated height longer by 0.11 cm (95% CI 0.08 to 0.15) per year earlier in pubertal landmark (menarche). In men leg length was non-significantly longer by 0.05 cm (95% CI −0.05 to 0.15) and seated height longer by 0.14 cm (95% CI 0.05 to 0.24) per year earlier in pubertal landmark.

Conclusions: Apart from providing new evidence concerning the association of male pubertal landmarks with components of height, our study shows that leg length may be a biomarker of different exposures in men and women particularly in developing countries where age at puberty is above its physiological minimum and still environmentally driven. Given that pre-pubertal and pubertal growth have different associations with adult diseases caution should be used in the interpretation of associations with components of height. Supported by The University of Hong Kong (HKSAR), Guangzhou Public Health Bureau (China), Guangzhou Science and Technology Bureau (China), The University of Birmingham (UK).

P-4B-116

Early exposure to infectious disease and human life-histories

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Objective: Exposure to infectious disease in early life has been suggested to have a negative effect on later-life survival[1,2], possibly through the induction of inflammatory responses[3].

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Alternatively, the high energetic costs of activation and maintenance of an immune system[4] may reduce the resources available for growth and development, which may have negative consequences for adult health. However, to date no studies have investigated whether other outcomes, such as reproductive performance, might also be affected by such an early life stressor. In this study, we use historical family data from the 18th and 19th century Krummhörn population (Ostfriesland, Germany) and focus on both survival and reproductive success. We hypothesize that both of these may be negatively affected by early exposure to disease.

Methods: Using Kaplan-Meier plots and t-tests we compare survival rates and lifetime reproductive success (measured as number of children, proportion of surviving children and grandchildren) between an exposed and a non-exposed group. The exposed group comprised those exposed to the smallpox epidemic in 1752–1754 for at least six months during early development.

Results: Males and females were affected differently by exposure to early-life infection. While females show no significant difference in postnatal mortality rates compared to the control group, in late adulthood (above the age of 40) survival probability of exposed males exceeds that of men of the control group (Breslow: $p = 0{,}017$). Concerning reproductive parameters females, but not males, show an effect of early exposure to infectious disease on their reproductive performance. Females have a reduced number of births (exposed: $\bar{x} = 3.95 \pm 3.20$, non-exposed: $\bar{x} = 4.90 \pm 2.93$; $\alpha = 0.037$) as well as a decline in the relation of children surviving to age 15 to number of births (exposed: $\bar{x} = 0.43 \pm 0.31$, non-exposed: $\bar{x} = 0.51 \pm 0.29$; $\alpha = 0.076$).

Conclusions: In the Krummhörn, early-life exposure to infection shows sex-specific differences. While exposure resulted in negative long-term consequences for female reproductive success there was no ascertainable negative effect for exposed males. In contrast to other studies [1], there was no indication that later-life survival of either sex was negatively affected by early-life stress. To our knowledge these results are the first to show an apparent effect of early disease exposure on reproductive function.

1. Bengtsson T, Lindström M: southern Sweden, 1760–1894. *Pop. Stud.* 54:263–277, 2000.
2. Catalano R, Bruckner T: *Int. J. Epidemiology* 35: 1264–1269, 2006.
3. Crimmins EM, Finch CE: *PNAS* 103:498–503, 2006.
4. Lochmiller RL, Deerenberg C: *OIKOS* 88:87–98, 2000.

P-4B-117

Searching for causes and effects of an increase in LBW children in Japan: The Hamamatsu Birth Cohort for Mothers and Children (HBC)

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Objective: The proportion of low birth weight (LBW: born less than 2,500g) has been steadily increasing for the last two to three decades in Japan.[1] As for the explanatory factors, an increase in young women with low body mass index and with lower weight gain than desired during pregnancy is a candidate.[2] In turn, LBW leads to adverse outcomes in infants' neurodevelopment such as deviance in cognitive developments and developmental disorders, e.g. autism.[3] An increase in the prevalence of autism in the last decades as shown in epidemiologic studies [4] is concordant with the concurrent the increase of LBW babies in Japan. To explore these postulated causes and effects of LBW, we initiated a novel, multi-purpose birth cohort study "the Hamamatsu Birth Cohort for Mothers and Children (HBC)" in November 2007. We provide backgrounds and a methodological perspective of this cohort.

	During pregnancy	Delivery	2 w	1 m	2 m	4 m	6 m	10 m	14 m	18 m	24 m
Medical checkups of mothers and children	+	+		+		+	+	+	+	+	+
Psychiatric & psychological checkups of mothers and children	+		+	+	+			+	+	+	+
Intention for pregnancy	+										
Nutrition				+		+	+	+	+	+	
Socio-economic status, parental smoking	+			+		+	+	+	+	+	+
Weight, height, head circumference		+		+		+	+	+	+	+	+
Gross/fine motor, visual, receptive/expressive language				+		+	+	+	+	+	+
Sociability, behaviours, temperament							+	+	+	+	+
Biological sample collection (smear, venous blood, cord blood, placenta)	+	+									

Methods: Approximately 500 pregnant women, consecutively referred to two maternity clinics in the north-eastern part of Hamamatsu City have participated in the study and given birth. More than 1,000 women and the children are expected to participate in the HBC by the end of 2010. Each of the cohort members is followed up until 4 years of age. All the

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participants are being directly evaluated by a well-trained interview team in a standardised fashion. Each child undergoes 8 separate assessments during the follow-up. Appropriate institutional ethics committee clearance and participants' consent were obtained.

Results: Assembled information covers a wide range of variables related to mother's characteristics and child development: biological, physiological, paediatric, obstetric, environmental, nutritional, psychological, psychiatric, sociological, and economical aspects (Table).

Conclusions: The comprehensiveness of the HBC approach with a representative sample in the community is expected to provide an informative data source that contributes to an improvement in health policies for mothers and children in Japan.

1. H. Takimoto *et al., J Obstet Gynaecol Res* 31:314–22, 2005.
2. H. Takimoto *et al., Int J Gynaecol Obstet* 92:272–8, 2006.
3. W. W. Eaton *et al., J Autism Dev Disord* 31:279–85, 2001.
4. S. Baron-Cohen *et al., Br J Psychiatry* 194:500–9, 2009.

P-4B-118

Effect of birth weight and length on the height of schoolchildren and young adults

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Objective: To determine mean height at school age and during adult age according to birth weight and length of the participants in a cohort of liveborn singletons delivered at hospitals in Ribeirão Preto in 1978/1979, and studied and at 23 to 25 years. An additional objective was to assess the associations of these birth variables with height at school age and with final height, controlled by biological and social characteristics during these ages.

Methods: This was a longitudinal and prospective cohort study. A total of 1147 individuals (561 men and 586 women) out of the sample of 6827 births with available weight and length measurements and with a questionnaire filled out by the mother were measured also at school age and during adult age. The variables studied at birth were: weight, length, sex, maternal schooling and smoking habit, birth order, maternal age and marital situation, and occupation of the family head. The variables studied at school age were z-score for height per age and type of school and those studied during adulthood were skin color, final schooling, and age at menarche for women. The mean and standard deviations for final height were calculated according to the variables determined at the three times in life. The associations between anthropometric birth variables and schoolchild and adult height were assessed by multiple linear regressions in separate models for weight and height. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: In non-adjusted analysis, children with higher birth weight and length, born to mothers with better schooling, first born, born to non-manual workers and studying in paid private schools were associated with higher height at school age for both genders. Maternal smoking during pregnancy and mothers with a companion were associated with lower height among girls. After adjustment, weight and height, maternal age and birth order continued to be associated in both genders, family head occupation continued to be associated among boys and maternal marital situation among girls. The factors associated with higher adult height in both genders were: higher birth weight and height, mothers with higher schooling, being a first born, older mothers, skilled occupation of the family head, studying in a paid private school, higher height at school age and higher final schooling, and late menarche in women. After adjustment, birth weight and length, schoolchild's height and age at menarche for women continued to be associated with higher height in both genders.

Conclusions: The effect of birth weight and length persisted on the height of the schoolchildren and young adults of this cohort even after adjustment, but the effect of the remaining variables on schoolchildren's height disappeared in the adult age models after adjustment of schoolchildren's height by the z-score. This result suggests that height at school age seems to incorporate the effects of factors present in previous phases of life on the growth profile during this initial period, thus becoming an important predictor of growth during later phases. Support: CNPq, FAPESP and FAEPA.

P-4B-119

Description and determinants of growth profiles in the first year of life: the EDEN prospective cohort study

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Objective: Rapid early postnatal growth has been associated with obesity, whereas slow growth is predictive of cardiometabolic risk in later life. We aimed at describing frequent patterns of weight and length growth during the first year of life in French infants and to study their potential determinants.

Methods: Of the 2002 mother-child pairs included in the EDEN prospective cohort study, we could obtain growth profiles for 1757 infants of whom 1468 had all of the following potential determinants recorded: age, weight and height for both parents; maternal plasma glucose during pregnancy; type of infant feeding; gestational age; primiparity; maternal education. Individual

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growth trajectories between birth and one year were predicted using two separate mixed four-effects Jenss models for weight and length respectively. From the eight coefficients and following a principal component analysis, we identified patterns by k-means clustering method performed for boys and girls separately. Factors associated with these profiles were tested using a multinomial logistic regression. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: We identified four frequent patterns of growth during the first year of life (see the figure in boys for weight growth which contributed more than length growth in the difference between the patterns): pattern I = low birth weight and constant weight gain; pattern II = constant rapid weight growth; pattern III = high birth weight and slow weight growth; pattern IV = rapid weight growth before three months and slow weight growth thereafter. Similar patterns were identified in boys and girls. The 572 infants (300 boys) following pattern II were less often exclusively breastfed in the first three months (18.7% versus 35% for the other patterns; $p < 0.0001$). Among the other profiles, the 225 infants (100 boys) following pattern I were more often premature (19.1% versus 3.4%; $p < 0.0001$). Finally, the main difference between the 537 infants (280 boys) following pattern III and the 423 infants (236 boys) following pattern IV – which presents a slowdown in growth after three months – was that the latter had less frequently an obese or overweight father compared to others (39.4 versus 47.7%; $p = 0.02$).

Conclusions: We described profiles of early growth which correspond to physiological situations observed in French contemporary infants. These profiles were differentially associated with the main groups of determinants of early postnatal growth (prenatal factors, postnatal nutrition and genetics), although not enough to be fully discriminated by them. Follow-up of these subjects could help to identify if they are also predictors of different heath status.

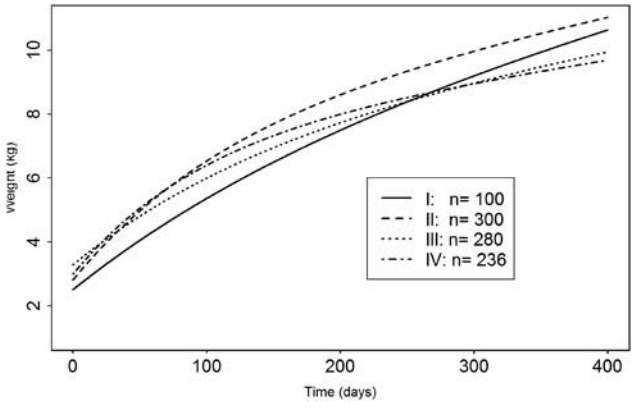

Figure: Weight growth profiles from birth to 1 year in boys.

P-4B-120

Influence of low birth weight on the nutritional status of children at 10–11 years old

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Objective: To assess the influence of low birth weight (LBW) and control variables at birth and at 10–11 years old on the nutritional status of school age children.

Methods: 874 school age children from a population-based birth cohort study carried out in 1994 in Ribeirao Preto, Brazil, were reevaluated in 2004/05. The parents were interviewed and children were submitted to anthropometric measurements (weight and height). Birth weight and other birth data were recovered from questionnaires applied to mothers after delivery. Intrauterine growth restriction (IUGR) was defined by the birth weight ratio (BWR–birth weight/mean weight for gestational age). Individuals with BWR < 0.85 were considered growth restricted. The nutritional status at school age was considered according to two references, Must *et al.*[1] and NCHS/CDC[2]. Children were undernourished when body index mass (BMI) was below the 5th percentile (P) and with weight excess when BMI ⩾ P85 in both references. LBW (birth weight < 2500g) and IUGR were the explanatory variables in a multinomial logistic regression analysis used to determine variables associated with BMI in separate models for each explanatory variable and for each reference of nutritional status[1,2], controlling for other variables from birth and school age. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Undernutrition was present in 10.5% and 11.1% according to Must *et al.* and NCHS/CDC references, respectively, and the proportions of weight excess were 28.7% and 24.2%. After adjustment LBW and IUGR were not associated to excess weight at 10–11 years old for both references. There was a positive association of IUGR with undernutrition for Must *et al.* (OR = 2.33; IC = 1.34–4.07) and CDC (OR = 2.23; IC = 1.29–3.84) and also a positive association of LBW with undernutrition for Must *et al.* (OR = 1.81; IC = 1.02–3.21) and NCHS/CDC (OR = 1.78; IC = 1.01–3.12). There was a negative association of IUGR with weight excess only for the NCHS/CDC reference (OR = 0.57; IC = 0.34; 0.93).

Conclusions: The weight excess as a risk factor for metabolic disorders in later life in individuals born with LBW and IUGR, seen in other studies, independent of the reference used to define nutritional status seems not to be yet present at 10–11 year olds. The results suggest that Ribeirao Preto is still experiencing nutritional transition. Possibly, due to the high rates of weight excess without reduction of undernutrition levels, the association of LBW and IUGR with excess weight has not yet occurred and is only observed for undernutrition in this setting. Acknowledgements: supported by FAPESP and FAEPA.

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1. A. Must *et al. Am. J. Clin. Nutr.*, 53: 839–46, 1991.
2. NCHS. Hyattsville, MD: National Center for Health Statistics, 2000: 1–28. (Vital and health statistics no. 314).

P-4B-121

Anthropometry from birth to 24 months among offspring of gestational diabetic mothers: 2004 Pelotas Birth Cohort

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Objective: The link between small birth weight and later obesity or later type 2 diabetes has been repeatedly documented. More recently, an association between offspring of gestational diabetic mothers (OGDM) and obesity or impaired glucose metabolism in early ages has also been documented, independently of size at birth. Less is known about the possible association between being born of a gestational diabetic mother and the growth pattern during the first years of life. The aim of this study was to describe the anthropometry, from birth to two years old, among offspring of women with and without gestational diabetes mellitus (GDM), in the 2004 Pelotas Birth Cohort.
Methods: Mothers that delivered their babies in 2004, in any of the five hospitals wards in Pelotas (a city in Southern Brazil), were interviewed at post-partum, by trained interviewers, using tested, pre-coded questionnaires. A medical diagnosis of GDM was self-reported. Weight, height and abdominal circumference of the newborns were collected and weight/age, length/age and weight/length Z scores calculated, from delivery to 3th, 12th and 24th months of life.
Results: 4239 children were studied. OGDM had lower gestational age ($p = 0.004$), higher weight ($p = 0.002$), and higher abdominal circumference at birth ($p < 0.001$) than offspring of mothers without GDM. Prevalence of large for gestational age (LGA) babies was threefold as higher in the OGDM (18.4% vs 6.8%) than in non-GDM group. Mean weight/age (-0.07 vs -0.37; $p = 0.01$) and weight/length (0.85 vs 0.47; $p < 0.001$) Z scores were also higher among OGDM at birth. During the first 3 months, there was an abrupt catch down among the OGDM that remained smaller than the non-DMG offspring until their 24th month of life. At birth, LGA OGDM were greater than LGA offspring from non-GDM mothers but they caught down steadily and at three months their size was similar of those born with appropriate weight for gestational age from non-GDM mothers (after adjustment for economic level, age and maternal BMI, child hospitalization and breastfeeding).
Conclusions: Following the Barker's hypothesis which establishes that the early years can program later life, the early and abrupt catch down pattern among LGA OGDM may be part of the causal pathway for obesity, impaired glucose tolerance and diabetes mellitus in the future.

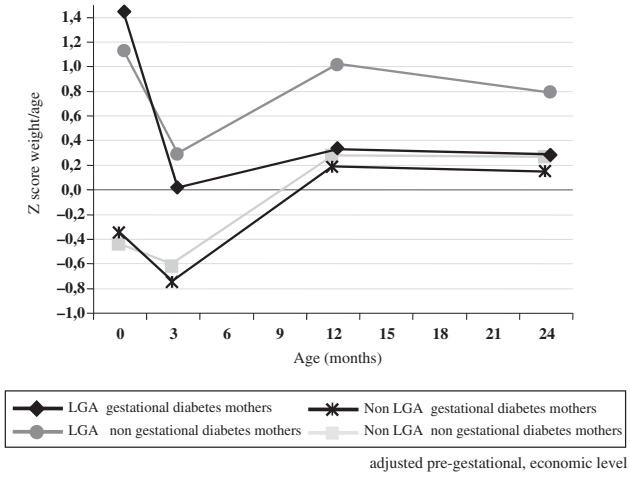

Figure 1-Z score weight/age from 0–24 months stratified by or gestational age.

P-4B-122

Transmission of risk of coronary heart disease risk across three generations in the Cohort of Norway (CONOR). Presentation of a multigenerational linkage within a large population based cohort

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Objective: Earlier studies have reported an inverse and stronger association between parental risk of coronary heart disease and offspring birthweight among mothers than among fathers in an attempt to disentangle intra uterine factors from common genetic factors shared between parents. There is sparse evidence adjusting for cardiovascular risk factors and looking at multiple generations. Here we investigated if there is a differential association between paternal and maternal risk of coronary heart disease (CHD) across three generations. This study is part of a multigenerational linkage within a large population based cohort including blood samples which will also be presented.
Methods: CONOR was linked to the Medical Birth Registry (MBR) and Cause of Death Registry, and from the full cohort two samples we identified parental offspring trios with grandparental data internally linked within CONOR cohort. Blood pressure, s-cholesterol, smoking, physical activity, length of education gestational length were used as covariates. The analysis was run in cox proportional hazards regression. Outcomes were birthweight adjusted for gestational length.
Results: CONOR includes 173,236 subjects. We were able to identify 19,848 parent offspring trios within CONOR. There was a decrease in risk of myocardial infarction among

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mothers per quintile increase in offspring birthweight, HR = 0.87 (0.77–0.98). Among fathers no similar effect was seen, HR = 0.94 (0.83–1.08). Adjusting for cardiovascular risk factors hardly changed the estimates. History of myocardial infarction among mothers mother was related to offspring birth weight, HR = 0.92 (0.88–0.97) but there was no effect for the other grandparents.

Conclusions: This study adds support to the role of intrauterine programming for the risk of cardiovascular disease in adulthood. Future studies of this cohort will investigate the mediating role of birth weight on subsequent cardiovascular outcomes in the offspring across generations. Appropriate institutional ethics committee clearance and participants' informed consent were obtained. The study was funded by our institutions. We would like to thank the participating health surveys and their universities who have contributed with data for the CONOR cohort.

1. Ø. Næss *et al.,* Cohort Profile: Cohort of Norway (CONOR). *Int J Epidemiol*, 37: 923–932, 2008.

P-4B-123

Socio-economic mobility and the risk of hypertension

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Objective: To investigate the association between socio-economic status (SES) at birth, in adulthood, SES mobility and the risk of hypertension.

Methods: By using data from the Swedish Twin Registry we formed a cohort of 23 547 like-sexed twins who were born in Sweden from 1926 to 1958. Our exposure measures were SES at birth measured by parental occupation, SES in adulthood measured by the participants own occupation and SES mobility that is a combination of these two. Our outcome measure was hypertension, defined as answering yes to the question of having hypertension and naming a prescribed antihypertensive drug. We used logistic regression analyses to estimate odds ratios (OR) with 95% confidence intervals (CI). Appropriate institutional ethics committee clearance was obtained.

Results: Compared with intermediate level white collar workers, the fully adjusted OR (95% CI) for hypertension in the unskilled blue collar worker group at birth and in adulthood amounted to 1.49 (95% CI; 1.15–1.93) and 1.11 (0.92–1.34), respectively. The association between low SES at birth and hypertension was independent of birth weight, while differences in body-mass index partly explained corresponding association with low SES in adulthood. Compared to those who were intermediate or high level white collar workers throughout life, blue collar or low level white collar workers throughout life had an increased risk of hypertension; OR 1.78 (1.37–2.33). Risks of hypertension were also increased among upward SES movers; (OR 1.50; 95% CI; 1.13–1.98) and downward SES movers (OR 1.39; 95% CI; 0.94–2.06) although significance was lost for downward movers after adjustment for BMI.

Conclusions: Those with persisting low SES have the highest risk of hypertension, but upward and downward SES movers have also increased risks. We could not disentangle the actual effects of social mobility from a cumulative effect of low SES. Support: Swedish Research Council and Swedish Council for Working Life and Social Research.

P-4B-124

Birthweight and mortality since the 1950s in a remote Australian Aboriginal community

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Objectives: Low birthweight is a risk factor for death in infants and children, and predisposes to chronic disease in adults. Birthweights have historically been low in remote Aboriginal Australia. We analysed associations of birthweight with deaths in one Aboriginal community against a background of rapidly changing mortality over time, due to better health services.

Methods: Participants were 996 people born alive between 1956 and 1985 to Aboriginal mothers in this community, and for whom birthweights were recorded. For birth intervals 1956–1965, 1966–1975 and 1976–1985, mean (SD) birthweights were 2.64(0.49), 2.69(0.54) and 2.87(0.52) kg respectively. Deaths were documented through 2006, and infant (0 to <1 year), childhood (1 to <15 year) and young adult deaths (15 to 36 years) were enumerated. Appropriate institutional ethics committee clearances were obtained for the studies generating these observations.

Results: Over the observation period there were dramatic falls in natural deaths in all age groups and across the entire birthweight spectrum**.** The death rates of infants born in the third birth interval were 10% the rates of those born in the first interval, while death rates of children born in the third interval were only 1.5% the rates of those born in the first interval. Against that background, birth weights below the median for each birth cohort were associated with higher mortality, with hazard ratios (HR, 95%CI) of 2.30 (1.3–4.7), 1.74 (1.03–3.1) and 2.69 (1.3–5.6) for natural deaths in infants, children and young adult respectively. Most strongly segregated among those of lower birthweight were "gastro-intestinal" deaths in infants, HR 5.2 (1.2–23), and "pulmonary" deaths in children HR 2.1 (0.94–4.7), while, in adults, pulmonary deaths were strongly associated with lower birthweights, 8.1(1.2–62), as was the combined endpoint of renal and/or cardiovascular deaths, HR 4.1(1.4–14).

Conclusions: The survival disadvantage associated with lower birthweight is confirmed for Aboriginal infants and children. It is also documented for the first time for Aboriginal adults, supporting the Barker hypothesis. Better services have dramatically reduced mortality of infants and children in all birthweight categories, which must be applauded. However, improved survival of low birthweight infants has resulted in a population of adults at higher risk for adult death. The current trend of improving birthweights promises further reductions in mortality.

P-4B-125

Infant growth during the first year of life and age at puberty onset: Hong Kong's "Children of 1997" birth cohort

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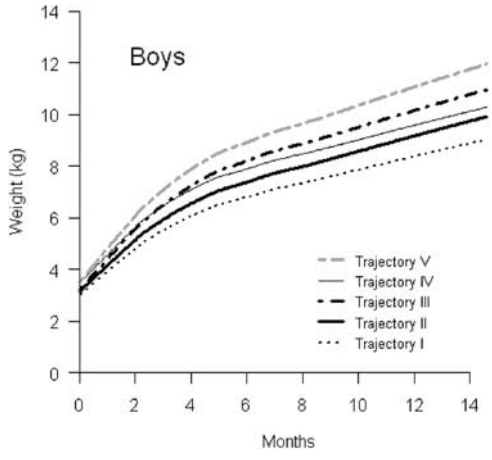
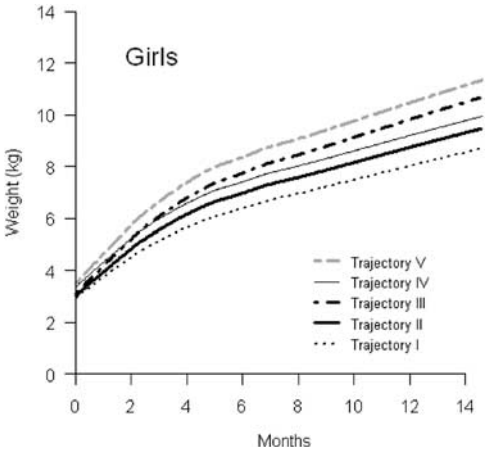
Objective: Younger age at puberty is associated with adult metabolic risk; it is unclear whether this is due to events at puberty or to the preceding growth trajectory as rapid infant growth is also associated with adult metabolic risk. To clarify this question, we examined the association of early growth with age at onset of puberty and whether the association was mediated by body mass index before puberty, at age 7 years. ***Methods:*** We used interval-censored survival analyses in term births (3369 boys and 3016 girls, 82% follow-up) from a Chinese birth cohort, "Children of 1997", comprising 88% of births in Hong Kong in April and May 1997, to examine the association of five sex-specific growth trajectories from birth to 12 months, derived from latent class analyses (figure), with clinically assessed age at onset of puberty (Tanner stage II for breast size or penis size). Confounders included were sex, gestational age and parental education.





Results: There was no evidence that infant growth trajectory had different associations with age at onset of puberty in boys and girls. Compared with infants born light who "tracked" (growth trajectory II), infants born light with slow growth (I) had older age at onset of puberty (Table). Children born heavy who grew fast during infancy (trajectory V) started puberty at the earliest age, about 4 months earlier than the children of same sex in trajectory I. However, the associations shown were negated by adjustment for body mass index at 7 years.

	Mean birth weight z-score	Infant Growth rate	Time ratio (95% CI)	Adjusted age at onset of puberty, years (95% CI)	
				Boys	Girls
Trajectory I	−0.71	Slow	1.01* (1.00, 1.03)	9.8 (9.7, 9.9)	12.0 (11.8, 12.2)
Trajectory II	−0.50	Tracked	Ref	9.6 (9.5, 9.7)	11.9 (11.7, 12.1)
Trajectory III	−0.24	Fast	0.99 (0.98, 1.00)	9.6 (9.5, 9.7)	11.7 (11.5, 11.9)
Trajectory IV	0.24	Tracked	1.00 (0.99, 1.01)	9.6 (9.5, 9.7)	11.9 (11.7, 12.1)
Trajectory V	0.44	Fast	0.98* (0.97, 1.00)	9.6 (9.5, 9.7)	11.6 (11.4, 11.7)

Conclusions: Earlier age at puberty appears to be a marker of more rapid pre-natal, infant and childhood growth. Whether the associations between rapid early growth and adult metabolic risk are in any way modified by the intensity or duration of puberty remains to be determined. Acknowledgements: We thank the Student Health Service and the Family Health Service of the Department of Health Hong Kong SAR, and grants HCPFC 216106 and HHSRF 03040711.

P-4B-126

Alberta Pregnancy Outcome and Nutrition (APrON) study

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Objectives: Nutrients are essential for brain growth and development, as well as for good physical and mental health. It is often assumed that people living in developed countries have adequate nutrient intake, yet deficiencies are common. The impact of poor diet is magnified by pregnancy, when a woman's nutrient needs increase and the fetus depletes maternal reserves. Studies have implicated the lasting effects of the uterine environment on the health of the fetus long after birth. Little is known, however, about the complex interplay between prenatal nutrition, maternal mental health, genetics, the uterine environment, and child development and health. The *purpose* of this study is to determine the relationship between maternal prenatal nutritional intake and status on the mother's mental and physical health, and the child's birth outcomes, neuro-development, behaviour, and mental/cognitive health.
Methods: This longitudinal cohort study will recruit 10,000 pregnant women in two large centre in Canada (Calgary and Edmonton, Alberta) to: a, Determine their nutrient intake with dietary questionnaires and by examining the nutrient status of their blood; b, Assess their mental health from pregnancy to 3 years post partum; c, Assess their anthropometrics (i.e. body measurements); d, Assess their thyroid function; e, Evaluate their obstetrical status, and neonatal outcomes; f, Evaluate their babies' neurodevelopment, behaviour, mental health/cognitive health, growth, anthropometrics, and feeding to age 3; g, Examine the composition of breast milk their babies receive; h, Develop a genetic biobank of women, biological fathers, and infants; i, Multiple time points for data collection through out pregnancy and postnatally.
Results: The results will be shared with health professionals, key community stake holders, industry leaders, educators, and the public.
Conclusions: Examples of potential applications of the study results might be: a, Ability to capture reliable data about the nature of the fetal environment, focused on nutritional intake and status, maternal physical and mental health during pregnancy and birth outcome as well as development of the offspring for minimal three years, but can be longer; b, Providing information to prenatal vitamin producers or informing baby formula and baby food producers for product design; c, Providing physicians, nutritionists, nurses and other educators information about the nutritional status of women and children in Alberta; d, Providing information to community groups to inform the most desired contents of food banks, possibly even leading to specially designed pregnancy-based food banks; e, Informing Canada's Food Guide content for pregnant women; f, Providing information to the public about optimal food choices during pregnancy and post partum; g, Educating school boards and daycares about optimal infant feeding and teaching practices; h, Providing agricultural groups information about what types of foods are being consumed in Alberta. Support: Alberta Heritage Foundation for Medical Research (AHFMR).

P-4B-127

Maternal nutrition and blood pressure in young adults from the 1982 Pelotas Birth Cohort

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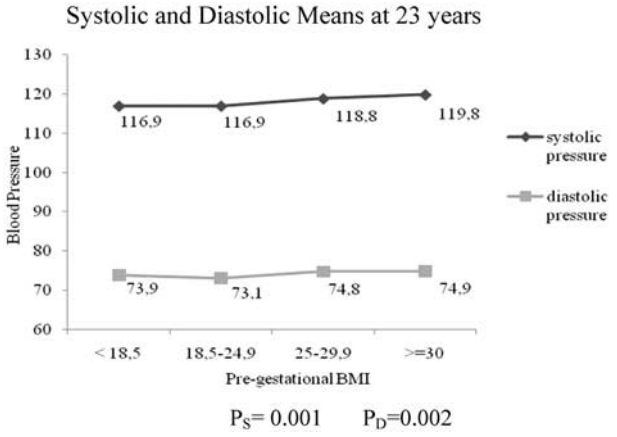
Objective: To assess the association between pre-gestational body mass index (BMI) and blood pressure in 23 year-old adults belonging to the Pelotas birth cohort.
Methods: In 1982, all births occurring in the city's maternity hospitals were included in a perinatal study. Newborns were weighed and their mothers were weighed and measured. Other variables related to child-mother health, demographic and socio-economic characteristics were also collected. Ten follow-ups were carried out between 1982 and 2004–5, when mothers and children/adolescents were interviewed, and the latter had their weight, height and blood pressure measured. This measure was taken twice through wrist digital sphygmomanometer. In this analysis variables collected at birth and in early adulthood were included, and ANOVA and linear regression were used in the analysis. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.



Results: At 23 years of age, mean systolic and diastolic pressure was higher in the cohort members whose mothers were overweight (118.8 ± 15.3 and 74.8 ± 11.2 mmHg) and obese before pregnancy (119.8 ± 15.8 and 74.9 ± 12.2 mmHg), than for those with underweight mothers (116.9 ± 15.3 and 73.9 ± 11.5 mmHg) (Figure). The association between pre-gestational BMI and blood pressure remained when adjustments were made for socioeconomic variables (family income, maternal schooling and asset index). Systolic pressure was 1.85 mmHg (0.52 to 2.18) higher if the mother was overweight before pregnancy compared to normal weight mothers. For diastolic pressure, the difference between

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overweight and normal weight mothers was 1.66 mmHg (0.65 to 2.67). In the analysis including birth weight as a mediating factor, the association remained the same, but when BMI at age 23 years of age was also included in the model, the positive association between pre-gestational BMI and blood pressure disappeared.
Conclusions: These results suggest that the effect of maternal pre-gestational BMI on blood pressure in adulthood is explained by the effect of maternal nutrition on anthropometric indices of the offspring. Acknowledgements: Wellcome Trust - Major Awards for Latin America on Health Consequences of Population Change.

P-4B-128

To what extent do birth weight and gestational age influence infant mortality when genetic and environmental factors are accounted for?

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Objective: Low birth weight and a short age of gestation are important risk factors for adverse health outcomes in infancy. Birth weight and gestational age have been associated with later-life adverse health and social outcomes, but it has been argued that these associations do not represent causal effects of early life exposures, but are partly or fully explained by confounding from genetic and environmental factors. Nearly all traits run in families and are shared by siblings to various degrees: This can be exploited to control for unobserved genetic and environmental confounding.
Methods: This paper examines the effect of birth weight and gestational age on infant mortality in a linked dataset consisting of all children born in Denmark 1977–2006 (n = 2,214,088). We examine the associations between birth weight, gestational age and mortality in ordinary cohort analyses and by comparing children that are discordant on birth weight or gestational age within sibships using two strategies: Comparing the outcomes of children whose mothers are siblings and by comparing children who are siblings. According to Danish legislation, the study was approved by the Danish Data Protection Agency.
Results: The cohort analyses reproduce the well-known associations between birth weight, gestational age and infant mortality, but out analysis of within-sibship data show that a substantial part of the associations seen in the cohort analyses are due to confounding from factors shared in families.
Conclusion: Our results suggest that some of the observed association between birth weight, gestational age and later health might not be due to a causal effect of birth weight and gestational age, but is due to unobserved common causes of birth weight, gestational age and later health. This has implications for observational research on the fetal origins hypothesis.

P-4B-129

Parental and grandparental cardiovascular mortality and offspring birthweight

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[1]*Division of Epidemiology, National Institute of Public Health, Oslo, Norway;* [2]*Institute of Health Management and Health Economics, University of Oslo, Norway;* [3]*Institute of Community Medicine, University of Tromsø, Norway;* [4]*Department of Social Medicine, University of Bristol, UK*

Objective: To investigate if there is a stronger maternal than paternal association in cardiovascular mortality risk with offspring birth weight and to investigate this across three generations.
Methods: A linkage between the Medical Birth Registry, Cause of death registry and the population registry in Norway. All offspring (F2) born between 1967–1996 were linked to their parents (F1) and grandparents (F0). Mortality follow-up among parents was from 1967 to 2006 and among grandparents from 1960–1980. The analysis was run in cox proportional hazards regression. Deaths by cause were followed over the period 1967 to 2006 for parents (F1) and 1960–1980 for grandparents (F0). All death certificates were registered with Statistics Norway, without any missing cases. Deaths were coded according to ICD-7 until 1969, ICD-8 1969-85, ICD-9 1986–95 and ICD-10 from 1996 onwards. Causes of death from different versions of ICD were coded according to the European short-list for causes of death: Circulatory (ICD-10: I00-I99), coronary heart disease (ICD-10: I20-I25), stroke (ICD-10: I60-I69), lung cancer (ICD-10: C32-C34), stomach cancer (ICD-10: C16) and accidents except suicide (ICD-10: V01-V99).
Results: 311,599 trios were identified. There was a stronger association between maternal risk of coronary heart disease, stroke and lung cancer. Per quartile decrease in hazard ratio among mothers was 0.73 compared to 0.93 among fathers for coronary heart disease. For stroke the similar figures were 0.80 (0.75–0.86) and 0.88 (0.82–0.94), respectively. Adjustment for parental length of education and housing conditions did not change these estimates. The association between grandparental risk of coronary heart disease and offspring birth weight was strongest for the maternal mother. The other grandparents had smaller effects but the paternal father (F0) no effect on offspring birth weight (F2).
Conclusions: In this first study to investigate differential associations between parental and grandparental cardiovascular mortality risk with offspring birth weight in three generations, stronger effects were seen through the maternal than the maternal line. This provides support for the role of the fetal environment on future cardiovascular disease risk. Appropriate institutional ethics committee clearance and participants' informed consent were obtained. The study was funded by our institutions. We would like to thank the Statistics Noway and the Medical Birth Register for making the data available.

P-4B-130

Hypertensive diseases of pregnancy and difficult babies: A large-scale cohort study

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Objective: Hypertensive diseases of pregnancy, including gestational hypertension and preeclampsia, continue to pose a serious threat to maternal and child perinatal health. In addition to the risks for the infant's physical health, such as intrauterine growth restriction, cerebral palsy and preterm birth, gestational hypertension was recently found to have long-lasting behavioural consequences for the child from age two to 14 years. This study aimed to assess whether hypertensive diseases of pregnancy are also linked with infant behaviour in the first year of life.
Methods: This was a prospective pregnancy cohort study of 2,900 pregnancies and 2,868 live births. Mothers completed a validated Australian adaptation of the Carey Toddler Temperament Scale (TTS) when the children were one year of age. Algorithms were used to classify children as difficult (arrhythmic, withdrawn, low adaptability, intense and negative in mood), or easy (the opposite characteristics). We used a multivariable logistic regression model and adjusted for known biomedical, sociodemographic and psychological factors from the pre- and postnatal period that may influence child behavioural development. These data were collected at 18 weeks gestation and included maternal age, maternal education, family income, the presence of the biological father in the family home, maternal smoking and maternal experience of stressful events in pregnancy. We also accounted for the appropriateness of fetal growth and gestational age. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: After adjusting for confounders, children born to mothers who were diagnosed with gestational hypertension (OR = 1.43, 95%CI = 1.12, 1.83) or preeclampsia (OR = 1.93, 95%CI = 1.05, 3.57) were more likely to be difficult babies in the first year of life than those born to mothers without either condition. We also found that toddler temperament was significantly correlated with later mental health outcomes in childhood through to adolescence.
Conclusions: These data suggest that the link between maternal gestational hypertension and child behavioural development begins in the first year of life with a negative impact on infant temperament. Temperament is considered to be an intrinsically biological construct, and therefore this study provides an indication of a biological pathway to explain the impact of maternal hypertensive diseases of pregnancy on child behavioural development. *Abbreviations:* OR (odds ratio), 95%CI (95% Confidence Intervals).

P-4B-131

Influence of birth weight on bone mineral mass at adult life: a systematic review

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Objective: A systematic literature review was conducted to investigate the possible association between birth weight and bone mineral mass at adult life.
Methods: The MEDLINE database was searched for English language papers using key words such as birth weight, birth size, bone mass, bone area, bone mineral content, bone mineral density and osteoporosis. Three hundred and ninety three papers were located in the initial search. An abstract review to identify clearly the adequate papers reduced this number to 14. Two authors read each paper and applied a checklist for methodological quality assessment proposed by Dawns & Black[1]. The maximum possible score for each paper was 20. None study was rejected by the attained score, however results from studies with higher score were considered more consistent. Kappa coefficient was applied to analyze the score consistency between readers.
Results: Birth weight has a crude association with adult bone mineral content and bone area. Some studies support these associations even after adjustment for adult body size. Regarding to bone mineral density, all crude associations observed lost significance after adjustment for adult body size. Thus the relation of birth weight with women's bone mineral density seems to be mediated by growth in early life. The fourteen papers mean score was 15.7 (range 10 to 19). In general, the recent studies (2005–2008) received a better score, denoting an improvement in data report. However, independently of publication year, questions that frequently fail were: planned sample representativeness, effective analyzed sample representativeness, adequate adjustment for confounding variables and tacking into account losses of follow-up in data analyses.
Conclusions: The reviewed studies argue that individuals with lower birth weight become adult with lower bone mass. Further research is needed to clarify the role of postnatal growth in the recovery of any deficit in peak bone mass, programmed during intrauterine development due some nutritional insult. Support: Coordenação de Apoio de Pessoal de Nível Superior – CAPES.

1. Downs SH, Black N. *J Epidemiol Community Health*, 52:377–384, 1998.

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P-4B-132

Early determinants of reproductive life in women from the 1982 Pelotas Birth Cohort

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Objectives: To describe the relationship between early exposures and menarche and fertility in the 1982 Pelotas Birth Cohort.
Methods: A population-based birth cohort carried out in Pelotas, Brazil. All newborns in the city's hospitals were enrolled in 1982. The subjects were followed-up on several occasions. In 2004–5 (mean age 23 years), we attempted to trace the whole cohort, and 2083 women were interviewed. Questions about age of menarche, parity, difficulty to get pregnant and regularity of menstrual cycles in the last 3 months were accessed from about 1980 non-pregnant women The multivariable analysis used Poisson regression and the model included, in a first hierarchic level, socioeconomic and demographic variables - maternal schooling, age and skin color, family income in 1982, change in income between 1982 and 2004–5, and women skin color. Next level included maternal variables (pre-gestational Body Mass Index (BMI) and smoking during gestation); in the third level birth weight, birth weight in z scores according to Williams' curves, prematurity and breastfeeding duration. Those variables with $p \leqslant 0.20$ were maintained in the models to control confusion. Appropriate institutional ethics committee clearance and participant's informed consent were obtained.
Results: The mean age of menarche was 12.4 yrs. At age 22–23, 40.5% of the women were mothers of one or more children. The prevalence of precocious menarche (⩽9 yrs) was 3.2% (N = 64). Early menarche (⩽11 years) was present in 24.4% of the women and was associated in the crude analysis only to maternal pre-gestational BMI Those mothers whose BMI was in the higher quartile showing 33% of early menarche ($p < 0.001$), compared to 19.6%, 22.8 and 32.1 in those whose mothers had pre-gestational BMI in the lower, second and third quartile, respectively. In the multivariable analysis, after controlling to smoking during the gestation, those subjects whose mother was of low BMI had 20% higher risk of early menarche, compared to those in the higher BMI. Regarding to difficulty to conceive, 5.4% of the women referred that had tried to conceive without success or had received medical treatment to get pregnant. This difficulty was more frequent in women from low income at birth, from low educated, younger, smoker mothers or those whose mother had a higher BMI. In controlled analysis, those women from lower tercile of family income had RR 2.47(CI 95%1.01–6.07) of report difficult to conceive than those from the higher tercile, a RR 3.22(CI 95% 1.00–10.34) in those of lower educated mothers and RR 2.63 (CI 95% 1.16–5.96) of those from higher BMI mothers. Skin color, birth weight, gestational age and breastfeeding were not associated with the outcomes studied.
Conclusions: In these 22–23 yrs. women from a birth cohort, we find a direct association of low socioeconomics with difficult to conceive. Low age of menarche was directly associated only with mothers of low BMI.

P-4B-133

Lifestyle and socioeconomic differences of Pakistani and white UK pregnant women: the Born in Bradford birth cohort

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Objective: To describe differences in lifestyle and socio-economic characteristics of a bi-ethnic cohort of UK pregnant women.
Methods: A birth cohort study – recruiting women, and husbands/partners, in the 26th week of pregnancy. The inclusion criteria is to be a pregnant woman in Bradford, one of the UK's most deprived cities, with 60% of babies born in Bradford being in the first quintile of area deprivation for England/Wales. Recruitment began in March 2007; 8,127 mothers, 6,932 babies and 1,865 fathers have been recruited (May 2008). The target is 10,000 babies with full baseline data – comprising permission to access routine health data, a questionnaire and biological samples from mother and baby. This presentation focuses on characteristics of 5,000 families. Ethnicity, country of birth of parents and grand-parents, occupation, socioeconomic position, smoking and alcohol consumption were obtained from questionnaires. Appropriate institutional ethics committee clearance and participant's informed consent were obtained.
Results: 48% of mothers in these 5,000 families were identified as Asian or Asian British Pakistani, half this group were born in the UK and half moved to the UK from Pakistan. There was a bimodal distribution of age at migration to the UK for mothers born in Pakistan with peaks at ages 1 and 18. 93% of the white British mothers were, or had been, in paid employment at the time of recruitment compared to 53% of the Pakistani mothers (prevalence ratio 1.74; 95% CI: 1.67–1.82). There were also marked differences in smoking (prevalence ratio comparing white British mothers to Pakistani mothers for ever smoked 7.16 (6.12, 8.37) and alcohol consumption (prevalence ratio for any alcohol consumption during pregnancy or 3 months before 179.1 (89.6, 357.8). Measures identifying material deprivation via ascertaining ownership of indicator items and

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subjective assessments of poverty show similarities between ethnic groups, with high levels of deprivation in both groups.
Conclusions: Recruitment of pregnant women is high in both ethnic groups, and appears representative of overall births in Bradford. The cohort exhibits high levels of material deprivation in both groups. Mothers of Pakistani origin are less likely to have ever worked and less likely to smoke or consume alcohol. The questionnaire data linked to obstetric medical records, to obtain information such as birthweight and gestational age, should by the date of the conference allow presentation of ethnic differences in birthweight and gestational age, together with the extent to which other characteristics might explain these at that time. Future work will explore differences in body composition (specifically evidence for the fat-thin insulin resistant phenotype) at birth between these ethnic groups and mechanisms for these differences.

P-4B-134

Effects of depressive symptoms during pregnancy on major obstetric and neonatal outcomes: results from a large prospective cohort study

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Objective: Maternal depression during pregnancy may negatively influence obstetric and neonatal outcomes, however, results are inconsistent. This large multiethnic prospective cohort study examined the associations between maternal depressive symptoms during pregnancy and four major adverse obstetric and neonatal outcomes: preterm birth, small for gestational age (SGA), child loss, and a low Apgar score. Additionally, we examined whether the associations were modified by ethnic background.
Methods: From January 2003 till March 2004, all pregnant women in Amsterdam were approached during their first antenatal visit (±13 weeks of gestation); 8266 women (response rate 67%) filled out a pregnancy questionnaire covering sociodemographic data, lifestyle and psychosocial health. To measure depressive symptoms, the CES-D scale was administered; we trichotomised the total score around the 50th and 90th percentile into low, moderate and high depressive symptoms. Obstetric and neonatal outcomes were obtained from two sources: the Youth Health Care Registration in Amsterdam and the Dutch Perinatal Registration. For this study, multiple births were excluded. Multiple logistic regression analyses were performed. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: In our sample, the prevalence of the obstetric and neonatal outcomes were respectively 5.3% (preterm birth), 12.3% (SGA), 1.4% (child loss), and 1.3% (low Apgar score). Univariate analyses showed that depressive symptoms (on a continuous as well as a categorical scale) were significantly associated with SGA (continuous scale: odds ratio [95% CI]: OR = 1.02[1.01–1.02]) and child loss (OR = 1.03[1.01–1.04]), but not with preterm birth (OR = 1.01[1.00–1.02]) and the Apgar score (OR = 1.02[0.99–1.04]). After adjustment for relevant covariates (maternal age, parity, education, ethnicity, maternal smoking and maternal pre-pregnancy BMI), significant associations between depressive symptoms and obstetric outcomes disappeared. The prevalence of high depressive symptoms and adverse obstetric outcomes was higher among ethnic minority groups compared to the Dutch group. The associations between depressive symptoms and obstetric outcomes were however not modified by ethnic background.
Conclusions: In one of the largest samples examining the effect of depressive symptoms on obstetric outcomes, maternal depressive symptoms during pregnancy were not significantly associated with four major obstetric and neonatal outcomes: preterm birth, SGA, child loss and a low Apgar score. Associations were not modified by ethnic background.

P-4B-135

Parental and lifetime socioeconomic status and multiple lifestyle risk factors in middle adulthood

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Objective: Early life social conditions have been associated with chronic disease risk in adulthood (e.g., cardiovascular disease and breast cancer), and more recently with single lifestyle risk factors (LRFs); however, little is known about the influences of childhood social factors on the co-occurrence of multiple LRFs among adults. We examined whether engagement in multiple LRFs in adulthood was associated with parental and lifetime socioeconomic status (SES).
Methods: Data were drawn from an adult follow-up study of a multiethnic birth cohort of US women (n = 262; age range = 38–46; mean = 41.8). We considered 4 lifestyle risk factors: current smoking (yes/no), excessive alcohol drinking (≥7 drinks/week), physical inactivity (<3 METs), and high BMI (>25 Kg/m^2). Using the number of LRFs as an unordered dependent variable, we conducted polytomous regression analyses to examine the associations of maternal education and paternal occupation at the time of participants'

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birth with the number of LRFs in which they engaged as adults. We also investigated the role of lifetime socioeconomic trajectories by creating 4 categories for each of the two SES indicators as follows: 1) persistent low SES (e.g., blue collar paternal and own occupation), 2) downward social mobility (e.g., white collar paternal occupation and blue collar own occupation), 3) upward social mobility (e.g., blue collar paternal occupation and white collar own occupation), and 4) persistent high SES (e.g., white collar paternal and own occupation). The study was approved by the Internal Review Board at Columbia Medical Center.

Results: No one had 4 LRFs, 34% of the sample had two or three LRFs, 45% had one LRF, and 21% had no LRF. Having a mother with less than a high school education and a father with a blue collar occupation were associated with a higher likelihood of engaging in any single LRF and in multiple LRFs, as compared with having no LRFs. After adjusting for participant's own education and occupation and race/ethnicity, the association of paternal occupation with engaging in 2 LRFs vs. 0 LRF remained strong and statistically significant (e.g., odds ratios [OR] (95% confidence intervals [CI]) of 2.6 (1.1, 6.0) for paternal blue collar vs. white collar occupation). When lifetime socioeconomic trajectories were examined, those with persistent low SES had the highest probability of engaging in multiple LRFs (e.g., OR (95% CI) for persistent low vs. persistent high educational trajectory: 2.9 (1.0, 8.4) and 6.3 (1.1, 36.8) for 2 and 3 LRFs vs. 0 LRF, respectively).

Conclusions: Our results provide some evidence for a long-term effect of childhood social conditions on the co-occurrence of multiple chronic disease lifestyle risk factors in middle adulthood. Support: The US Department of Defense Breast Cancer Research Program and the National Cancer Institute.

P-4B-136

Maternal licorice consumption during pregnancy alters diurnal and stress-induced hypothalamic-pituitary-adrenocortical axis function in 8–year-old children

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Objective: Overexposure to glucocorticoids has been proposed as a mechanism by which prenatal adversity 'programs' the function of the hypothalamic-pituitary-adrenocortical axis (HPAA) thereby increasing risk for adult disease. Glycyrrhizin, a natural constituent of licorice, inhibits placental 11β-hydroxysteroid dehydrogenase type 2, the feto-placental barrier to higher levels of cortisol in the maternal circulation. We studied if prenatal exposure to glycyrrhizin in licorice exerts detrimental effects on the HPAA activity in 8-year-old children.

Methods: A longitudinal urban cohort. Children ($n = 321$, mean age = 8.1, SD = 1.4 years) born as healthy singletons between 35 to 42 weeks of gestation, whose mothers consumed high (>500 mg/week), moderate (250–499 mg/week) and zero-low (0–249 mg/week) amounts of glycyrrhizin in licorice during pregnancy. The main outcome measures were diurnal salivary cortisol and salivary cortisol in response to Trier Social Stress test for Children (TSST-C).

Results: In comparison to the zero-low exposure-level group, children in the high exposure-level group had higher salivary cortisol awakening peak ($P < 0.03$), awakening slope ($P < 0.05$) and awakening area under the curve ($P < 0.05$). Further, they had higher TSST-C baseline salivary cortisol ($P < 0.003$), and their salivary cortisol increased less in response to the TSST-C (mixed model $P < 0.006$). These effects appeared dose-related. The results were not confounded by factors implicated as risks for pregnancy and HPAA function.

Conclusions: Our findings lend support to prenatal 'programming' by overexposure to glucocorticoids and caution against excessive use of glycyrrhizin-containing products during pregnancy.

P-4C-137

Impact of developmental environments on adult reproduction: A migrant model

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Objective: Investigating the impact of developmental environments on reproductive function across the life course using a model of migrant Bangladeshis to the UK.
Method: Cross-sectional studies of Bangladeshi men (aged 18–80) and women (aged 18–60). Participants include: a) subjects in Bangladesh (sedentees); b) first-generation migrants who moved to the UK at different life-stages (childhood or adulthood); c) second-generation British-Bangladeshis, and d) comparative groups of Britons of European origin. Collection of saliva/blood samples for analyses of reproductive hormones, anthropometrics, and questionnaires concerning migration, reproductive and health histories, nutritional intake, exercise, household and life stressors.
Results: Bangladeshis represent an ideal population for a migrant model. Migration has been continuous since the 1960s. Ninety five percent of migrants originate from Sylhet (northeast Bangladesh), are uniformly Muslim and middle class. Inter-marriage with other groups is rare. These factors act as natural controls for potential confounders. *Ovarian function:* women who develop in Bangladesh have significantly lower levels of salivary progesterone, later puberty, lower rates of ovulation and indicators of an earlier menopause compared to women who develop in the UK (both migrants and European women). Women migrating to the UK prior to age eight (the slow growth period) have significantly higher levels of salivary progesterone as adults compared to adult migrants or sedentees. Length of time in the UK as an adult migrant has no impact on levels of salivary progesterone, but does lead to differences in hormone levels reflecting ovarian reserve (in women >35y): namely, higher levels of inhibin B and anti-Mullerian hormone (AMH) and lower levels of follicle-stimulating hormone (FSH) compared to age-matched sedentees in Bangladesh. *Testicular function*: levels of salivary testosterone (men aged <40y) are significantly higher among adult male migrants to the UK compared to sedentees, and fat free muscle mass increases significantly following migration.
Conclusion: Life history theory posits that energy is divided between growth, maintenance and reproduction. Constraints on growth in Bangladesh may result from differential exposure to diseases and poorer health care compared to the UK; these constraints affect males and females differently via gonadal function. The rise in gonadal steroid levels and accompanying muscle mass for migrant Bangladeshi men (18–40y) suggests a more facultative response to a positive change in environment compared to females. For the latter, our data point to a critical window *during childhood* when levels of ovarian reproductive steroids are set on a trajectory for adulthood. Other aspects of female reproductive function appear more malleable: preliminary data for inhibin B, AMH and FSH suggest women living in Bangladesh deplete their follicular pool and approach menopause at a significantly earlier age than European women. Levels of these hormones for adult migrants, however, are intermediate between sedentees and Europeans, suggesting an effect of the UK environment in adulthood. Lower levels of salivary progesterone in adult migrants are associated with lower ovulation rates per cycle, but lifetime fertility could be enhanced by an extension of the reproductive lifespan. Further research will illuminate these issues. Support: CONACyT (Mexico); ESRC/MRC (UK), Prostate (UK), Royal Society (UK), NSF (USA).

P-4C-138

Secular trend for age at menarche and associated factors in indigenous and non-indigenous women in the Araucanía region

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Objective: To estimate the secular trend of age at menarche in indigenous (Mapuche) and non-indigenous women and its association with socio-demographics, family and nutritional factors.
Method: Retrospective cohort study, with observational design of historical cohorts of 688 women (10–46 years old) establishing four non-concurrent birth cohorts (1990–1996; 1980–1989; 1970–1979; 1960–1969). Data were collected from girls and women from health and educational public systems through structured interviews. Menarcheal age was collected by recall. Nutritional status at menarche, sociodemographic and family data were requested. Weight/height at birth and growth data were not accessible due to lack of records for many women. Final statistical analyses used multiple linear regression and Cox's proportional hazard regression models. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Age at menarche had decreased 11,2 months (CI, 95%; 14,6–7,7) from the oldest cohort to the youngest, with no ethnic differences, adjusted by level income, sibling number and parents cohabitation during childhood. Age at menarche had decreased 3,7 months per decade since 1960 to 1990. The youngest cohort had approximately double the risk 2,2 (CI, 95%; 1,6–2,7) for an earlier menarche in comparison with the oldest. The indigenous women had maintained a later menarcheal age than the non-indigenous through time; however, this difference is significant only among younger cohorts.
Conclusions: Better income level, fewer siblings and cohabitation with one partner during childhood are the factors that had more influence on the secular trend in age at menarche, similar to other studies[1]. In indigenous women, an accelerated pubertal maturation might arise from two different mechanisms: either an improvement in life conditions or subjugation to psychosocial stressors [2,3] . These aspects need more study. In non-indigenous women,

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nutritional factors had influenced the younger cohort modelling trends in the last years[4]. A secular trend towards earlier menarche is relevant because of its association with non- communicable chronic diseases[5,6]. In Chile, changes in age at menarche in less prosperous regions and within indigenous populations have been unknown. Mapuche women have their own health risk factors due to gender, ethnic and social class health disparities. Therefore, it is important to know if this pubertal trend will turn into another health risk in the future. Fondecyt Project N° 1060884.

1. I. Wronka, R. Pawlinska-Chmara. *Ann Hum Biol,* 32: 630–638, 2005.
2. D.A. Coall, J.S. Chisholm. *Am J Hum Biol,* 00:0–0 (early view in www3.interscience.wiley.com), 2009.
3. P.D. Gluckman, M.A. Hanson. *Mol Cell Endocrinol,* 254–255:26–31, 2006.
4. M.I. Hernández *et al. Rev Med Chile,* 135:1429–1436, 2007.
5. K. McPherson *et al. BMJ*, 321:624–628, 2000.
6. Y. Feng. *et al. Atherosclerosis*, 196:590–597, 2008.

P-4C-139

Development of secondary sex characters and secular trend of age at menarche among Chinese girls in Beijing

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Objective: Study of sexual development and secular trend of age at menarche between the 1940s and 2000s among Chinese females in Beijing.
Methods: We used two large data sets, a 2004 survey and a 60-year cohort study. We investigated pubertal development of 9,778 Beijing girls aged 6–18 years participating in the Beijing Child and Adolescent Metabolic Syndrome (BCAMS) study, a cross-sectional survey of a large representative sample conducted in 2004. Tanner stages of breast and pubic hair development were assessed through visual inspection by physicians and nurses. Palpation was implemented when assessing breast stages in order to distinguish adiposity from breast tissue. Self-reported age at menarche was collected. Tanner Stage 2 of breast (B2) development and pubic hair (PH2) were used to indicate onset of puberty. Median ages at menarche (MAM), B2 and PH2, respectively were calculated. The FOAD (Fetal Origins of Adult Disease) cohort study collected age at menarche of 4,094 mothers and 481 female children based on original obstetric records collected during 1948–1954, and a follow-up study from 1995 to 2001, respectively, which represent the MAM of girls in the 1930s and 1960s in Beijing. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: For the 2004 sample, 5,040 girls (51.5%) lived in urban areas. The MAM was 0.6 years (7.2 months) earlier in urban than rural girls (11.9 yrs vs 12.5 yrs). In all urban and rural girls, the median age at B2 was 9.5 ± 1.2 years, (9.4 ± 1.1, and 9.6 ± 1.2 respectively). For PH2, it was 11.1 ± 1.1 (10.8 ± 1.1, and 11.4 ± 1.1 respectively). In the FOAD cohort, maternal MAM was 14.9 ± 1.7 years in the 1940s and 13.4 ± 1.4 years in the 1960s. Thus, MAM of girls in Beijing was advanced by 1.5 years between 1965 and 1985, an average of 0.8 years (9.6 months) per decade, and the rate decreased to 0.4 years (4.8 months) per decade between 1985 and 2004.
Conclusions: Urban girls in Beijing have experienced an earlier onset of menarche over the past six decades, but the rate of change decreased from ten months/per year during the 1960s−1980s to five months in the 1980s–2000s. Among contemporary girls in Beijing, the urban group had an earlier onset of secondary sexual characteristics and menarche than their rural counterparts. Acknowledgements: We are grateful to all participants for taking part in this study. Support: NSFC (30872165) and BMSTC (D08050700320801) in China.

P-4C-140

Gestationally programmed leptin and early reproductive dysfunction in low birth weight female rats

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Objective: Obesity in women is associated with reproductive dysfunction. We have shown that maternal undernutrition in pregnancy results in low birth weight (LBW) newborns with hypoleptinemia. When nursed normally, LBW females develop pre-pubescent hyperleptinemia prior to the onset of adult obesity and reproductive dysfunction with early anovulation. Thus, the underlying primary mechanism for obesity-related reproductive dysfunction may involve leptin, which is essential for maturation and action of the hypothalamic–pituitary-gonadal (HPG) axis. Centrally, leptin influences secretion of hypothalamic gonadotropin-releasing hormone (GnRH) and pituitary gonadotropins, whereas peripheral leptin effects are mediated via the ovary, though both are mediated by binding to the leptin receptor (ObRb). We hypothesized that programmed alterations in leptin and leptin signaling impacts the HPG axis, leading to reproductive dysfunction in LBW female offspring.
Methods: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to 21. All pups were nursed by Control dams and weaned at 3 weeks to ad libitum feed. At 1 day of age, female hypothalamic and ovarian protein expression of ObRb and estrogen receptor-alpha (ERα) and hypothalamic expression

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of GnRH were analyzed by Western Blot. Data were normalized to β-actin.

Results: Consistent with our previous studies, 1 day old LBW females had significantly reduced body weights (6.1 ± 0.1 vs. 6.9 ± 0.1 g) and plasma leptin levels (0.7 ± 0.1 vs. 1.6 ± ng/ml) as compared to Controls. LBW female pups showed significant upregulation of hypothalamic ObRb (2.1-fold) and ERα (3.2-fold), though downregulation of GnRH (0.5-fold). Ovarian ObRb expression was significantly increased (1.6-fold), though ovarian ERα protein level was significantly decreased (0.6-fold).

Conclusions: LBW hypoleptinemic newborns demonstrate upregulation of hypothalamic and ovarian ObRb receptors, in association with marked dysfunction in basal hypothalamic GnRH, and both hypothalamic and ovarian estrogen receptor expression. These results suggest that early reduced leptin levels in LBW newborns alters growth, maturation and function of HPG axis which later leads to reproductive dysfunction.

P-4C-141

Early reproductive aging in intrauterine growth restricted female rats

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Objective: Reproductive aging in rats is marked by three phases comparable to menopause in women. These phases are characterized by (1) persistent estrus - small, polyfollicular ovaries with large and sometimes cystic follicles, and very few corpora lutea (CL), (2) persistent diestrus - enlarged ovaries with many CL and very few large antral follicles, and (3) anestrus - small, atrophic ovaries with very few follicular or CL elements. We have shown that food restriction in rat pregnancy results in intrauterine growth restricted pups (IUGR) with hypoleptinemia. When nursed normally, IUGR females develop pre-pubescent hyperleptinemia prior to the onset of adult obesity. Leptin, which is associated with obesity, is essential for maturation and action of the hypothalamic-pituitary-gonadal axis (HPA). We thus hypothesized that elevated leptin levels prior to puberty causes premature activation of HPA axis, ultimately leading to early reproductive aging in IUGR females.

Methods: From gestational day 10 to 21 (term), control dams received ad libitum food, whereas study dams were 50% food restricted to produce IUGR pups. All pups were nursed by control dams and weaned to ad libitum feed. At 8 months of age, reproductive cycles were assessed by daily examination of vaginal smear and EC40 estrus cycle monitor. At 10 months of age, plasma estradiol and luteinizing hormone levels were analyzed using RIA. Ovaries were paraffin embedded, sectioned (10 μm) and stained with hematoxylin and eosin. Every 10th section of the ovary was examined to determine the relative quantity of CL, and small, medium and large follicles.

Results: At 8 months of age, all control females had normal estrous cycles. Additionally, control ovaries demonstrated greater number of small follicles (20 ± 1), followed by CL (7 ± 1) with very few large antral follicles (2 ± 1), indicative of normal reproductive cycling and ovulation. In contrast, among age-matched IUGR offspring, 80% of females had persistent cornified vaginal epithelium, indicative of the first phase of reproductive aging. By 10 months of age, 63% of the IUGR females were in persistent estrus, 37% were in persistent diestrus, and none showed normal cycling. The IUGR females in persistent estrus had ovaries with significant reduction in the number of small follicles (15 ± 2) and CL (3 ± 1), though with increased number of large, cyst-like follicles (5 ± 1) indicative of anovulation. Ovaries of IUGR females in persistent diestrus showed a significantly decreased number of small follicles (9 ± 2) with increased CL (11 ± 1), consistent with continued reproductive aging. Furthermore, IUGR offspring had decreased estradiol (11 ± 2 vs. 18 ± 1 pg/ml, $p < 0.05$) and increased luteinizing hormone (0.58 ± 0.1 vs. 0.28 ± 0.05 ng/ml, $p < 0.05$), indicating anovulation.

Conclusions: IUGR females at 8 months of age exhibit early anovulation as evident by persistent estrus phase with absence of normal cycling. These findings suggest that prenatal undernutrition combined with increased postnatal leptin levels and adiposity programs premature reproductive aging in IUGR females.

P-4C-142

Birth weight as a potential predictor of sex hormones and related factors in men at age 49–51 years: The Newcastle Thousand Families Study

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Although a number of associations have been shown between early growth and later levels of reproductive hormones in women, far less is known regarding the potential for such associations in men.

Objective: The objective of this study was to investigate whether birth weight was associated with a range of reproductive hormones in men in the Newcastle Thousand Families birth cohort.

Methods: The Newcastle Thousand Families Study is a prospective study initiated in 1947 when all 1142 children

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born to mothers resident in the city of Newcastle upon Tyne in northern England were recruited. Detailed information on many aspects of their lives was collected prospectively during childhood, including early growth, illnesses and socio-economic conditions. At age 49–51, 574 study members returned detailed self-completion questionnaires and 412 attended for clinical examination, including 172 men in whom the following reproductive factors were measured: oestradiol, follicle stimulating hormone (FSH), luteinising hormone (LH), testosterone and sex hormone binding globulin (SHBG). Measures of free testosterone, the free androgen index (FAI) and the free oestrogen index (FOI) were also calculated. These data were analysed, using multiple linear regression, in relation to birth weight (standardised for gestational age and sex), with adjustment for body mass index measured at the same time as when the samples were taken. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Birth weight showed near significant positive univariate associations with oestradiol ($r^2 = 0.02$, $p = 0.066$) and SHBG ($r^2 = 0.02$, $p = 0.052$). After adjustment for contemporary BMI, the association with SHBG increased in significance ($p = 0.024$), the association with oestradiol lost some significance ($p = 0.082$) and an association with testosterone approached significance ($p = 0.074$). No other significant or near-significant associations were found in these data.

Conclusion: Despite the relatively low statistical power of this study, our findings suggest that birth weight may be positively associated with levels of reproductive hormones and related factors in men. These findings are independent of contemporary BMI. Given the links between reproductive hormones and related factors, particularly SHBG and testosterone, and disease outcomes such as type II diabetes and osteoporosis, it is possible that reproductive hormones may play a mediating role in the associations between growth in early life and later risk of disease.

P-4C-143

Neonatal exposure to lipopolysaccharide programs reproductive function in the female rat

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Objective: Neonatal exposure to immunological challenge (e.g. lipopolysaccharide: LPS) increases the activity of hypothalamic-pituitary-adrenal (HPA) axis[2] and sensitises the gonadotrophin-releasing hormone pulse generator to the inhibitory effects of stress in adult female rats[1]. The aims of the present study were to determine whether neonatal exposure to LPS also exerted programming effects on the timing of puberty, ovarian cyclicity and ovarian sympathetic tone, the letter by evaluating nerve growth factor receptor (p75NGFR) and tyrosine hydroxylase (TH) immunoreactivity in the ovary of immediate post-pubertal and adult rats.

Methods: Female Sprague-Dawley rats were administered saline or LPS (Salmonella enteritidis) at a dosage of 50 μg/kg, i.p. on postnatal days 3 and 5. Body weight was measured weekly until the end of the experiment. Vaginal opening and first oestrus were monitored as markers of puberty. Oestrous cyclicity was monitored by vaginal cytology for 2 consecutive weeks after vaginal opening and again at 8–10 weeks, followed by ovariectomy. In a separate group of neonatal saline or LPS treated rats, ovaries were removed immediately post-puberty. All ovaries were sectioned at 4 μm and the thickness of theca interna was measured. Additionally, the presence of p75NGFR and TH was detected immunohistochemically in the theca interna. All animal procedures were undertaken in accordance with the UK Home Office regulations.

Results: Neonatal exposure to LPS delayed puberty (vaginal opening: LPS 40.6 ± 0.7 vs control 38.6 ± 0.6, $p < 0.05$; first vaginal oestrus: LPS 41.0 ± 0.5 vs control 38.7 ± 0.5, $p < 0.05$) without affecting growth rate, and disrupted estrous cyclicity both at puberty (regular cycles: LPS 26.1% vs control 66.8%) and in adulthood (regular cycle: LPS 37.5% vs control 77.7%; $p < 0.05$). The thickness of the theca interna was increased in the immediate post-puberty and adult rats treated neonatally with LPS. The intensity of immunostaining for p75NGFR and TH showed no difference in neonatal saline-treated controls, but the immunohistochemical staining for both was significantly enhanced in the theca interna cells of neonatal-LPS treated rats immediately post-puberty and across all stages of the oestrous cycle in adulthood ($p < 0.05$).

Conclusions: These data suggest that exposure to LPS during critical developmental windows in the rat can delay puberty and have life-long dysfunctional effect on the reproductive system at the level of the ovary, which might involve in part increased ovarian sympathetic tone. Acknowledgement: Wellcome Trust and BBRSC. XQW supported by K.C. Wong Scholarship.

1. Li XF, *Endocrinology,* 148: 5984–5990.
2. - Shanks N, *Proc Natl Acad Sci USA*, 97:5645–5650.

P-4C-144

Abstract withdrawn by authors.

P-4C-145

Human chorionic gonadotropin and reproductive failure: genetic variation and disease-specific gene expression

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Human chorionic gonadotropin (hCG) is one of the first proteins synthesized by fetus. Besides its luteotropic function, hCG regulates implantation and immunomodulation at the maternal-fetal interface. Low level of hCG is related to miscarriage and extrauterine pregnancy whereas high hormone level may indicate a trophoblastic disease. HCG hormone consists of alpha- and beta-subunit. Critical for hCG function is the beta-subunit coded by *hCG beta* genes (*CGB, CGB5, CGB7, CGB8*) that share a common gene cluster with highly homologous luteinizing hormone beta gene and two *beta*-subunit non-coding genes (*CGB1, CGB2).* Aberrations in the expression profile and genetic variation of the hCG beta coding genes may lead to the reproductive failure.
Objectives: The aim of the study was to (i) determine the expressional profile of *CGB* genes during the normal and complicated pregnancy; and (ii) find variants in *hCG beta* coding genes that are related to recurrent miscarriage (RM, $\geqslant$3 consecutive pregnancy losses).
Methods: The expression of *CGB* genes in trophoblastic tissues from normal and complicated pregnancies (RM, ectopic pregnancy-EP, molar pregnancy) was determined by real-time PCR. *CGB5* and *CGB8* genes that contribute the most to hCG hormone production were fully resequenced in Estonian and Finnish RM patients (n = 184) and fertile women (n = 195). Study was approved by Ethics Committee of the University of Tartu (Estonia) and Helsinki University Central Hospital (Finland); an informed consent was obtained from every patient.
Results: In the cases of RM, the expression level of *CGB* genes is low and correlates with reduced hCG hormone level indicating their possible involvement in the pathogenesis of miscarriage. In EP, *CGB* genes were highly expressed but the hormone concentration in serum was significantly reduced. In molar pregnancies both hCG level in serum and expression of *CGB* genes were at the upper range of the distribution compared to normal first trimester pregnancies. As a result of the resequencing study, six polymorphisms (SNPs) showed significant protective effect towards RM in *CGB5* and *CGB8* genes ($p = 0.042–0.007$) decreasing the risk to miscarry up to1.8-fold. The minor allele frequency of these SNPs was 12.05%–14.36% in fertile females compared to 7.10%–8.15% in RM group. Three non-synonymous amino acid changes in *CGB5* and *CGB8* and a rare promoter polymorphism located within transcription initiator element of *CGB8* were identified only in RM cases as possible risk variants for RM.
Conclusions: Comparison of expression levels of the *CGB* genes and hCG hormone levels in serum indicate a different contribution of *CGB* genes in the miscarriage, ectopic and molar pregnancies Changes in the expression levels of *CGB* genes may be caused by genetic variants as demonstrated in case of *CGB5* and *CGB8* with variants that either increase or decrease the risk of miscarriage. The results provide evidence that genetic factor contributes to the pathogenesis of RM.
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P-4C-146

Ovarian gene expression levels and follicle populations in rat offspring are modified by maternal nutrition

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Objective: We have previously shown that moderate maternal high fat (HF) nutrition advances pubertal age in female offspring. It is not known however, whether maternal diet has persistent effects on pituitary-gonadal function. Therefore in the present study we determined the impact of maternal HF nutrition on circulating hormones associated with reproductive function and mRNA expression levels of genes regulating ovarian steroidogenesis.
Methods: Wistar rats were time-mated at 100 days of age. Three maternal backgrounds were investigated: 1) control dams fed standard rat chow from weaning to conception and throughout pregnancy and lactation (CONT), 2) dams fed a HF diet (45% kcals as fat) from weaning to conception and throughout pregnancy and lactation (MHF) and 3) dams fed a HF diet throughout pregnancy and lactation only (PLHF). Litter size was standardised to 8 pups and offspring were placed on either a chow or HF diet from weaning (21 days of age). Offspring were killed at 160 days of age; blood was collected for plasma analyses and ovaries were dissected and either fixed for follicle analyses or snap frozen in liquid nitrogen after which RNA was extracted for qPCR. All females were investigated at proestrus. PLHF, but not MHF offspring demonstrated higher progesterone levels than those offspring born to CONT mothers. A post-weaning HF diet had no further effect.
Results: Maternal and postnatal HF diets did not alter either plasma inhibin B or plasma follicle stimulating hormone (FSH) levels. Although ovarian weight (% of body weight) was similar between groups, MHF, but not PLHF offspring, demonstrated higher numbers of primordial follicles/mm^3 in adulthood ($p < 0.01$). Other follicle populations (primary, secondary and antral follicles) were not altered by maternal diet or postnatal nutrition. MHF offspring also demonstrated significantly lower levels of total ovarian leptin receptor (ObRb) and 3βHSD mRNA levels ($p < 0.01$). Ovarian gene expression levels in PLHF offspring were not different from CONT offspring.
Conclusions: These data suggest that in the rat, maternal HF nutrition results in persistent changes in ovarian mRNA levels of key factors regulating steroidogenesis but not in pituitary secretion of FSH, a key gonadotrophin regulating follicular

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growth. These effects were dependent upon the timing of maternal HF exposure. We speculate that elevated numbers of primordial follicles but not later stage growing follicles could be suggestive of either altered follicular atresia or changes in other drivers of primordial-primary follicle transition not currently measured. These data have important implications for the reproductive function, and potentially reproductive capacity, in subsequent generations.

P-4C-147

Early life infection impairs reproductive success

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It has been well established that early exposure to a bacterial agent such as lipopolysaccharide (LPS) results in alterations to the HPA axis. However, despite the regulatory influence of this system on the hypothalamic-pituitary-gonadal (HPG) axis, the question of whether subclinical early life infection has implications for reproductive success remains largely unexplored.
Objective: We investigated whether neonatal exposure to LPS impairs sexual development, sexual decline, as well as reproductive behaviour in later life.
Methods: Animals were exposed to either LPS (*Salmonella* enteritidis, 0.05 mg/kg, i.p.) or non-pyrogenic saline (equivolume) on days 3 and 5 of life (birth = day 1). Animals were monitored daily for pubertal markers of vaginal opening in females, or preputial separation in males from weaning (day 22). Blood was collected across 3 time points during the pubescent period to monitor surges of lutropin in females and testosterone in males. Given that the cumulative effects of perinatal stress have been shown to be amplified in the presence of a subsequent stressor in later life, animals were exposed to either restraint and isolation stress or no stress in adulthood prior to behavioural testing. Following "stress" or "no stress" in adulthood, animals were paired with a naïve partner of the opposite sex (either proven male studs or experimentally naïve females in proestrous) and sexual behaviour was monitored. Blood was collected at baseline and immediately following behavioural testing to assess corticosterone and gonadal hormone responses. To confirm success of mating, females were checked for sperm using vaginal smears. Between 9 and 12 months of age blood was collected to assess testosterone and lutropin decline in aged rats.
Results: Developmental data indicated that neonatal LPS exposure disrupted the normal weight-to-age ratio of vaginal opening in females, and preputial separation in males. Neonatal LPS exposure impaired sexual performance in adulthood on all measures, including initiatory and receptive behaviours compared to saline controls ($p < .05$ for all). This was reflected in the low sperm presence of LPS-treated matings. No differences were observed in testosterone or lutropin levels during puberty, nor was there a difference in the timing of decline in these hormones in aged rats. However, testosterone and lutropin surges during sexual behaviour were significantly reduced in LPS-treated animals compared to controls, which reflected the observed hypersecretion of corticosterone during copulation for these animals ($p < .05$ for all).
Conclusions: This research demonstrates the long-term impact of neonatal infection on reproductive success. Early life exposure to infection disrupted puberty onset and sexual performance, in turn leading to lower reproductive success. Associated changes in neuroendocrine functioning suggest a possible mechanism through which individuals may exhibit previously unknown complications with fertility.

P-4C-148

Ovarian response after ovarian stimulation treatment is modulated by periconceptional folic acid use

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Objective: The B vitamin folate is implicated in reproductive performance, including pregnancies established by in vitro fertilization (IVF). We investigated the effects of folic acid use on ovarian response to mild and conventional forms of stimulation in women.
Methods: In a randomized controlled trial among subfertile women, 24 subjects received conventional ovarian stimulation and 26 subjects underwent mild ovarian stimulation. Blood samples were obtained for determination of serum oestradiol and serum folate before treatment was commenced and on the day of hCG administration. Folic acid use was determined by questionnaire and serum folate concentrations. Ovarian follicles were visualised, counted and diameters recorded using transvaginal ultrasound. Linear regression analysis was applied with adjustments for potential confounders.
Results: Oestradiol response after conventional ovarian stimulation treatment is modulated by serum folate levels (unstandardized $\beta^{interaction} = 0.52$ and standardized $\beta^{interaction} = 0.36$; $P = 0.03$). In the conventional protocol, mean follicle number was greater in non-folic acid users women compared to the folic acid users group (14.1 vs. 8.9, $P = 0.03$). Also, homocysteine levels are independently affected by the stimulation protocol (unstandardized $\beta^{protocol} = 0.15$, standardized $\beta^{protocol} = 0.24$; $P = <0.01$). Follicle number and serum oestradiol concentrations on the day of hCG were correlated ($r = 0.78$, $P < 0.01$).

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Conclusion: Folic acid use modulates follicular and related endocrine responses to conventional ovarian stimulation therapy. Mechanisms of interest for future study are aberrant DNA methylation of ovarian regulatory proteins, modulation of folate expressed proteins and the production of reactive oxygen species.

P-4C-149

The effect of growth and body composition on the onset of puberty: longitudinal observations in Afro-Caribbean children

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Objective: Childhood growth and body composition may influence the onset of puberty. We therefore examined the effects of birth size, growth rates throughout childhood and body composition on the onset of puberty in Afro-Caribbean children.
Methods: The Vulnerable Windows Cohort Study is a longitudinal observational study of Jamaican mother/child pairs recruited during the antenatal period. Anthropometry (weight, height, skinfold measurements, waist circumference) of the children was measured at birth, at 6 weeks, 3 monthly to 2 years and then every 6 months. Tanner staging for puberty and orchidometry were performed 6 monthly starting at age 8 years. Bioelectrical impedance was done at mean age 11 years. For this report, data analysis was confined to the 140 girls and 119 boys who were seen at all scheduled visits between birth and age 11 years. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: In the girls, thelarche, pubarche and menarche occurred at median ages of 8.8, 9.9 and 12.0 years, respectively. Pubarche in boys occurred at a median age of 11.3 years when the median testicular volume was 2.8 ml.Faster weight gain during infancy (age 0 to 6 months) and childhood, but not birth size, were associated with more advanced puberty (p-values < 0.05). Fat mass at age 8 years was associated with more advanced puberty (p-values <0.001) in both sexes. At age 11 years, lean mass, but not fat mass, is associated with more advanced puberty (p-values <0.001). Waist circumference in girls at age 11 years was not associated with the stage of pubarche or thelarche, or the age of menarche (p-values >0.4). However, the waist circumference in boys was related to stage of pubarche ($r = 0.33$; $p = 0.0003$) and testicular volume ($r = 0.27$; $p = 0.003$).
Conclusions: Faster growth throughout childhood, especially with fat mass accretion, is associated with earlier onset of sexual maturation. With the onset of puberty, lean mass accretion significantly increases. More advanced puberty in boys is associated with greater waist circumference measurement.

P-4C-150

Is there association between intrauterine growth restriction and early age of menarche?

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Objective: To tested the hypothesis of association between intrauterine growth restriction and early age of menarche.
Methods: Follow-up data (n = 1056) from the population based live birth cohort study of Ribeirão Preto of 19789 were analyzed. Early menarche was defined as having the first menstrual event before 12 years-old and intrauterine growth restriction was defined by three measurements: low birthweight (<2500 grs), small for gestational age (<10% Alexander growth curve) and fetal growth ratio (<0.85 mean weight for gestational age). Relative risks were estimated by generalized estimation equations (Poisson distribution) with robust method for estimation of standard errors. Analyzes were adjusted for maternal age, education and marital status, number of siblings, birth length and preterm. Body mass index was tested as intervenient or interaction factor in a subsample of the cohort examined at 9 yrs-old.
Results: Body mass index was tested as intervenient or interaction factor in a subsample of the cohort examined at 9 yrs-old. The mean age of menarche was 12.3 years (Standard Deviation = 1.5). Early menarche was observed for 27.7% for the entire cohort and 29.1% for the sub-sample. Negative association was observed between intrauterine growth restriction and early menarche. The adjusted relative risks and respective confidence intervals (95% CI) for low birth weight, small for gestational age and fetal growth ratio were respectively: 0.47 (95% CI: 0.26–0.84), 0.57 (95% CI: 0.37–0.89), and 0.65 (95%CI: 0.47–0.92). No evidence that body mass index was an intermediate or interaction factor was observed.
Conclusions: this study showed a negative association between intrauterine growth restriction and anticipation of age of menarche. Support: FAPESP and CNPq.

P-5A-151

Short term leptin supplementation during early neonatal period improves pig viability of very low birth weight piglets

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Objective: In mammals, including human infants, intrauterine growth retardation (IUGR) is associated with low birth weight and a decrease in perinatal survival. IUGR is also linked to a high risk for the development of metabolic diseases during adult life. In a previous study (1), we have shown that neonatal leptin supplementation enhanced the development of several organs and partially reverse the IUGR phenotype by correcting growth rate and development. In this study, we aimed to test whether neonatal leptin administration ameliorates the viability of very low birth weight piglets and improves their survival before weaning.

Methods: At day 1 after birth, control (1.51 kg ± 0.07; n = 13) and IUGR piglet (0.97 kg ± 0.04; n = 26) received intramuscularly either saline (IUGRsal) (0.9 kg ± 0.1; n = 13) or 0.5 mg/kg body weight recombinant porcine leptin (IUGRlep) (1.02 kg ± 0.1; n = 13) for 7 days. Cholesterol, triglycerides, glucose and serum leptin levels were measured at birth and day 7. Body temperature was followed during leptin treatment. Piglets were sacrificed at weaning (day 21). Several organs were weighted and the endocrine profile determined.

Results: 1) in both control and IUGR group, glucose, triglycerides and total cholesterol levels increased significantly from day 2 to day 7; thereafter a significant decrease was observed at weaning. 2) Leptin treatment enhanced notably daily weight gain (217 g ± 0.2; 203 g ± 0.4; 160 g ± 0.4 in control, IUGRsal and IUGRlep, respectively), as well as corporal body temperature 72 hours after birth (37.5 ± 1; 36.8 ± 1.6; 35.2 ± 1.5 in control, IUGRsal and IUGRlep respectively, $p < 0.05$). 3) No differences in the level of cholesterol, cortisol and T4 were observed between the groups while a significant decrease of triglycerides levels was observed at day 7 and day 21 after birth in the IUGRlep group (3 ± 0.7; 3.11 ± 0.7; 2.3 ± 0.7 in control, IUGRsal and IUGRlep, respectively at day 7, $p < 0.05$) and (2.1 ± 0.6; 2.4 ± 0.5 and 1.5 ± 0.3 in control, IUGRsal and IUGRlep respectively at day 21, $p < 0.05$) and in 4) the mortality rate from day 2 to weaning was significantly higher in IUGR group as compared to control group (9% and 33% in control and IUGR respectively $p < 0.05$). Leptin treatment significantly improved pig viability by 20%.

Conclusions: These results suggest that neonatal leptin treatment protects against postnatal outcomes and ameliorates viability by improving energetic metabolism and thermoregulation in very low birth weight piglets. A potential effect of leptin supply on UCP (Uncoupling proteins) and PPR (peroxisome proliferator-activated receptor) expressions in liver and adipose tissue is under investigation.

1. L. Attig *et al.*, *Am J Physiol*, 295: E1117–E1125, 2008.

P-5A-152

Undernutrition *in uteri*: role of renin-angiotensin system on adults cardiac remodeling

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Nutritional insults during embryonic and foetal periods can be associated to impaired maturation of physiological functions and cardiovascular diseases in adulthood. Alterations in nutrition and endocrine status during the embryonic, foetal and neonatal periods can trigger developmental predictive adaptative responses, causing permanent structural, physiological and metabolic changes, thereby predisposing and individual to cardiovascular, metabolic and endocrine diseases in adult life. The rennin-angiotensin system (RAS) plays an important role in primary and secondary forms of hypertension. Components of the RAS, such as angiotensina-converting enzyme (ACE) and angiotensin II are locally produced in the cardiac tissues, and are primary candidates for the factors promoting remodelling, mainly cardiac myocite hypertrophy and increased extracellular fibrosis, thereby deteriorating cardiac functions.

Objective: The present study aims to investigate the effects in arterial blood pressure (AP) and expression of different RAS compounds in adult rats submitted to undernutrition *in uteri* by maternal food restriction.

Methods: Pregnant rats were divided into two groups. The daily food supply of one group (FR50) was restricted to 50% of the food consumed by the other group (NF), fed *ad libitum*. Only male pups were used in the study. Ano-genital distance (AGD) was measured in the day of birth and body weight (BW) of the offspring was measured every week. Arterial blood pressure (AP) was measured weekly, since the 42nd day of age. In the age of 90th days, their hearts were rapidly removed and weighed. The samples were stored in −80°C biofreezer to western blotting analysis. One rat of each mother in both groups had the heart ventricles removed and 5-μm-thick sections were used to immunohistochemistry. Both techniques analyzed the expression of AT1, AT2, ERK1 and PI3K receptors.

Results: FR50 offspring presented significant reduction in BW (5.67 ± 0.16 v 6.84 ± 0.13 in NF, $p < 0.001$) and higher AGD (2.56 ± 0.11 v 1.81 v 0.05, $p < 0.001$ in NP). AP also increased from 6th to 12nd week (6th, 149.1 ± 3.4 v 125.1 ± 3.2 in NF, $p < 0.001$; 12nd, 164.4 ± 4.9 v 144.0 ± 3.3 in NF, $p = 0.02$). Expression of AT1, AT2 and ERK1 were increased in FR50 (AT1, 136.43 ± 8.66 v 89.32 ± 7.35 in NF; AT2, 79.26 ± 7.64 v 10.44 ± 4.35 in NF; ERK1, 200.80 ± 7935 v 155.10 ± 9.820 in NF,

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$p \leqslant 0.05$), however, PI3 K hasn't show any significant difference (NF 121.50 ± 7.098 v FR50 120.80 ± 12.720).
Conclusion: Since maternal steroids cross the placenta, raised maternal androgen levels during gestation may contribute to the increased AGD in male pups of food-restricted mothers. It might be possible that enhanced AGD may, precociously, indicate that alterations of the intrauterine environment can result in development of disease later in life. AP values confirm that RAS modifications in embryonic and early life may modulate adulthood blood pressure. High levels of ERK1 and AT1 may be involved in cardiac remodeling. Besides, such a high increase in AT2 expression may suggest RAS adaptive response, since AT1 and AT2 receptors plays antagonistic roles on hypertensive responses. Support: FAPESP.

P-5A-153

Impaired glucose-induced insulin secretion on pancreatic beta cell from perinatal protein of malnourished rats is improved by islets graft in diabetic rats

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Objective: Altered nutrition, over- or malnutrition, during fetal and perinatal life, may modulate metabolism and induces the onset of Type 2 diabetes. It has been reported that low body weight and/or small length of neonate babies may develop obesity during adulthood. Protein malnourishment during early lactation provokes impaired glycemic control in adult rats, which is not reversible by nutritional recovery. Low parasympathetic activity in rats fed by poor protein milk has also been observed. Adult rats with perinatal protein malnourishment presented low blood insulin levels and their pancreatic islets still secreted low insulin after high glucose demand.
Methods: Islets from protein malnourished rats were grafted in diabetic recipient rats to know whether islets injuries were permanent and to evaluate the capacity of islets in regulating blood glucose concentration. Hyperglycemic (22–34 mM) rats were obtained with streptozotocin (STZ) treatment and used as recipient. Islet came from adult rats, which during the first 2/3 of lactation mothers received a 4% protein diet (LP). Control group received normal diet (23% protein) (NP). After protein restriction, all mothers received normal diets. Grafts consisted of 1000–1200 islets transplanted on diabetic rats via portal vein. Fed blood glucose was monitored. In the 5^{th} day after graft, animals were killed after 12h-period of fasting and their blood samples were used to measure glucose and insulin concentration; retroperitoneal fat pads were isolated and weighed to estimate body fatness.
Results: Transplanted islets from LP rats decreased 34% of fed glucose levels on diabetic rats ($p < 0.05$); however glucose level still remained 2 fold higher than that of intact control ones ($p < 0.05$). Similar to LP-islets, graft with islets from control rats provoked same effects on diabetic rats. High fasting blood glucose and low insulin level of diabetic rats were corrected by islet grafts. Transplantations were able to recover 40% of fatness in diabetic rats.
Conclusions: Results demonstrate that islets from perinatal protein poor nourished rats improve blood glucose concentration in diabetic rats and suggest that beta cell function after graft may be recovered.

P-5A-154

Gestational protein restriction: expression and localization of angiotensin signaling proteins in the heart of male offspring

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The maternal protein restriction may lead to a reduction in the number of cardiomyocytes of the offspring and may be a risk factor for cardiovascular disorders in adulthood. The renin-angiotensin system (RAS) play a pivotal role in cardiac remodeling, *i.e.* fibrosis and hypertrophy. In the heart all compounds of RAS, including angiotensin II (Ang II) and its receptors type I (AT1R) and type 2 (AT2R) are expressed.
Objective: The aim of this study was investigate whether gestational protein restriction alters the expression and localization of AT1R and AT2R and RAS signaling pathway proteins (ERK1/2, PI3K, JAK2 and STAT3) in parallel with left ventricle hypertrophy and collagen distribution and systemic hypertension in 16- wk old male offspring.
Methods: Female Wistar rats were put with male Wistar rats. At the day that we observed the presence of sperm in the vaginal smear, the dams were kept, ad libitum, on normal (NP, 17% protein), or low (LP, 6% protein) protein diet over all pregnancy phase. The experiments were performed only in male pups. The systolic blood pressure (SBP) was measured in conscious 6, 8, 10 12, 14 and 16-wk-old rats by an indirect plethysmographic tail-cuff method. With 16 wk-old, the animals were killed and left ventricle was waiting and processed for immunoblotting and immunohistochemistry.
Results: The results were expressed as mean ± SD. The LP offspring showed significant reduction in body weight when compared to NP group (NP: 6.724 ± 0.409g vs. LP: 6.157 ± 0.157g; $P = 0.05$). The SBP increased significantly in LP rats from 6 to 16 weeks of age (NP 127.3 ± 1.3 mmHg; LP 133 ± 1.0 mmHg). The mass and left

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ventricle volume was significantly greater in 16-wk old LP male offspring when compared to NP animals. We also verified the presence of perivascular fibrosis widespread throughout the heart tissue. Furthermore, in the current study the analysis by immunoblotting confirmed by immunohistochemistry demonstrated in LP offspring a significantly enhance in cardiomiocyte expression of AT1R (NP, 1227 ± 138.2 vs LP, 1998 ± 165.9, $P = 0.01$) and ERK1 (NP, 1403 ± 6.46 vs LP, 1623 ± 6.355, $P = 0.001$). On the other hand, the expression of PI3 K in LP was significantly reduced in cardiomyocytes and intramural coronaries wall (NP, 20.52 ± 0.795 vs LP, 11.21 ± 0.34, $P = 0.008$) which in turn the heart AT2R expression was unchanged when compared to NP group (NP 121.40 ± 0.974 vs. LP 120.2 ± 7.23, $P = 0.8$). The study by immunohistochemical analysis we verified that LP expression of JAK2 and STAT3 are reduced in both cardiomyocytes and coronary endothelium.

Conclusion: The present results support that early, approximately 4 weeks of arterial hypertension development, occurs cardiomiocyte hypertrophy despite an unaltered heart collagen deposition. In the current study, we may hypothesize a presumable AT1R transactivation as consequence of crosstalk between RAS and another growth factor pathway. These findings may contribute to elucidation of basic mechanisms of epidemiological studies and could indicate that maternal underfeeding associated with low birth weight offspring may result in increased risk of morbidity of cardiovascular diseases in adulthood. Support: FAPESP.

P-5A-155

Dissociation between *in vivo* mitochondrial function and peripheral insulin action in low birth weight subjects during high-fat overfeeding

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Objective: Low birth weight (LBW) has consistently been associated with an increased risk of insulin resistance and type 2 diabetes. The aim of this study was to clarify whether LBW subjects may be more susceptible to develop insulin resistance and signs of mitochondrial dysfunction upon short-term high-fat overfeeding as compared with matched control subjects.

Methods: The effects of 5 days intake of a diet high in fat (60%) and calories (+50%) and a control diet were studied in a randomized crossover setting. We included young (24–27 years), lean and healthy subjects where 20 had LBW and 26 normal birth weight (NBW). State-of-the-art procedures were used including measurements of *in vivo* insulin secretion, insulin action, hepatic glucose production, ^{31}PMRS determinations of muscle mitochondrial function and quantitative real-time PCR was performed on muscle biopsy tissue for selected oxidative phosphorylation (OXPHOS) genes. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The LBW, but not the NBW subjects developed peripheral insulin resistance, which was due to impairment of exogenous glucose storage most likely reflecting decreased muscle glycogen synthesis. Interestingly, despite similar plasma FFA levels in LBW and controls, the LBW subjects showed significantly increased fat oxidation during insulin infusion as compared with NBW controls. Although the development of insulin resistance in the LBW subjects in response to high-fat feeding was associated with relative reductions of OXPHOS gene expression, *in vivo* mitochondrial function was not impaired in the LBW subjects after overfeeding. Finally, the LBW subjects were unable to increase plasma leptin and GIP levels to the same extent as the NBW subjects in response to overfeeding.

Conclusions: Young, lean and healthy men born with LBW are more susceptible to a dietary challenge of 5-days high-fat overfeeding, as evident by development of peripheral insulin resistance compared to age and BMI matched control subjects. Our data does not support the current hypothesis that high-fat diet induces alterations in mitochondrial function – not even in subjects at risk of developing insulin resistance and type 2 diabetes. Accordingly, we propose that peripheral insulin resistance precede, and potentially may be a cause rather than an effect of mitochondrial dysfunction. Finally, the LBW subjects were unable to increase plasma leptin levels when exposed to overfeeding, suggesting that they do not experience the same degree of appetite reduction as control subjects after overfeeding. This may promote further overeating and eventually obesity, insulin resistance and development of overt type 2 diabetes.

P-5A-156

High fat exposure in early development primes the development of adult non-alcoholic fatty liver disease

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Objectives: Non-alcoholic fatty liver disease (NAFLD) describes an increasingly prevalent spectrum of liver disorders associated with obesity and the metabolic syndrome, which constitutes a considerable health burden to western society.

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However, it is uncertain why steatosis (simple fat accumulation) occurs in some individuals, whilst non-alcoholic steatohepatitis (more severe steatosis with inflammation-NASH) occurs in others. Evidence suggests that poor maternal nutrition increases susceptibility of the adult offspring to develop the metabolic syndrome, therefore we hypothesize that poor nutrition during early development may increase susceptibility to develop NAFLD in adult life.
Methods: We have generated a novel mouse model to test our hypothesis. Female mice were fed either a high fat (HF) or control chow (C) diet prior to and during gestation and lactation. Resulting offspring were either fed a C or HF diet post weaning to generate four offspring groups; HF/HF, HF/C, C/HF, C/C.
Results: At 15 weeks of age liver histology was normal in both the C/C and HF/C offspring. Kleiner scoring revealed that whilst the C/HF offspring developed NAFLD, the HF/HF offspring developed NASH. At 30 weeks histological analysis and Kleiner scoring revealed that both the HF/C and C/HF groups had NAFLD whilst the HF/HF had a more severe form of NASH. Therefore, exposure to a HF diet *in utero* and during lactation contributes towards NAFLD progression. We investigated the mechanisms by which this developmental priming is mediated. At 15 weeks of age, hepatic mitochondrial electron transport chain (ETC) enzyme complex activity (I, II/III and IV) was reduced in both groups of offspring from HF fed mothers (HF/C and HF/HF). We also observed reduced levels of D-3-Hydroxybutyrate, a marker of β-oxidation status, in the HF/HF offspring ($p < 0.001$) vs. the C/C animals. Microarray analysis demonstrated upregulated mRNA levels of genes involved in lipogenesis, oxidative stress, and inflammatory pathways. Real-time PCR analysis validated the expression of the *rate limiting* enzymes in mitochondrial and peroxisomal β-oxidation (*Cpt1* and *Acox1* respectively), which remain unchanged between offspring groups. Genes associated with Triacylglycerol (TAG) and fatty acid synthesis (*Dgat* and *Fas* respectively) were upregulated in the HF/HF offspring, and therefore may contribute to the severe hepatic steatosis observed in these animals.
Conclusions: Collectively these data suggest that exposure to a HF diet during early development increases susceptibility to develop NAFLD in adulthood by decreasing mitochondrial complex activity and modulating expression of key genes controlling lipogenesis.

P-5A-157

Genetic influences on metabolic traits by the glucocorticoid receptor gene and confirmation of modification effects

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Objective: To explore and replicate our previously reported glucocorticoid receptor gene (*NR3C1*) haplotype association to metabolic traits and further the finding that birth weight modified the association between glucose tolerance in adulthood and *NR3C1* haplotypes[1].
Methods: We studied 1982 individuals of Finnish origin included in the Helsinki Birth Cohort study. Appropriate institutional ethics committee clearance and participants' informed consent were obtained. We genotyped ten tagSNPs of *NR3C1* using MALDI-TOF technology (Sequenom) and observed one region of high linkage disequilibrium, including four SNPs from rs6195 to rs258747. This region spans exon 2 of *NR3C1* all the way downstream of the last exon. The additional tagSNPs are located upstream of exon 2, within the region of alternative promoters and exons, and these showed a lower degree of pair wise linkage disequilibrium. Within the haplotype block, five major haplotypes of frequencies above 1% were observed.
Results: In carriers of the H3 haplotype, there was extended linkage disequilibrium in the upstream regulatory region, outside the haplotype block. A similar pattern was not found when evaluating the linkage disequilibrium pattern of carriers of a haplotype with a similar frequency (H2). We could confirm the previously reported[1] H3 modification trend of size at birth on the relation between *NR3C1* haplotypes and glucose intolerance, which was most pronounced when studying the interaction between the effects of birth weight and haplotype H3 on diabetes ($P = 0.02$). The overall association patterns between glucocorticoid receptor haplotypes and metabolic traits were complex, where sex influenced several of the associations. In particular, the H4 haplotype showed male-specific associations such that H4 increased prevalence of diabetes ($P = 0.02$) and contributed to increased fasting insulin levels ($P = 0.005$) in males.
Conclusions: We report that glucocorticoid receptor haplotypes are associated with metabolic traits in a complex manner. These effects are modified by factors such as size at birth and sex. In order to exhaustively evaluate glucocorticoid receptor regulation on metabolic traits, we plan to study DNA methylation in the alternative promoter region, where no genetic influences on phenotypic traits were found.

1. A. Rautanen, *et al. JCEM* 91:4544–4551, 2006.

P-5A-158

Evaluation and assessment of the nutritional component of the comprehensive perinatal services program in overweight and obese women: the effectiveness of nutritional intervention on pregnancy weight gain and neonatal birthweight

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Objective: To determine the efficacy of the Comprehensive Perinatal Services Program (CPSP) program in the identification of overweight and obese women in pregnancy that could benefit from nutritional counseling and referral, and the effects of such interventions on maternal weight gain and neonatal birth weight.

Methods: A retrospective cohort of overweight (BMI 25.0–29.9) and obese (BMI ⩾30.0) women, initiating prenatal care and receiving intake through the CPSP program at Harbor-UCLA Medical Center Faculty Perinatal Practice, were evaluated by chart review and data abstraction. CPSP is a comprehensive assessment program developed by the department of public health in California to evaluate, educate and assess pregnant patients in areas of nutrition, health education and psychology. For this study, we evaluated: pre-pregnancy weight; final pregnancy weight; nutritional counseling (yes/no); number of visits to the nutritionist; active exercise (yes/no); and neonatal birthweight. We excluded women with BMI ⩽ 25.0, those presenting for care ⩾24 weeks gestation, women with multi-fetal gestation, or those found with pre-gestational or gestational diabetes. Comparisons were made between overweight and obese patients, with pregnancy weight gain goals established by the Institute of Medicine[1], and macrosomia defined by birthweight ⩾4,000 grams[2]. Chi-square, T-test, and logistic regression were utilized for statistical analyses, with multivariate logistic regression performed to adjust for potential confounders. Appropriate institutional ethics committee clearance was obtained, and as a retrospective chart review this study was classified as exempt by the Humans Subjects Committee at Harbor-UCLA Medical Center.

Results: One hundred and thirty two consecutive charts were reviewed for women meeting inclusion criteria. Utilizing pre-pregnancy BMI, 63 women were obese and 64 were overweight, with data missing for 5 subjects. Obese women were more likely to meet the 2009 IOM guidelines than overweight women (74% vs. 51%, p = 0.029; OR 2.70, 95% CI 1.09–6.65). This difference persisted even after multivariate logistic regression to adjust for nutritionist visits and active exercise (OR 2.60, 95% CI 1.05–6.45, p = 0.04). However, despite obese women meeting IOM guidelines more often than overweight women, there was no significant difference in the incidence of macrosomia (15.2% vs. 9.3%, p = 0.36) or in mean infant birthweight (3447 g vs. 3403 g, p = 0.66) for obese and overweight women, respectively.

Conclusions: The nutritional component of the CPSP program is effective in identifying obese women in pregnancy that may benefit from nutritional counseling and referral. Non-diabetic, obese women more often met IOM guidelines for weight gain in pregnancy, compared to overweight women. However, differences in weight gain between obese and overweight women were not explained by nutritionist counseling or active exercise. Additionally, improvement in maternal weight gain parameters did not result in reduced infant birth weights or macrosomia. The optimal pregnancy intervention for the prevention of LGA infants and macrosomia is yet to be determined.

1. IOM (Institute of Medicine). *Weight Gain During Pregnancy: Reexamining the Guidelines*. Washington, D.C.: The National Academic Press, 2009.
2. ACOG (The American College of Obstetrics and Gynecology). *ACOG Practice Bulletin*: *Fetal Macrosomia.* Number 22,Washington, D.C, 2000.

P-5A-159

Elevated late gestational blood pressure and subsequent abnormal glucose tolerance: The Savitaipale Follow-up Study

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Diabetes is one of the leading causes of premature mortality from cardiovascular complications in women; early identification of women at high risk of insulin resistance would therefore allow for intervention and significant reduction in cardiovascular mortality. A growing body of evidence indicates an association between pregnancy-induced hypertension (PIH), including gestational hypertension and pre-eclampsia, and insulin resistance. Insulin resistance is the earliest detectable form of type 2 diabetes (T2D). Hyperinsulinaemia persists for a length of time post-pregnancy, leading to the hypothesis that PIH may be associated with an abnormal glucose tolerance (AGT) or diabetes at a later age.

Objective: To determine the association between elevated, lower than hypertension level blood pressure during gestation and consequent AGT (including T2D, impaired glucose tolerance (IGT) and impaired fasting glucose (IFG)).

Methods: A prospective, population-based study of nulliparous women (n = 216) born 1933–1956 and living in the municipality of Savitaipale, Finland. Collected data included background demographic and clinical data, anthropometric and cardiovascular measurements and venous blood samples. Prevalence of AGT was determined using an oral glucose tolerance test (OGTT) in 1996, repeated in 2007. Glucose tolerance status was classified according to the WHO criteria. A retrospective analysis of antenatal clinic records was carried out to assess a series of gestational blood pressure measurements. Participants provided written consent and the study was approved by the local ethics committee.

Results: Follow-up with two OGTTs was completed by 77.3% (n = 167) women. The rates of IGT, IFG and T2D were 17.6%, 0.5% and 5.5%, respectively, in 1996 and

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higher: 27.5%, 15.6% and 3.0%, respectively, in 2007 for this female population. There was an increase in the rate of AGT over the 10-year follow up-period. Women with AGT were shown to be statistically older ($p = 0.015$) and to have a higher BMI ($p < 0.001$) at the time of the OGTT. A significant association was observed between elevated systolic blood pressure in late pregnancy and AGT during the initial, and the follow-up study ($r = 0.169$, $p = 0.044$), independent of age and BMI at the time of OGTT.

Conclusions: Longitudinal analysis of nulliparous women shows an increased risk of abnormal glucose tolerance associated with even below hypertension-level late gestational blood pressure measurements. Our results are in keeping with previous reports of an association between gestational hypertension and insulin resistance. Results from this longitudinal follow-up study confirm the findings from the initial cross-sectional study, the first report of the adverse effects of lower than hypertension-level blood pressures. Further analysis is required to identify the high risk group of young women that may benefit from intervention. This unique cohort with extensive background data and an exceptionally high follow-up rate provides a valuable resource for further studies into the long-term effects of an altered gestational metabolic status.

P-5A-160

Differential effects of maternal nutrient restriction between early-to-mid gestation on cardiac and skeletal muscle in the young adult offspring following juvenile onset obesity

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Objectives: Reduced nutrient supply during early organogenesis is established to have long term adverse outcomes in metabolically active organs of the offspring following obesity. Skeletal and cardiac muscle both have a high energetic demand for glucose and lipid that is regulated, in part, by the peroxisome proliferator activated receptor (PPAR)-γ and its coactivator (PGC)1α. It is hypothesized that a re-setting of muscle metabolism during obesity would result in an exacerbated adverse metabolic response in those offspring born to nutrient restricted mothers.

Methods: Pregnant sheep ($n = 26$) were randomly assigned to a normal (7 MJ/day) or nutrient restricted diet (NR, 3.5 MJ/day) from 30 to 80 d gestation (term = 147 days) and fed to requirements at all other times. Following weaning at 10 weeks postnatal age, offspring were reared in an environment of restricted activity and increased availability of energy dense food to promote fat deposition and, thus, juvenile obesity. Two groups of offspring were exposed to this postnatal intervention, those born to prenatal nutrient restricted (NR-O, $n = 11$) and control fed mothers (O, $n = 7$). A further group of control-fed offspring ($n = 8$) remained on pasture, and were therefore designated as lean (L). At ~one year of age all sheep were humanely euthanased and the Longissimus Dorsi (LD) muscle and heart sampled, from which total RNA was extracted and mRNA abundance determined. All results were calculated using the $2^{-\Delta CT}$ method and expressed as in arbitrary units (a.u.) as a % of a L reference. Appropriate institutional animal ethics committee approval was obtained.

Results: The triglyceride content of the LD was raised with obesity irrespective of the prenatal nutritional environment (L 23.9 ± 1.2; O 35.7 ± 2.9 mg/g ($P < 0.05$)) whilst this was only raised in the hearts of offspring born to nutrient restricted mothers (O 7.1 ± 2.0; NR-O 20.2 ± 3.4 mg/g ($P < 0.05$)). PPARγ gene expression was raised with obesity in the heart only (L 1.00 ± 0.15; O 2.21 ± 0.72 a.u. ($P<0.05$)) but decreased in both the LD (NR-O 0.77 ± 0.12 a.u. ($P < 0.05$)) and heart of the NR-O group. Pre-feeding plasma glucose was negatively correlated (r^2 0.984 ($P < 0.001$)) with LD PPARγ gene expression in obese offspring but positively correlated (r^2 0.823 ($P = 0.0125$)) in NR-O offspring. In addition, although mRNA abundance for PGC-1α in the heart was decreased with obesity (L 1.00 ± 0.08; O 0.55 ± 0.10 a.u. ($P < 0.05$)) it was unaffected by maternal diet, whereas prenatal nutrient restriction reduced gene expression in the LD.

Conclusions: In conclusion, the excess lipid accumulation in nutritionally programmed offspring following obesity may be mediated in part by a reduced capacity for LD to deposit triglycerides following obesity. This effect may be mediated by a re-setting of PPARγ action.

P-5A-161

Enhanced adipose tissue desaturation activity promotes programmed obese phenotype in intrauterine growth restricted newborns

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Objective: Intrauterine growth restriction (IUGR) leads to increased risk of adult obesity and lipid abnormalities. Targets for prevention and treatment of obesity include stearoyl-CoA desaturase enzyme 1 (SCD1) which is expressed in metabolically active organs. In adipose tissue, liver and muscle, SCD1 converts stearate to oleate (C18:0 to C18:1) and palmitate to palmitoleate (C16:0 to C16:1). Further, oleate modulates central appetite suppression, which is impaired in IUGR offspring. Maternal undernutrition during rat pregnancy results in IUGR newborns. When allowed rapid catch-up growth, IUGR offspring develop hypertrophic adipocytes at 3 weeks of age prior to the development of

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hypertriglyceridemia and overt obesity, implicating adipose tissue as a primary source of these abnormalities. We thus hypothesized that upregulated SCD1 in IUGR offspring leads to increased desaturation indices in adipose tissue prior to onset of obesity. The desaturation indices (ratios of oleate/stearate and palmitoleate/palmitate) which represent a measure of SCD1 activity was studied in 3 week male IUGR and Control offspring.

Methods: Control dams received ad libitum food from day 10 to 21 of gestation, and study dams were 50% food-restricted to produce IUGR pups. All pups were nursed by Control dams and male offspring were studied at 3 weeks of age. Adipose tissue (non-visceral subcutaneous and visceral retroperitoneal), liver, muscle, and plasma samples were saponified, fatty acids extracted, and GC/MS performed. Desaturation indices were determined for the oleate to stearate ratio and palmitoleate to palmitate ratio from the relative intensities of gas chromatogram peaks. Values are means ± SE.

Results: IUGR males exhibited increased SCD1 activity in *adipose tissue,* evidenced by significantly increased oleate/stearate desaturation index in subcutaneous fat (3.5 ± 0.1 vs. 3.2 ± 0.1, $p < 0.05$) and retroperitoneal fat (3.2 ± 0.1 vs. 2.8 ± 0.1, $p < 0.05$). Similarly, palmitoleate/palmitate desaturation index was increased in both fat depots of IUGR as compared to Controls (subcutaneous: 0.06 ± 0.01 vs. 0.04 ± 0.01, $p < 0.05$; retroperitoneal: 0.04 ± 0.01 vs. 0.03 ± 0.01, $p < 0.05$). In contrast to adipose tissue, IUGR males showed significantly decreased *liver* oleate/stearate desaturation index (0.19 ± 0.01 vs. 0.25 ± 0.03, $P < 0.01$). *Muscle* and *plasma* desaturation indices were comparable to those of the Controls and the palmitoleate/palmitate desaturation index was undetectable in liver, muscle and plasma. Lastly, the overall liver stearate to palmitate ratio was significantly increased in IUGR males as compared to Controls (1.51 ± 0.02 vs. 1.38 ± 0.01, $p < 0.01$).

Conclusions: In IUGR male offspring, the reduced liver desaturation index together with elevated stearate to palmitate ratio indicates augmented stearate accumulation, either from increased production or decreased desaturation. Additionally, the higher desaturation index in IUGR adipocytes reflects increased propensity toward fat accrual owing to the ability of adipose tissue to store more oleate than stearate. These findings suggest that programmed changes in adipose tissue may be the major contributory factor leading to adult obesity in IUGR offspring. As these finding occur prior to the development of obesity, preventative approaches may be applicable during early postnatal life.

P-5A-162

DNA methylation in type 2 diabetes risk: A targeted approach using genes identified in genome wide association studies

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Objectives: Epigenetic mechanisms are believed to be important in capturing early life exposures at a cellular level and mediating changes in gene expression that have long term effects on health. One important consideration when confirming a role of epigenetic mechanisms in developmental programming is to establish the relationship between epigenetic variation and disease phenotype. The aim of this study was to identify whether variation in DNA methylation patterns are associated with risk of type 2 diabetes (T2D). A targeted approach was adopted in which genes harbouring SNPs associated with T2D, identified by genome wide association studies (GWAS), were interrogated for putative epigenetic perturbation.

Methods: Genes associated with T2D (and related traits) were selected utilising evidence from published GWAS reports. Accurate and sensitive quantification of DNA methylation was conducted in 19 candidate genes (including *FTO*, *KCNQ1*, *PPAR gamma* and *KCNJ11*) utilising the Sequenom® EpiTYPER®, a high-throughput MALDI-TOF based system. DNA samples were derived from the *Relationship between Insulin Sensitivity and Cardiovascular Disease* (RISC) cohort; a group of healthy individuals aged between 30–60 years of age, with DNA samples collected at baseline and 3 year follow-up. DNA samples from 11 subjects were used to identify inter-individual variation in DNA methylation with further analysis in 48 samples for the *KCNQ1* locus. Numerous biological, physiological and lifestyle measures were also collected (including height, weight, body fat, insulin sensitivity by euglycaemic hyperinsulinaemic clamp measurements, oral glucose tolerance test, lipid measurements, smoking and family history of disease). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: DNA methylation was analysed at 941 CpG sites (up to 52 CpG sites per gene) in a total of 40 amplicons in 19 genes. Methylation analysis using novel amplicons was successful in 30 (75%) of the amplicons assayed. Fifteen (37.5%) of the amplicons (in a total of 12 genes) demonstrated variable methylation levels which were selected for further analysis within the main RISC cohort. Regression analysis of DNA methylation against T2D traits revealed a number of statistically significant observations including an inverse relationship between methylation of the *KCNQ1* locus and HOMA-IR (insulin resistance) (regression coefficient indicating change in HOMA-IR per unit change in DNA methylation; −0.03 [95% CI −0.05, −0.001] $p = 0.008$).

Conclusions: These data provide evidence that the Sequenom® EpiTYPER® platform is a valuable high-throughput system for population-based studies to assess

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variation in methylation in DNA derived from leucocytes. A number of candidate genes showed inter-individual variation in methylation and in turn were associated with T2D-related traits within the RISC cohort. Further work is required to establish the temporal relationship between methylation and early life exposures. Acknowledgements: We thank EU FP5 and Astra Zeneca for funding to support RISC and the Newcastle Healthcare Charity for funding to support DNA methylation analysis.

P-5A-163

Gene expression profiling in the Cynomolgus macaque *Macaca fascicularis* following spontaneous intrauterine growth restriction

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Objective: Although an adverse early-life environment has been linked to an increased risk for development of the metabolic syndrome, the molecular mechanisms underlying altered disease susceptibility are largely unknown. To understand the possible molecular mechanisms involved we have utilised a non-human primate model, the *Macaca fascicularis* (Cynomolgus macaque) as they share with humans the same progressive history of the metabolic syndrome. In the present study we used birth weight as a surrogate marker indicating a suboptimal early life environment in order to identify potential early indicators of altered metabolic function and studied the effect of low birth weight on gene expression in key tissues collected from Cynomolgus macaque neonates.
Methods: Tissues were harvested from umbilical cord, hepatic and skeletal muscle tissue in 4 appropriate and 4 growth restricted female neonates. Liver and skeletal muscle samples were collected at 5 days following birth. RNA was isolated and used to hybridize the Agilent macaque gene expression micro array.
Results: Using two-way factorial ANOVA, we have identified 1973 genes which were differentially expressed in the three tissue types between the normal and low birth weight animals ($P < 0.05$). Of these 1973 genes we have identified 250 genes differently expressed ($P < 0.05$, >1.5 fold difference) in liver, 850 genes differently expressed ($P < 0.05$, >1.5 fold difference) in skeletal muscle and 891 genes differently expressed ($P < 0.05$, >1.5 fold difference) in cord samples. Of particular interest, four genes were common but differentially regulated between the tissues (up regulated in liver and muscle but down regulated in cords). Gene ontology analysis identified that these genes were involved in different metabolic processes such as cellular lipid metabolism, cellular biosynthesis, cellular macromolecule synthesis, cellular nitrogen metabolism, cellular carbohydrate metabolism, cellular catabolism, nucleotide and nucleic acid metabolism, biological adhesion and development.
Conclusions: Gene expression profiling of metabolic tissues and cord samples from low birth weight macaques have identified novel genes which could be potential prognostic markers for later life disease risk. Further detailed analyses of these may improve our understanding of how alterations in such genes due to adverse early life environment predisposes towards metabolic syndrome. Support: BSE, RK and PDG are supported by the Agency for Science, Technology and Research (Singapore). PDG, DMS and MHV are funded by the National Research Centre for Growth and Development (New Zealand).

P-5A-164

Differences in adult height and waist circumference explain gender differences in 2-hour plasma glucose levels

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Objective: Low adult height is strongly related to low birth weight, so effects of height on glucose metabolism may be related to intra-uterine growth. The prevalence of impaired glucose tolerance (IGT) is higher in women than in men. Currently, it is debated whether this higher prevalence in women is caused by hormonal factors or whether it is simply due to differences in body size between men and women and thereby differences in the ability to dispose of the fixed amount of 75 g glucose during an oral glucose tolerance test (OGTT). The aim of this study was therefore to test the hypothesis that men and women of same height and waist circumference have similar 2-hour post OGTT plasma glucose (2hPG) levels.
Methods: We used baseline data from 6,111 non-diabetic men ($n = 3,007$) and women ($n = 3,104$) of the Inter99 study with available information on height, waist circumference (WC), and 2hPG levels. Univariate analyses between 2hPG and height and between 2hPG and WC were performed stratified by gender. Furthermore, a linear regression model with 2hPG level as response variable and height, WC, and gender as explanatory variables was tested. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The mean ± SD 2hPG levels were 5.99 ± 1.83 for men and 6.17 ± 1.69 for women ($p < 0.001$ for diff.). In both men and women we found associations between 2hPG and WC ($p < 0.001$) and inverse associations between 2hPG

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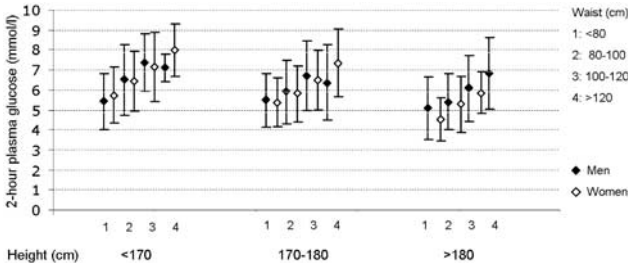
and height ($p < 0.001$). When height, WC, and gender were included as explanatory variables, there was no difference in 2hPG levels between men and women ($p = 0.881$), whereas both height and WC were still significantly associated with 2hPG levels ($p < 0.001$). The figure illustrates 2hPG levels for men and women by height and WC strata.

Conclusions: Gender differences in 2hPG levels can be explained by differences in body size between men and women. This finding questions the validity of using the same standard 75 g-OGTT for classifying IGT in men and women of different body size. *Acknowledgements:* The Danish Medical Research Council, DACEHTA, Novo Nordisk, the Danish Heart Foundation, Danish Pharmaceutical Association, as well as Augustinus, Ib Henriksen, and Becket Foundations.



P-5A-165

Adrenal renin-angiotensin system signaling pathways in maternal protein-restricted offspring: effect on sympathoadrenal cell lineage differentiation and arterial blood pressure

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During normal pregnancy, fetus is protected from higher maternal glucocorticoid levels by placental 11β-hydroxysteroid dehydrogenase (11β-HSD), and the major mechanism involved in the genesis of fetal programming is related with decreased placental enzyme activity, causing overexposure to maternal glucocorticoids. Glucocorticoids have been involved in cromaffin cell differentiation as well as induction of the enzyme tyrosine hydroxylase (TH). There are several studies showing that some degree of gestational protein malnutrition reduce 11β-HSD activity in parallel with development of hypertension in the adult offspring. Otherwise, gestational protein restriction affects offspring sympathoadrenal activity and this activity is clearly implicated in the hypertension pathogenesis. Additionally, adrenal compounds of renin-angiotensin (AngII) system (RAS) are involved in adrenal function as well as blood pressure control. The mechanism by which glucocorticoid exert this effect is, however, unknown.

Objective: Our purpose was to determine possible alterations in adrenal structure and localization/expression of TH and RAS signaling pathway proteins in gestational protein restricted male offspring at 16 weeks old.

Methods: Female Wistar rats were put with male Wistar rats. At the day that we observed the presence of sperm in the vaginal smear, the dams were submitted a normal (NP, 17% protein), or low (LP, 6% protein) protein diet ad libitum during all pregnancy. To our experiment we used only male pups that, in the day of birth, were weighted and ano-genital distance (AGD) measured. The systolic blood pressure (SBP) was measured in conscious 6, 8, 10 12, 14 and 16-week-old rats by an indirect tail-cuff method. With 16 weeks-old, the animals were killed and adrenals processed for immunoblotting and immunohistochemistry and morphometric analysis.

Results: We found significant reduction in LP birth weight (5.7 ± 0.1 vs. 6.3 ± 0.1, $p = 0.008$) at 16-wk old (315.4 ± 22 vs. 343.1 ± 23, $p = 0.006$) offspring. The higher AGD was observed in LP (0.52 ± 0.02 vs. 0.43 ± 0.01, $p = 0.0061$). In LP, increased AP was verified from 10-wk old (155.4 ± 7.8 vs. 133.6 ± 2.5, $p = 0.02$) to 16-wk old (174.1 ± 2.4 vs. 151.1 ± 2.64, $p = 0.0001$) rats. No difference was found in adrenal total area despite enhanced medullar/cortical ratio in LP with a higher medullar area (14.36 ± 0.6776 vs. 11.01 ± 0.7903, $p = 0.0031$), reciprocally to decreased TH expression in the 16-wk old offspring (55.38 ± 79.04 vs. 105.10 ± 15.07, $p = 0.017$). By blotting, AngII receptors were increased in LP (AT1R, $552.5 \pm 25.9\%$, $P < 0.05$ and AT2R, $124.3 \pm 22.4\%$, $P < 0.05$) compared with controls. Also, RAS signaling pathways proteins (JAK-2, $195.6 \pm 21.4\%$, $P < 0.05$ and SOCS-3, $69.2 \pm 13.9\%$, $P < 0.05$) were enhanced in LP compared to controls. These findings were confirmed by immunohistochemistry in all adrenal cortical zones and in medulla.

Conclusion: Protein restriction *in uteri* results in hypertension associated with increased RAS activity and higher adrenal medullar area. This effect may occur accompanied by adrenergic hyperactivity in part responsible by genesis and maintenance of hypertension. The paradoxical TH fall could also be related to down-regulated action of elevated adrenal production of catecholamine. Support: FAPESP.

P-5A-166

Polymorphisms in genes within the HPA-axis are associated with birth-weight and insulin resistance during adolescence

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Objective: Suboptimal antenatal and postnatal environments are associated with the development of the metabolic syndrome in human populations and multiple species. Since the hypothalamic-pituitary-adrenal axis (HPA-axis) has been implicated in this developmental programming, we analysed single nucleotide polymorphisms (SNPs) in HPA-axis genes for associations with fetal growth trajectories and insulin resistance (IR) during adolescence.

Methods: 1415 population-based adolescents from the Western Australian Pregnancy Cohort (Raine Cohort) previously underwent serial ultrasound biometry of fetal head (HC) and abdominal circumference (AC) and femur length (FL) between 18–38 weeks' gestation. Anthropometry was measured at birth and the homeostasis model assessment of insulin resistance (HOMA-IR) was measured at 14-years. 545 tagged SNPs within 45 genes that regulate or interact with HPA-axis function were identified and 499 of these were successfully genotyped. Associations between HPA-axis SNPs and HOMA-IR at 14-years were investigated using multivariate linear modelling including terms for diet, exercise, maternal smoking and socioeconomic status. Gene:environment interactions involving sex and birth-weight were explored. SNPs with significant associations with HOMA-IR were tested for association with antenatal growth trajectories. Linear mixed effects models were used to analyse ultrasound anthropometrics (HC, AC, FL and HC/AC) and random effects were fitted for each individual for slope and intercept. Institutional ethics committee clearance and participants' informed consent were obtained.

Results: HOMA-IR was elevated in 29% of the cohort (3.5–3.9) with levels similar to those seen in adults with type-2 diabetes. 27 SNPs within 10 HPA-axis genes were associated with HOMA-IR in males and 55 SNPs within 22 genes were associated with HOMA-IR in females: five genes were common to both sexes (IGF1R, IRS2, NR3C2, PCSK2 and SLC2A1). Only one SNP was common to both males and females, RS16999070, in PCSK2 where the minor allele was associated with increased insulin resistance. In males, of 16 SNPs associated with increased HOMA-IR (all $p < 0.05$), three were associated with decreased ponderal index at birth: RS9341105 in IGFBP2 ($p = 0.032$); RS2740210 in OXT ($p = 0.030$); and RS11696561 in PCSK2 ($p = 0.005$). Further, of 11 SNPs associated with decreased HOMA-IR ($p < 0.05$), two were associated with fetal AC growth trajectories: RS2871865 in IGF1R ($p = 0.007$) and RS7686433 in NR3C2 ($p = 0.001$). In females, of 31 SNPs associated with increased HOMA-IR ($p < 0.05$), four were associated with reduced antenatal growth trajectories: RS7649121 in ADIPOQ ($p = 0.021$); RS975537 in CRHR2 ($p = 0.045$); RS12916884 in IGF1R ($p = 0.048$); and RS11763517 in LEP (0.001). One was associated with decreased ponderal index at birth: RS822391 in ADIPOQ ($p = 0.002$). Further, of the 23 SNPs associated with decreased HOMA-IR (all $p < 0.05$), four were associated with increased antenatal growth trajectories: RS10735380 ($p = 0.027$), RS10860865 ($p = 0.027$) and RS2162679 ($p = 0.036$) in IGF1; and RS17412368 in LEPR ($p = 0.027$). Complex interactions were identified between birth-weight, SNP and HOMA-IR at 14-years for three SNPs in males and six SNPs in females such that effects of the SNPs on HOMA-IR were reduced in low birth-weight males and increased in low birth-weight females.

Conclusion: These data demonstrate significant gene: environment interactions between SNPs in HPA-axis genes, intrauterine growth and HOMA-IR in adolescence which require further evaluation and replication in other pregnancy cohorts.

P-5A-167

Birthweight and mortality since the 1950s in a remote Australian Aboriginal community

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Objectives: Low birthweight is a risk factor for death in infants and children, and predisposes to chronic disease in adults. Birthweights have historically been low in remote Aboriginal Australia. We analysed associations of birthweight with deaths in one Aboriginal community against a background of rapidly changing mortality over time, due to better health services.

Methods: Participants were 996 people born alive between 1956 and 1985 to Aboriginal mothers in this community, and for whom birthweights were recorded. For birth intervals 1956–1965, 1966–1975 and 1976–1985, mean (SD) birthweights were 2.64(0.49), 2.69(0.54) and 2.87(0.52) kg respectively. Deaths were documented through 2006, and infant (0 to <1 year), childhood (1 to <15 year) and young adult deaths (15 to 36 years) were enumerated. Appropriate institutional ethics committee clearances were obtained for the studies generating these observations.

Results: Over the observation period there were dramatic falls in natural deaths in all age groups and across the entire birthweight spectrum**.** The death rates of infants born in the third birth interval were 10% the rates of those born in the first interval, while death rates of children born in the third interval were only 1.5% the rates of those born in the first interval. Against that background, birth weights below the median for each birth cohort were associated with higher mortality, with hazard ratios (HR, 95%CI) of 2.30 (1.3–4.7),

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1.74 (1.03–3.1) and 2.69 (1.3–5.6) for natural deaths in infants, children and young adult respectively. Most strongly segregated among those of lower birthweight were "gastro-intestinal" deaths in infants, HR 5.2 (1.2–23), and "pulmonary" deaths in children HR 2.1 (0.94–4.7), while, in adults, pulmonary deaths were strongly associated with lower birth-weights, 8.1(1.2–62), as was the combined endpoint of renal and/or cardiovascular deaths, HR 4.1(1.4–14).

Conclusions: The survival disadvantage associated with lower birthweight is confirmed for Aboriginal infants and children. It is also documented for the first time for Aboriginal adults, supporting the Barker hypothesis. Better services have dramatically reduced mortality of infants and children in all birthweight categories, which must be applauded. However, improved survival of low birthweight infants has resulted in a population of adults at higher risk for adult death. The current trend of improving birthweights promises further reductions in mortality.

P-5A-168

Differential response in global DNA methylation patterns by high fat overfeeding in low birth weight subjects

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Objective: For decades the association between low birth weight (LBW) and increased risk of metabolic diseases such as type 2 diabetes, cardiovascular disease and hypertension, has been proposed to involve epigenetic mechanisms, including DNA methylation[1]. Recent research indicates a time dependent role of epigenetic programming of LBW subjects *in utero*[2], and environmental factors appear to have a modulating role as epigenetic patterns diverge in monozygotic twin pairs with increasing age[3]. As unhealthy diets are associated with an increased risk of metabolic disease a dietary challenge could unmask a potential role of DNA methylations in the development of disease. Based on these findings, we investigated differences in global DNA methylation levels between LBW and normal birth weight (NBW) subjects. Additionally, we challenged the subjects with a 5-day high-fat overfeeding diet to clarify whether LBW subjects respond differently to the deleterious effects of an unhealthy diet. Furthermore, we investigated the correlation between DNA methylation and gene expression.

Methods: Seventeen young LBW men and 22 matched controls with NBW, received both a 5-day high-fat (60E%) overfeeding (+50% energy) and a weight maintaining control diet in a randomized crossover fashion. DNA was extracted from skeletal muscle biopsies and global DNA methylation measured at 27,578 CpG sites covering 14,475 gene promoters by the Illumina Infinium Bead Array. Gene expression was studied in a subset of genes by low density arrays and quantitative real-time PCR. Appropriate institutional ethnic committee clearance and participant's informed consent were obtained.

Results: Few differences in DNA methylation patterns were observed between NBW and LBW subjects during the control (2.7%) diet. An increased proportion of genes changed in DNA methylation between NBW and LBW subjects during the overfeeding diet (5.9%) (X^2: $p < 0.001$). The largest response to the high fat overfeeding diet was observed in NBW subjects were 25.1% of the genes changed in DNA methylation, whereas only 5.7% changed in the LBW subjects (X^2: $p < 0.001$). The maximum diet induced change in methylation was 8.5% within the NBW and 8.2% within the LBW subjects, whereas methylation differences between NBW and LBW subjects were 10.2% during the control and 10.9% during the overfeeding diet. Week correlations were observed between DNA methylation and gene expression.

Conclusions: The similar DNA methylation patterns observed between NBW and LBW subjects indicate only few epigenetic effects of *in utero* programming. However, high fat overfeeding revealed dynamic changes in DNA methylation patterns in NBW, but not LBW subjects. We speculate that the limited response in LBW subjects, unmasked by the unhealthy diet, can have implications for the increased risk of metabolic disease throughout life.

1. J.C. Mathers, *Forum Nutr.*, 60:42–8, 2007.
2. B.T. Heijmans, *Proc. Natl. Acad. Sci. USA*, 105: 17046–17049, 2008.
3. M.F. Fraga, *Proc. Natl. Acad. Sci. USA*, 102:10604–10609, 2005.

P-5A-169

Transgenerational programming of insulin resistance by early post-natal overfeeding in mice

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Developmental Origins of Adult Health and Disease Hypothesis proposes that alterations of the peri-natal environment increases the prevalence of metabolic syndrome

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in adulthood. In humans it has been well documented that many rural populations are rapidly transitioning to environments where nutrition is highly affluent during childhood and infancy. Thus, this 'nutritional transition' may have deleterious consequences on adult health by affecting postnatal early development. Even more, there is evidence suggesting that these effects may perpetuate and manifest into the following generation (i.e. transgenerational programming of disease). Despite this, few studies have examined adaptive molecular mechanisms during early post-natal development when offspring is exposed to over-nutrition.

Objectives: Therefore, our specific aims are: (1) First, to develop a mouse model of early postnatal overnutrition-associated diabetes to understand molecular mechanisms of disease. (2) Second, to explore whether postnatal overnutrition may influence second generation offspring metabolism.

Methods: Here we have developed a mouse model of immediate postnatal overfeeding by reducing the size of the litter during the suckling period to 4 pups/ dam. At weaning, all mice are maintained on a regular chow diet until sacrificed.

Results: We show that male mice overfed during the suckling period (over-nutrition group, O-N) develop many features of the metabolic syndrome by age four months: Fed and fasted hyperinsulinemia (Control = 1.39 ± 0.36 ng/ml, O-N = 9.7 ± 3.55 ng/ml; $p < 0.05$), insulin resistance as assessed by intraperitoneal insulin tolerance test (Area Under the Curve; C = 3.9, O-N = 4.4; $p = 0.02$) and obesity (% epididymal fat; C = 1.34 ± 0.28%, O-N = 2.32 ± 0.38; $p = 0.05$). Next, we explored whether metabolic dysregulation, in males, progresses to next generation offspring in the absence of any other additional nutritional interventions. Strikingly, O-N-F2 male mice also develop both fed and fasting hyperinsulinemia by age four months as compared to controls (Fed insulin: C-F2 = 1.89 ± 0.36 ng/ml; O-N-F2 = 3.0 ± 0.73 ng/ml; $p = 0.08$) (Fasting insulin: C-F2 = 0.27 ± 0.02 ng/ml; O-N-F2 = 0.45 ± 0.06 ng/ml; $p = 0.02$).

Conclusions: Thus, this data supports that insulin resistance progresses from first to second generation offspring through the paternal lineage. This effect is specific to glucose-insulin metabolism, since the obese phenotype does not progress to second generation offspring: Body weight and % body fat are similar in both groups. Together, our data suggests that postnatal overfeeding, prior to weaning, predisposes individuals to insulin resistance and obesity during adulthood. In our mouse model, insulin resistance, but not obesity, progresses to next generation offspring through the paternal lineage. This data implicates that a complex array of molecular events might underlay transgenerational progression of adult phenotypes. In particular, we propose that trans-paternal inheritance of hyperinsulinemia might be mediated by epigenetic mechanisms, primarily DNA methylation, since males contribute to second generation offspring through transmission of DNA only. In summary, we demonstrate that early nutrition may influence across-generations metabolism, thereby influencing adult health and disease in, at least, one generation later.

P-5A-170

Preterm birth: a risk factor for type 2 diabetes? – The Helsinki Birth Cohort Study

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Objective: The risk of type 2 diabetes is increased in people with a lower birth weight. Low birth weight can be a consequence of slow fetal growth or short gestation, or both. While the link of slow fetal growth with type 2 diabetes is well established, recent studies have suggested that people born preterm could also carry an increased risk. We assessed this question in the Helsinki Birth Cohort Study.

Methods: The Helsinki Birth Cohort includes 13345 men and women born in Helsinki between 1934 and 1944 and resident in Finland in 1971. Of them, 12598 had adequate data for gestational age at birth, based on the mother's last menstrual period, and were included in the present study. We based the diagnosis of diabetes on special medication reimbursement, which in Finland is granted on the basis of a physician at National Social Insurance Institution reviewing each case history. Data were analysed with logistic regression. The study protocol was accepted by the local Ethics Committee.

Results: 761 people (5.7%) had received special reimbursement for medication for diabetes. They were characterised by slower fetal growth: the odds ratio for one SD lower birth weight relative to duration of gestation was 1.19 (95% CI 1.10 to 1.29). Independent of this association, the risk of diabetes was also higher in people who were born before 35 completed weeks of gestation; this is shown in the Table.

Conclusions: Preterm birth before 35 weeks of gestation is associated with an increased risk of type 2 diabetes in adult life. The risk is independent of that associated with slow fetal growth.

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Table. Odds ratios for diabetes according to gestational age at birth.

	<35 weeks	35 to <37 weeks	37 to <42 weeks (Term)	⩾42 weeks
N of subjects with diabetes/total N	21/173 (12.1%)	20/496 (4.0%)	606/10783 (5.6%)	84/1146 (7.3%)
Model 1	2.23 (1.40 to 3.55)	0.69 (0.43 to 1.08)	1.0	1.25 (0.99 to 1.59)
Model 2	2.12 (1.24 to 3.63)	0.72 (0.44 to 1.18)	1.0	1.20 (0.93 to 1.54)

Model 1: adjusted for sex and year of birth. Model 2: adjusted for sex, year of birth, mother's BMI during late pregnancy, socio-economic status in childhood, parity and birth weight relative to duration of gestation.

P-5A-171

Intergenerational effects on ischemic heart disease risk: The Guangzhou Biobank Cohort Study

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Background: Sexual divergence in premature morbidity and mortality from ischemic heart disease (IHD) widens with economic development. We have previously hypothesized and shown in recently developed or developing populations that pre-adult environment has sex-specific impacts on IHD risk, perhaps because of environmentally driven increases in pubertal sex-steroids. Both maternal and contemporaneous pubertal environment may affect pubertal sex-steroids. Here, in a rapidly developing southern Chinese population, we tested the hypothesis that maternal environment distinct from paternal environment (both proxied by literacy) also has sex-specific impacts on IHD risk (proxied by Framingham score).
Methods: In 19,748 older (⩾50 years) adults from The Guangzhou Biobank Cohort Study (phases 2 and 3) examined in 2005–8, we used multivariable linear regression to assess the adjusted associations of maternal and paternal literacy with Framingham score and whether these associations varied by sex.
Results: Maternal, but not paternal, literacy had different associations with Framingham score by sex. Maternal literacy was associated with lower Framingham score in women (−0.19, 95% confidence interval (CI) −0.32 to −0.07) but not in men (0.09, CI −0.04 to 0.21) adjusted for age, study phase, leg length, seated height, age of menarche (women), life course socio-economic position and paternal literacy.
Conclusions: Intergenerational environmental conditions may have sex-specific impacts on IHD risk, perhaps driven by maternal sex-steroids. To what extent increasing levels of sex-steroids with economic development underlie corresponding changes in patterns of IHD or explain observed intergenerational effects, such as the negative association between birth weight and IHD, remains to be determined.

P-5A-172

Prediction of body fat percentage from skin-fold and bio-impedance measurements in Indian school children

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Objective: Developmental origins research often requires measurement of body composition in large numbers of participants. 'Bedside' methods such as anthropometry and bio-impedance analysis (BIA) are frequently used. South Asians have a more adipose body composition than other ethnic groups for a given Body Mass Index. There are currently no equations developed using a primary reference method for calculating body fat percentage (BF%) from skin-folds or BIA in South Asian children. Our objective was to investigate the agreement between BF% from a primary reference method and that predicted from published skin-fold and BIA equations in Indian children.
Method: We measured BF% using primary reference methods in two groups of Indian children. In Pune, 698 children aged 6 years underwent DEXA scans. We administered Doubly Labelled Water (DLW) to 59 children aged 9 years living in Mysore and from this derived BF% using the equation: BF % = 100 × (Weight − TBW/0.77)/Weight, where TBW = Total Body Water. In both groups, at the time of BF% assessment, we measured sub-scapular and triceps skin-fold, weight, height, and bio-impedance at 50 kHz using standardised methods. We used the published equations of Shaikh (SH)[1] and Slaughter (SL) [2] to calculate BF% from skin-folds and the 'Bodystat' manufacturer's equation to do the same for BIA measurements. We tested the agreement between these calculated values of BF% and those derived from DEXA and DLW. Appropriate institutional ethics committee clearance and informed consent were obtained.
Results: In Pune the mean (SD) weight was 16.2 kg (2.2) and height was 110.0 cm (6.2). The mean (SD) BF% derived from DEXA was 18.2% (4.5) for boys and 21.2 (5.2) for girls. Mean (SD) weight of the Mysore children was 24.1 kg (3.5) and height was 128.2 cm (5.6). BF% from DLW was

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21.6% for boys (n = 30) and 29.2% for girls (n = 29). Scatter-plots and Bland-Altman analysis showed poor agreement between the BF% values derived from the primary reference methods and those from the SH equations in both populations. The SH equations over-predicted body fat at lower levels of BF% and under-predicted at higher levels. The SL equations under-predicted BF% of all children except those with a BF% < = 10 as measured by DEXA. There was no systematic bias for the BIA equations, although the limits of agreement (LoA) were wide (in Pune mean bias: +4.2LoA −6.6, 16.0; in Mysore mean bias +1.95 LoA –7.84, 11.74). ***Conclusion:*** Currently available equations for calculating body fat percentage from skin-folds in children do not accurately predict body fat percentage in these two groups of Indian children. We recommend that equations be specifically developed for South Asian children. The BIA equation predicts BF% most accurately at the group level and may be useful for investigating differences between populations or changes over time.

1. S. Shaikh, D. Mahalanabis. *Am J Hum Biol,* 16:278–288, 2004.
2. M.H. Slaughter *et al. Hum Biol*, 60:709–723, 1988.

P-5A-173

Adiposity, insulin resistance and CVD risk factors in 9–10 year old Indian children: Relationships with birth size and postnatal growth

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Objective: Fetal undernutrition, resulting in lower birth size and poor infant growth, may have adverse implications for the subsequent development of cardiovascular risk factors even during childhood. We aimed to examine the associations of birth measurements, current size and postnatal growth on the risks of insulin resistance and other cardiovascular risk markers in 9–10 year old Indian children.
Methods: Five hundred and eighty nine children born to mothers with normal glucose tolerance during the index pregnancy had detailed measurements taken at birth and every 6–12 months thereafter until the age of 9.5 years. At 9.5 years, 479 children (255 boys), available for follow-up, underwent a 2-hr oral glucose tolerance test, and systolic (SBP) and diastolic blood pressure (DBP; automated BP monitor) and plasma lipid concentrations were measured. The study was approved by the Holdsworth Memorial Hospital, Mysore, research ethics committee and informed consent was obtained from parents and children.
Results: There were strong positive associations between adiposity at birth (triceps and subscapular skinfold thickness), and at 9.5 years. The children's current size, especially subscapular skinfold measurements, were positively associated with glucose ($P < 0.05$) and insulin concentrations ($P < 0.001$), HOMA insulin resistance ($P < 0.001$), SBP ($P < 0.001$) and DBP ($P = 0.04$). Lower birthweight was associated with higher fasting glucose concentrations ($\beta = -0.13$, $P = 0.003$, adjusted for age and sex), independent of current subscapular skinfolds ($P = 0.002$). Shorter length at birth was also associated with higher fasting glucose ($P = 0.002$) and HOMA ($P = 0.04$) after adjusting for current adiposity. Lower birthweight was associated with higher insulin concentrations ($P < 0.05$ for all), HOMA ($P < 0.001$) and higher SBP ($P = 0.002$) after adjusting for current weight. The highest values for HOMA were observed in children who had lower birthweight and higher 9.5-year weight, height or adiposity (Figure 1). However, there were no significant interactions between birthweight and current size for any outcomes. Increase in size, particularly conditional height growth from birth to 2, 2–5 and 5–9.5 years was associated with higher HOMA and SBP.
Conclusions: Greater postnatal growth and higher current adiposity are associated with higher cardiovascular risk factors in 9-10 year old Indian children. Our study gives some evidence for an association between reduced birth size and altered glucose and insulin metabolism, independent of current adiposity. Support: The Parthenon Trust, Switzerland, the Wellcome Trust and the Medical Research Council, UK.

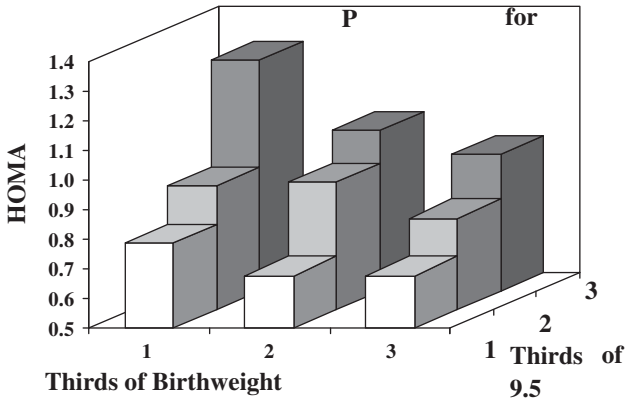

Figure 1. HOMA insulin resistance of Mysore children according to thirds of birthweight and current weight.

P-5A-174

Duration of breastfeeding and the development of cardiovascular disease risk factors in young adulthood in Cebu, Philippines

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Objective: The aim of this study is to examine the association between the duration of breastfeeding and development of hypertension, insulin resistance and dyslipidemia in young adulthood in a lower-income country such as the Philippines. In addition, we aim to explore whether these associations differ by gender.
Methods: We used data from 1,616 participants (901 males and 715 females) of the Cebu Longitudinal Health and Nutrition Survey who were born between 1983 to 1984. Breastfeeding data were collected prospectively from the mother every 2 months from birth to two years. Duration of any breastfeeding was categorized into <6 months and $\geqslant 6$ months. Outcome measures were collected in 2005 and included: hypertension (systolic blood pressure $\geqslant 140$ mmHg or diastolic blood pressure $\geqslant 90$ mmHg), insulin resistance (homeostasis model assessment for insulin resistance (HOMA-IR) index >4.65) and dyslipidemia (triglycerides $\geqslant 150$ mg/dl). To estimate the association between breastfeeding duration and the outcomes, we used logistic regression models adjusted for birthweight, baseline maternal characteristics (age, number of prior pregnancies, and education), household SES (income, assets), environmental hygiene, and urbanicity. Weight at the time of outcome measurement may be a mediating factor of the effect of breastfeeding duration on the outcomes; thus, we did not adjust for adult weight to capture the overall effect of breastfeeding duration. Heterogeneity of associations by gender were determined using likelihood ratio tests (alpha set at $P < 0.10$). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: About 24.4% were breastfed for less than 6 months and in 2005, 6.9% had hypertension, 6.5% had insulin resistance and 14.6% had high triglyceride values. Gender was a significant modifier of the effect of breastfeeding duration on insulin resistance ($p < 0.05$) and dyslipidemia ($p < 0.01$) but not on its effect on hypertension. Breastfeeding for at least 6 months was associated with lower odds of insulin resistance (OR: 0.41, CI 0.22–0.76, $p < 0.01$) and lower odds of having high triglyceride levels (OR: 0.62, CI 0.42–0.91, $p = 0.01$) among males but not among females (OR: 1.25, CI 0.62–2.52, $p = 0.53$ and OR: 1.87, CI 0.91–3.84, $p = 0.09$, respectively). Breastfeeding duration was not associated with developing hypertension in young adulthood.
Conclusions: Results suggest that breastfeeding for at least 6 months may decrease the likelihood of developing insulin resistance and dyslipidemia among males. These support the intensification of programs that promote breastfeeding to potentially lower cardiovascular disease prevalence, especially in resource-poor countries. Possible mechanisms that may explain the heterogeneity of effect by gender need to be investigated. Support: NIH/NICHHD 1R01HD054501-01A2 (Modeling the Developmental Origins of Adult Disease Risk Factors).

P-5A-175

The counts reference ranges of neutrophils from peripheral blood and primary utility in diagnosis of septicemia in very-low-birth-weight infants in southwest China

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Objective: to build a counts reference ranges of neutrophils from peripheral blood, analyze the influencing factors and evaluate the utility in the diagnosis of sepsis in very-low-birth-weight (BLBW) infants.
Method: clinic datum were collected without perinatal and neonatal complication, the reference ranges for absolute total neutrophil count (ATN), absolute total immature neutrophil count (ATI) and proportion of immature neutrophils to total neutrophils (I/T ratio) from peripheral blood were built and some influencing factors were analyzed by group comparison.
Result: The peak value of white blood cell count (WBC) occurred between 12 and 18 hours after birth with a upper limit of 24,000/mm^3. At birth, the neutrophil percentage of WBC was 0.63, and maintained the same proportion with lymphocyte percentage during 8 to 15days. Afterward, the lymphocyte occupied a proportion of 0.60 in the WBC. ATN reference range was showed a peak at about 10 hours after birth, with a upper limit of 19,000/mm^3 and a lower limit of 5,700/mm^3, which remained unchanged after 60 hours, with a upper limit of 8,200/mm^3 and a lower limit of 2,000/mm^3. Only one case (3.3%) of ATI and I/T values obtained from the ''normal'' VLBW neonates was outside the previously abroad reference ranges. VLBW infants of the females, single birth and earlier gestational age had significantly higher ATN counts ($p < 0.05$). One of 3 cases of definite neonatal septicemia was demonstrated neutropenia, and the others had neutrophilia with elevated ATI and I/T ratio.
Conclusion: the domestic reference ranges for ATN differ from the abroad ones, and the utility of this reference ranges in the diagnosis of neonatal septicemia requires further study.

P-5A-176

Prenatal food and micronutrient supplementation have effects on metabolic status in early childhood, a randomised trial in rural Bangladesh (the MINIMat trial)

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Objective: In a setting where poor nutrition is common we aim to investigate the effects of prenatal food and micronutrient supplementation on metabolic status in early childhood.
Methods: A randomised intervention trial was performed in 4436 pregnant women in rural Bangladesh (the MINIMat trial). Women were randomly allocated to receive promotion to start food supplementation early in pregnancy or no such promotion and to receive multiple micronutrients or iron-folate supplements (30 or 60 mg of iron). In a randomly selected subset of the offspring at 4 years (n = 1335) blood glucose was measured and plasma samples analysed for apoA1, apoB, cholesterol, HDL, LDL, triglycerides, and insulin. Intent-to-treat analysis was used to evaluate the effects of supplementations. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Children born to women in the group introduced to early food supplementation had lower apoB ($p < 0.05$), cholesterol ($p < 0.05$), glucose ($p < 0.001$), and LDL ($p < 0.05$) than children born to women receiving food supplements later in pregnancy. In addition, multiple micronutrients resulted in lower glucose than supplementation with folate and 60 mg of iron ($p < 0.01$) as well as lower insulin compared to supplementation with folate and 30 mg of iron ($p < 0.05$).
Conclusions: An intervention of food supplementation and multiple micronutrients starting early in pregnancy had favourable effects on blood glucose and lipid profile in 4-year old children in Bangladesh.

P-5A-177

Food restriction during gestation and impaired fasting glucose or glucose tolerance and type 2 diabetes mellitus in adulthood: evidence from the Dutch Hunger Winter Families Study

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Objective: Several studies have reported inverse associations between birth weight and risk of type 2 diabetes mellitus (DM2), but few have related these observations to maternal undernutrition. We use the circumstances of the Dutch Hunger Winter of 1944–45 to relate maternal undernutrition in pregnancy to impaired fasting glucose (IFG, 100–125 mg/dl), impaired glucose tolerance (IGT, 2-h post-challenge glucose 140–199 mg/dl) and to type 2 diabetes (DM2, diabetes history or fasting glucose >= 126 mg/dl or 2-h glucose >= 200 mg/dl) among adult offspring evaluated by detailed history and conventional oral glucose tolerance tests.
Methods: We studied adults born in one of three hospitals in affected cities in the western Netherlands to mothers exposed to famine immediately prior to or during pregnancy (n = 359) or born in the same hospitals to mothers not exposed to famine during pregnancy (n = 299); and non-exposed same-sex siblings of individuals in the above two groups (n = 313). We defined partially overlapping intervals of famine exposure in gestational weeks 1–10, 11–20, 21–30, or 31 through delivery based on available rations of <900 kcal/day throughout the gestational-age interval. Offspring exposed in at least one such interval were considered to have "any" exposure. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The examined offspring included 956 adults (mean age 58 y, 46% men) with adequate information to assign each individual to one of the mutually exclusive states of glucose regulation as follows: 116 had isolated IFG, 58 had isolated IGT, 44 had both IFG and IGT (IFG + IGT), 131 had DM2, and 607 were normoglycemic. Compared to the normoglycemic reference group, the age and gender-adjusted odds ratio (OR) of any famine exposure for isolated IFG was 0.93 (95%CI: 0.60–1.43), for isolated IGT 0.60 (CI: 0.32–1.13), for IFG + IGT 1.19 (CI: 0.62–2.28), and for DM2 1.88 (CI: 1.25–2.82; $p = 0.002$). These OR point estimates were each attenuated by ~15% with additional adjustment for waist circumference, but the famine association with DM2 remained statistically significant (OR 1.64; CI: 1.07–2.51; $p = 0.02$). The adjusted OR relative to normoglycemia for the combined states of isolated IFG or isolated IGT was 0.82 (CI: 0.56–1.18) and for the combined states of IFG + IGT or DM2 was 1.69 (CI: 1.18–2.42; $p = 0.005$). We found no clear differences in ORs across categories of 10-week gestational-age exposures to famine.
Conclusions: Famine exposure in pregnancy was associated with an increased likelihood of offspring DM2 but not of isolated IFG or isolated IGT. The likelihood of offspring IFG + IGT among famine-exposed individuals was intermediate. Our data suggest that prenatal undernutrition may particularly affect the more severe manifestations of glucose dysregulation, involving the IFG and IGT pathways in combination.

P-5A-178

Blood pressure in $2\frac{1}{2}$-year-old children born extremely preterm

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Objective: Adolescents and young adults born preterm have elevated blood pressure (BP).[1,2] Little is known about the emergence of high BP in young children surviving extremely preterm birth (EXPT).

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Methods: Population-based study of 45 EXPT (gestational age 23–26 weeks, mean birth weight 773 [169] g) surviving to a corrected postnatal age of 32 [1.7] months, and 42 matched controls born at term (BW 3540 [441] g). After 15 min acclimatization in the room and 5 min rest in sitting position, systolic (SBP) and diastolic (DBP) BP were measured in the upright sitting position using a validated oscillometric BP-device (Omron HEM-907). Data are mean [SD] or proportions (%). Appropriate institutional ethics committee clearance and parents' informed consent were obtained.

Results: Blood pressures were successfully measured in 75 of 87 children (86%). There were no differences in postnatal age, gender distribution or resting heart rate (mean 103 and 102 min^{-1}, respectively) between EXPT and controls. In addition, SBP (mean 100 [11] vs 97 [8.1] mmHg, $p = 0.16$) did not differ significantly between EXPT and controls. However, DBP was higher in EXPT (mean 69 [12] compared to controls 62 [9.2] mmHg, $p = 0.004$) despite that EXPT were on average 2.1 kg lighter ($p < 0.001$) and 5.7 cm shorter ($p < 0.001$) than controls. The proportion of boys with an age, gender and height adjusted SBP >90th percentile[3] was 16/21 (76%) in EXPT and 6/19 (31%) in controls ($p = 0.004$). The corresponding proportions for girls were 7/16 (44%) and 4/17 (24%) ($p = 0.22$).

Conclusions: A large proportion of children, especially boys, born extremely preterm have elevated systolic and diastolic blood pressures already at a corrected age of $2\frac{1}{2}$ years.

1. Edstedt Bonamy A.K. *et al. Pediatr Res* 2005;58, 845–849, 2005.
2. Johansson S. *et al. Circulation* 2005; 112:3430–3436, 2005.
3. The Forth Report on the Diagnoses, Evaluation and Treatment of High Blood Pressure in Chidren and Adolescents, National High Blood Pressure Education Program Working group on High pressure in Children and Adolescents. *Pediatrics*, 114: 555–576, 2004.

P-5A-179

Understanding the developmental pathways to ill-health: associations of size at birth and weight gain in infancy and childhood with adult diabetes risk in five low- or middle-income country birth cohorts

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Objective: The aim of this study is to investigate associations of size at birth and infant and child growth with adult diabetes among 5 low- or middle- income country cohorts. Methods: Data from 6501 participants (47% female) aged 15–32 years were pooled from COHORTS (Consortium on Health-Orientated Research in Transitional Societies - Brazil, Guatemala, India, Philippines, South Africa). Exposure measures were birth size (z-score; WHO 2006) and infant and child growth (conditional weight at 24 and 48 months). Outcome measures were impaired fasting glucose (IFG; glucose $\geq$6.1 mmol/l and <7.0 mmol/l) and diabetes (glucose $\geq$7.0 mmol/l and/or prior medical diagnosis). We combined these into one outcome measure (IFG/diabetes). We adjusted for age, sex, socio-economic status at birth, maternal education, adult height and adult waist circumference (WC). Each cohort study was approved by an appropriate institutional ethics committee and participants provided informed consent.

Results: The prevalence of IFG ranged from 1% (Philippines) to 17% (India), and diabetes ranged from 0% (Philippines) to 4% (Brazil) across the 5 cohorts. Birth weight, after adjusting for confounders other than adult height and WC, was inversely associated with prevalence of IFG/diabetes (OR = 0.92 per kg; 0.86–0.99; $p = 0.02$). Conditional weight at 24mo (OR = 0.98; 0.90–1.06; $p = 0.58$) and at 48mo (OR = 1.0; 0.93–1.08; $p = 0.98$) were not associated with prevalent IFG/diabetes. After adjusting for adult height and WC, birth weight (OR = 0.88; 0.82–0.95; $p < 0.001$), conditional weight at 24 mo (OR = 0.90; 0.82–0.98; $p = 0.02$) and at 48 mo (OR = 0.92; 0.85–0.99; $p = 0.04$) were each inversely associated with IFG/diabetes. There was no evidence of heterogeneity by gender or cohort in the associations.

Conclusion: Low birth weight is a risk factor, while conditional weight at age 2 and 4 years are not directly related to adult diabetes. The inverse associations observed only after adjustment for adult size suggest that there may be one component of infant/early childhood weight gain that leads to greater adult abdominal adiposity and IFG/diabetes risk, and another component that (like larger birth weight) protects against IFG/diabetes. Understanding these components of early weight gain is important in the formulation of effective public health policy and life-course interventions.

P-5A-180

Cortisol response to AVP + CRH challenge is suppressed in adult offspring of ewes undernourished around the time of conception

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Objective: Maternal periconceptional undernutrition, resulting in a 10–15% decrease in maternal body weight, accelerates development of the fetal hypothalamic-pituitary-adrenal axis (HPAA) axis. The aim of this study is to investigate postnatal HPAA function through to adulthood (18 months of age) in the same animals.
Methods: Ewes were undernourished from 61 d before to 30 d after mating (PCUN) or well fed (N). Singleton and twin offspring of both sex were challenged at 4, 10 and 18 months of age with an equimolar dose of CRH (0.5 μg/kg) and AVP (0.1 μg/kg), with plasma samples collected over 60 minutes. Plasma cortisol was measured by mass spectrometry. Area under the curve (AUC) for cortisol response was calculated. Data were analysed by ANOVA and multiple regression and are expressed as means ± SEM.
Results: Weight at birth was not affected by maternal PCUN (N; 5.4 ± 0.1 vs. PCUN; 5.3 ± 0.2 kg), sex or single/twin status. At 4, 10 and 18 months rams were heavier than ewes ($p < 0.05$) but there was no effect of maternal PCUN or twinning on weight, in either sex. Baseline plasma cortisol concentrations were not affected by twinning, birth weight or current weight but were higher in ewes than rams at 18 months (4.5 ± 0.5 vs. 2.1 ± 0.4 ng/ml, $p < 0.005$), but not at younger ages. Plasma cortisol AUC response to CRH + AVP challenge was reduced in PCUN rams at 4 months (Table, $p < 0.05$), both PCUN rams and ewes at 10 months (both $p < 0.05$), but only in PCUN ewes at 18 months ($p < 0.005$). Plasma cortisol AUC response decreased between 10 and 18 months in N rams only ($p < 0.05$).
Conclusion: Maternal PCUN results in accelerated maturation of the fetal HPA but early in postnatal life, cortisol responses to CRH+AVP co-challenge are actually diminished. In rams, the suppressive effects of PCUN appeared before puberty, remained at 10 months but had diminished by 18 months of age. This change at 18 months was most likely due to a decreased cortisol response in N rams. In ewes, the effect of PCUN on cortisol response appears after puberty and persists into adulthood. Further studies will investigate whether the suppressive effect PCUN has on postnatal cortisol responses to CRH+AVP involves hypothalamic/pituitary feedback, adrenal post receptor mechanisms or steriodogenic regulatory factors.

Age in months	N (ng/ml.min)		PCUN (ng/ml.min)	
	Rams	Ewes	Rams	Ewes
4†	921±60(17)	1012±38(21)	734±35(13)*	942±61(18)
10†	1771±134(17)‡	2545±145(22)‡	1376±54(11)*‡	2023±91(18)*‡
18†	1389±112(16)‡	2443±102(24)	1173±113(9)	1996±82(15)*

Cortisol AUC values are means ± SEM, numbers per group in brackets. *, within sex PCUN effect ($p < 0.05$). †, sex effect ($p < 0.05$). ‡, change from previous age ($p < 0.05$).

P-5A-181

Pre and postnatal determinants of gene expression of inflammatory markers in subcutaneous adipose tissue

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Objectives: Changes in both the pre and postnatal nutritional environment can have a long term impact on adipose tissue function in the offspring. This includes a resetting of inflammatory and related responses as a consequence of changes in gene expression. The aim of the present study was, therefore, to determine the extent to which exposure to a nutrient restricted diet in late gestation, with or without accelerated postnatal growth, determines gene expression of inflammatory markers in subcutaneous adipose tissue of young adult offspring.
Methods: Pregnant twin-bearing sheep were either fed to requirements (R; n = 20) or nutrient restricted to 60% of this amount (N; n = 20) from 110 days up to term (~ 147 days). Ten offspring in each group were then reared by their mother as singletons in order to promote postnatal growth (accelerated weight gain – A). Ten twin offspring from each group were reared by their mother together in order to restrict postnatal growth (standard weight gain – S). After weaning, offspring were either kept in a control indoor environment in order to promote obesity or reared in an unrestricted environment and remained lean. Offspring were humanely euthanased at 17 months of age, adipose tissue was sampled and stored at −80°C until analysis of mRNA abundance for the genes encoding adiponectin, interleukin (IL)s-6 and -18, monocyte chemoattractant protein (MCP)1, fat mass and obesity-associated (FTO) gene and glucose-responsive protein (GRP)78 using real-time PCR. Appropriate institutional animal ethics committee approval was obtained.
Results: Gene expression for adiponectin was increased with accelerated postnatal growth (NS: 0.34 ± 0.08, NA: 1.3 ± 0.41, ($p < 0.05$)) whereas mRNA abundance for the FTO and IL-6 genes were both reduced by maternal nutrient restriction (e.g. FTO - RA: 6 ± 1, NA: 3 ± 0.2, ($p < 0.05$)). Although IL-18 gene expression was downregulated with obesity (L: 2 ± 0.7, O: 0.6 ± 0.2, ($p < 0.05$)), it was unaffected by the postnatal environment. Surprisingly, there were no changes in mRNA abundance for GRP-78 or MCP-1 with any of the interventions.
Conclusion: Subcutaneous adipose tissue exhibits limited changes in gene expression following manipulation of either the pre and postnatal nutritional environments. The main changes are related to increased postnatal growth and coincide

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with the period in which significant amounts of subcutaneous adipose tissue is being deposited. The extent to which these changes differ from those in central fat depots is currently being examined, together with the mechanisms by which they may act to protect an individual from excess fat deposition and the metabolic complications that can accompany obesity.

P-5A-182

Gene-environment interactions underlying the development of non-alcoholic fatty liver disease in adolescence are influenced by gender

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Non-alcoholic fatty liver disease (NAFLD) is a complex liver disorder. Previous reports describe gender, familial, racial and ethnic differences in prevalence of NAFLD, suggesting that gene-environment interactions influence the phenotypic expression of NAFLD. Single nucleotide polymorphisms (SNPs) in genes associated with lipid metabolism and cardiovascular disease have been associated with NAFLD in adults; however, the impact of these genes during early life is unknown.

Objective: The aim of this study was to investigate the association between SNPs in adiponectin and C-reactive protein (CRP) with NAFLD during adolescence controlling for gender, early life events and insulin resistance (IR).

Methods: 1162 population-based adolescents from the Western Australian Pregnancy Cohort (Raine Cohort) underwent detailed questionnaire, hepatic ultrasound, anthropometric and biochemical phenotypic characterisation at 17-years of age. DNA was extracted and polymorphisms in adiponectin and the CRP genes were genotyped. Associations between an ultrasound diagnosis of NAFLD and SNPs were investigated using multivariate modelling including terms for diet, waist circumference, HOMA-IR and dyslipidemia. Analyses focused on 740 adolescents with ultrasound and genetic data. Genetic modelling was stratified by gender. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: NAFLD was diagnosed by ultrasound in 13% of the Raine cohort at 17-years of age (9% male, 16% female). Adolescents with NAFLD had significantly higher weight, BMI, waist circumference, ALT, HOMA-IR, triglycerides and HDL-cholesterol than adolescents without NAFLD (all $p < 0.005$). Further, males with NAFLD had significantly higher weight, BMI, waist circumference, fasting glucose and ALT but lower HDL cholesterol than females with NAFLD (all $p < 0.05$). The inverse association between the duration of breast feeding and the development of NAFLD at 17-years was seen in males but not females. No other associations between nutrition in childhood and adolescence and NAFLD were identified. In univariate analyses, CRP levels were higher in adolescents with NAFLD than those without NAFLD (mean $\pm$ SEM: 3.2 ± 0.58 mg/L vs. 1.6 ± 0.18 mg/L; $p < 0.001$); however, when multivariate modelling considered early life nutrition and waist circumference, the relationship between CRP and NAFLD was not significant ($p = 0.337$). In multivariate modelling two SNPs in the adiponectin gene (RS266729; RS182052) and two SNPS in the CRP gene (RS1572970; RS876538) were associated with increased risk of NAFLD (all $p < 0.01$) in males but not females. Further, one SNP in the adiponectin gene (RS12495941) and one SNP in the CRP gene (RS2027471) were associated with decreased risk of NAFLD (both $p < 0.05$), again in males but not females. These associations were independent of waist circumference, BMI, alcohol, CRP level, HOMA-IR and dyslipidemia.

Conclusions: In the Raine cohort early life nutrition and polymorphisms in the adiponectin and CRP genes are associated with the development of NAFLD in males during adolescence, consistent with the hypothesis that complex interactions between genes and the environment modulate the expression of NAFLD. Further, these data suggest that future studies investigating the influence of genetics and the environment on the development of NAFLD should stratify their analyses by gender.

P-5A-183

Programmed enhanced adipogenesis contributes to adult obesity in growth restricted offspring: Evidence from ex vivo adipose cell culture

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Objective: Insulin, a potent inducer of adipogenesis and lipogenesis, acts via stimulation of the adipogenic transcription factor (peroxisome-proliferator-activated-receptor, PPARγ) lipogenic transcription factor (sterol regulatory element binding-protein, SREBP1) and lipogenic enzyme (fatty acid synthase, FAS). Obese individuals exhibit upregulation of PPARγ, SREBP1 and FAS, and develop cellular resistance to insulin. In rats, maternal food restriction in pregnancy results in IUGR newborns which develop adult obesity, hyperinsulinemia and insulin resistance. IUGR newborns have low plasma insulin levels, but paradoxically *increased* adipose PPARγ expression. To determine if IUGR adipose tissue has a programmed propensity to adipogenesis

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and lipogenesis, in the absence of systemic factors, we examined IUGR and Control adipose tissue proliferation, differentiation and signaling in an ex vivo tissue culture.

Methods: Control dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to term to produce IUGR newborns. Adipose tissue from 1 day old IUGR and Controls was collected, preadipocytes isolated and grown in absence of insulin. After 48 h, preadipocytes were treated with insulin (5, 20 or 40 mg/ml) for 24 h. Cell proliferation rate (MTT) was determined and cells were extracted and measured for protein expression (Western Blot) of insulin signal molecules. Additionally, preadipocytes were allowed to differentiate and treated with insulin (as above). Adipocyte lipid content (oil-red) and protein expression of PPARγ, SREBP1c and FAS was determined.

Results: The basal *preadipocyte* proliferation was significantly increased in IUGR newborns (2-fold). Consistent with this, IUGR preadipocytes exhibited significant upregulation of insulin signaling pathway as evident by increased protein expression of insulin receptorb (1.5-fold) insulin substrates (IRS1, 9-fold; IRS2, 8-fold) and AKT (4-fold). In response to insulin, both Controls and IUGR showed dose-dependent increment in proliferation though at all times, the IUGR exhibited enhanced preadipocyte proliferation as compared to Controls. In *differentiated adipocytes,* the basal lipid content was significantly increased in IUGR newborns (1.5-fold). In concert with this, IUGR adipocytes had significantly increased protein expression of PPARγ (5-fold), SREBP1 (1.5-fold) and FAS (6-fold) as compared to Control adipocytes. In response to insulin, IUGR adipocytes continued to exhibit increased lipid content with upregulated PPARγ, SREBP and FAS.

Conclusions: The basal upregulation of adipogenic and lipogenic factors in newborn IUGR adipose tissue indicates a programmed obesity phenotype, independent of systemic endocrine influences. Furthermore, IUGR preadipocytes and differentiated adipocytes have enhanced insulin sensitivity, which facilitate increased adipocyte proliferation and differentiation with enhanced propensity for lipid storage. These results indicate that programmed enhanced adipogenesis contributes importantly to the development of adult obesity in IUGR offspring.

P-5A-184

In middle aged Danish Citizens an increase in birth weight of 1 kg reduces the risk of type 2 diabetes by 45%.

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Objective: Since maternal under nutrition is uncommon in Scandinavia the relevance of low birth weight in respect to risk of type 2 diabetes has been questioned. This study was set to determine the clinical importance of birth weight on the risk of type 2 diabetes in a group of middle aged Danish citizens.

Methods: In a population-based prospective intervention study (The Inter99 study; n = 6,784), measures of body composition were recorded and an oral glucose tolerance tests (OGTT) was conducted at baseline prior to any intervention. Birth records on participants born in Denmark in the period from 1939–1969 (n = 4,654) were collected using national archives, and birth weight, birth length, parity and prematurity were extracted. Diabetes was defined according to the WHO 1999 criteria. Insulin sensitivity was assessed by the Homeostasis Model Assessment (HOMA-IS). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The mean birth weight was 3,479 ± 526 grams for men (n = 2,151) and 3,331 ± 512 grams for women (n = 2,503). Birth weight was positively associated with measures of anthropometrics. An increase in birth weight of 1 kg was associated with an increase in adult height (2.9 cm; 95% CI: 2.8–3.1), weight (4.2 kg; 95% CI: 3.8–4.6), BMI (0.44 kg/m^2; 95% CI: 0.31–0.57), waist circumference (1.6 cm; 95% CI: 1.3–1.9) and hip circumference (1.8 cm; 95% CI: 1.6–2.1). Out of the 4,654 singleton participants 76 had known diabetes and 174 had screen detected diabetes. An increase in birth weight of 1 kg was associated with a reduced risk of type 2 diabetes of 45% after adjusting for age, BMI and gender (OR 0.55; 95% CI: 0.43–0.72), but we also found a marked non-linear effect with an increase in risk from birth weigth of 4200 grams. Birth weight was positively associated with insulin sensitivity and inversely associated with the area under the OGTT-derived curve for glucose (−23.4 mmol/l × min/kg birth weight; 95% CI: −6.1; −40.7; p = 0.007) and insulin (−2,055 pmol/l × min/kg birth weight; 95% CI: −479:−3, 631; p = 0.009).

Conclusion: Birth weight is associated with adult body composition (positive), glucose tolerance (positive) and insulin secretion (inverse), and consequently, among middle aged Danish Citizens, an increase in birth weight of 1 kg reduces the risk of type 2 diabetes with 45%.

P-5A-185

A maternal low protein diet programs glucose and fatty acid metabolism differentially in adult male and female mouse offspring

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Nutritional conditions during human fetal life can influence the risk to develop cardiovascular diseases and the metabolic syndrome in adult life ('metabolic programming'). Dysregulated fatty acid metabolism and impaired glucose tolerance are hallmarks of the metabolic syndrome.

Objective: We aimed to establish a mouse model of metabolic programming focusing on the effects of a maternal low protein diet during gestation on glucose and lipid metabolism in the adult offspring.

Methods: Pregnant C57BL/6J mice received a control or a low protein diet (18% vs. 9% casein) throughout gestation. Offspring received a low fat diet or a high fat diet from 6–22 weeks of age. Glucose metabolism was studied with a whole-body-glucose test using [6,6-^{2}H]-glucose. Hepatic gene expression was characterized by microarray.

Results: Maternal low-protein-diet did not affect glucose metabolism in male offspring. Male offspring showed lower insulin sensitivity after receiving a high fat diet than female offspring, regardless of the diet of the dam. Female offspring from normal-protein fed dams was relatively resistant to diet-induced metabolic dysregulation. Maternal low-protein-diet during gestation led to deteriorated insulin sensitivity upon high-fat feeding in female offspring, as determined by biochemical and microarray analyses.

Conclusions: We conclude that, in mice, maternal protein restriction during gestation does not change the glucose response to a high fat diet in male offspring. However, gestational protein restriction changes fatty acid and glucose metabolism in female offspring in such a way that it resembles male metabolism. Our study shows limited effects of fetal malnutrition in male mouse offspring. On the contrary, females presented a masculinized reaction to a high-fat challenge when derived from a protein-restricted dam. Support: The Dutch Heart Foundation, grant 2004T048 to T.P.

P-5A-186

Early–life environment and the development of metabolic syndrome in adulthood

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Objective: Prevailing conditions during prenatal and early postnatal life predict long-term health[1]. Rats that receive reduced levels of maternal licking during the first days of life show an increased response to acute stress in adulthood[2]. Individual differences in maternal care also modulate the basal glucocorticoid tone across the circadian rhythm such that the offspring from low licking/grooming (Low-LG) dams release more corticosterone across the day (Dhir *et al.*, unpublished observations). Increased exposure to glucocorticoids is implicated in the development of metabolic syndrome[3,4]. Thus, we hypothesized that the offspring from Low-LG dams may have a greater predisposition for the development of metabolic syndrome with increasing age.

Methods: Maternal licking/grooming of pups was quantified across the first six days of postnatal life. Litters were categorized as either Low-LG (LG < -1 SD below the mean) or High-LG (LG $> +1$ SD above the mean). At 7 months of age, ad libitum fed male rats were weighed and injected IP with saline or insulin (5 U/ Kg). Trunk blood was collected fifteen minutes after for glucose measurement. Pan AKT and Phosphorylated AKT (AKP and pAKT respectively) were quantified in the muscle using western blot, and the total amount of abdominal fat was weighed.

Results: Low-LG rats had a tendency to be heavier than high-LG rats (Low-LG: 743.1 ± 29.5 g; High-LG: 673.3 ± 23.3 g; $P = 0.073$). There was a significant difference in the abdominal adiposity index (Low-LG: $6.65 \pm 0.37\%$; High-LG: $5.56 \pm 0.34\%$; $P = 0.04$) such that Low-LG rats had a higher level of abdominal fat compared to high LG rats. Maternal care did not have a significant influence on the glucose response to the insulin challenge, but Low-LG rats had significantly higher glucose levels than High-LG rats (Two Way ANOVA, drug $P < 0.001$, group $P = 0.036$ and drug versus group interaction $P = 0.45$). Low-LG rats also had lower pAKT to AKT ratio following insulin challenge as compared to the High-LG group (Two Way ANOVA, drug $P = 0.001$, group $P = 0.083$ and drug versus group interaction $P = 0.044$).

Conclusions: Natural variations in maternal care during early-life may thus have a long-term influence on the development of metabolic syndrome in adulthood. Low-LG rats had a significantly higher propensity to develop central adiposity and showed evidence of muscle insulin resistance in middle life. The programming of the HPA axis by variations in maternal care may be contributing to the development of metabolic syndrome in adulthood.

1. C.N. Hales, D.J. Barker. *Br Med Bull*, 60:5–20, 2001.
2. D. Liu *et al. Science*, 277:1659–1662, 1997.
3. M. Salehi *et al. Horm Metab Res*, 37:193–197, 2005.
4. D. Qi, B. Rodrigues. *Am J Physiol,* 292:E654–E667, 2007.

P-5A-187

Higher serum C-peptide concentrations are associated with slower growth rate in the first year of life in girls

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Objective: At birth, girls have higher cord blood concentration of insulin or C-peptide and may be more insulin resistant than boys. We aimed at analysing the associations of cord blood serum C-peptide and IGF-1, with anthropometry at birth and growth in the first year of life in boys and girls separately.
Methods: Cord blood C-peptide and IGF-1 of 378 neonates born in the EDEN mother-child cohort that recruited non-diabetic pregnant French women were assayed. Offspring weight, length, head, arm, wrist and abdominal circumferences, subscapular and tricipital skinfolds were measured at birth and 1 year. Additional measurements were obtained from personal child health records and used to model early growth in order to compute instant weight and length growth velocities at 1 and 3 months. C-peptide and IGF-1 were log-transformed, adjusted for each other in partial correlations with anthropometry, which also accounted for potential confounders. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: At birth, even after accounting for subcutaneous skinfolds, girls had higher C-peptide concentrations (+12%, $p = 0.03$) than boys despite a lower birthweight (−153 g, $p = 0.002$). In both sexes, there was no association between C-peptide and anthropometrics after accounting for IGF-1, which was positively associated with weight ($r = 0.44$, $p < .0001$), length ($r = 0.26$, $p < .0001$), head circumference ($r = 0.21$, $p = 0.0002$) and ponderal index ($r = 0.22$, $p = 0.0001$) after adjustement for gestational age. A positive association between IGF-1 and subscapular skinfold persisted after additional adjustment for birthweight and length ($r = 0.11$, $p = 0.05$). In the early postnatal period, cord C-peptide was not associated with early growth in males while there was a strong negative association in females with weight growth velocity at 1 and 3 months (eg. 1 month: $r = -0.28$, $p = 0.002$) and with length growth velocity ($r = -0.29$, $p = 0.002$) and weight ($r = -0.24$, $p = 0.01$) at 3 months. Cord IGF-1 was positively associated with weight and length at 1 and 3 months in males and females but not with growth velocities. At 1 year, there was no association between cord C-peptide or IGF-1 and weight or length in males whether or not the analysis was adjusted for corresponding variables at birth. In girls, IGF-1 was not associated with anthropometrics but there was a strong negative association between C-peptide and weight ($r = -0.28$, $p = 0.003$), length ($r = -0.20$, $p = 0.03$), head ($r = -0.20$, $p = 0.03$), arm ($r = -0.22$, $p = 0.02$) and abdominal circumference after adjustement for birth anthropometry and 1-hour maternal blood glucose at screening test for gestional diabetes. Girls in the higher tertile of cord C-peptide were 490 g lighter compared to those in the lower tertile. The relationship with abdominal circumference persisted after additional adjustment for weight and length at 1 year ($r = -0.17$, $p = 0.06$).
Conclusions: Our results suggest that insulin resistance is associated with slower early postnatal growth in girls only. Support: AFD-PNRD, ALFEDIAM, Nestlé, ANR-non-thematic-program.

P-5A-188

Transcriptional profiling in a model of programmed metabolic disease: use of gene network and pathway mapping tools to investigate the protective effects of maternal leptin administration from later development of metabolic syndrome

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Objectives: This study aimed to identify some of the molecular pathways and metabolic networks through which maternal leptin administration acts to alleviate the effects of developmental programming of metabolic syndrome. By hybridising RNA prepared from micro-dissected hypothalamic tissue to Affymetrix rat genome 230 2.0 microarrays, we sought to investigate the transcriptional consequences of leptin administration *in utero* and during lactation in offspring born to malnourished rat dams. To identify the molecular mechanisms that predispose an individual to later metabolic disease, researchers have traditionally focussed on candidate genes or pathways. Although genome-wide transcriptional profiling facilitates the analysis of a great many genes, simple fold-change analysis or ontology enrichment provides only limited insight into the complex interactions that are associated with whole-body physiology. Increasingly, it is understood that nutrition *in utero* and during early life influences the likelihood of the eventual development of metabolic disease, for example obesity. Moreover, early postnatal growth patterns have long-term consequences, with maternal health (in particular nutrition) being a key determinant.
Methods: Microarray data were examined using gene/protein interaction network and multiple biochemical pathway analysis tools (both open source and proprietary) in order to generate physiologically relevant results. This allowed the creation of an "appetite network" populated by a collection of important neuropeptides, and both autocrine and paracrine receptors with both orexigenic and anorexigenic effects. In contrast to existing enrichment analysis, our innovative analysis pipeline combined pre-processing, gene network, ontology and pathway mapping tools, such as Matlab, GenMAPP, Reactome, Ingenuity Pathway Analysis, BioCyc and BioPython.
Results: Data analysis necessitated a more pragmatic approach to fold-change thresholds to create our enriched "appetite network" and so allow the identification of key biological processes subject to regulation by leptin, including

gastrin, oxytocin, cortisol and neuropeptide Y (NPY) pathways. Leptin treatment produced positive effects on gastrin releasing peptide receptor expression, which potentiates the release of gastrin, in turn positively feeding back into the cholecystokinin/gastrin receptor axis. The chemokine receptors such as Ccr2 and Ccr3 are also positively affected by leptin. Powerful orexigens such as NPY receptors (NPY1 and NPY5) were observed to be consistently down-regulated. Finally, oxytocins and vasopressin receptors were also found to be positively affected by leptin.

Conclusion: Our novel analysis pipeline improved our understanding of the complex phenotype mediated by leptin's direct and indirect effects on biological processes, or nodes, such as those involving oxytocin, proopiomelanocortin, norepinephrine and NPY. The analysis also revealed that leptin was involved in energy balance regulation via modification of bombesin and gastrin receptors, chemokine pathways, and via modulating the function of adrenocorticotropic hormone and cortisol.

P-5A-189

Developing overweight when stunted: study of metabolic mechanisms in preschool children of Yaoundé, Cameroon

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Objective: Animal and human studies suggest the existence of energy sparing mechanisms related to stunting, including metabolic and behavioural pathways[1]. Among them, impaired fat oxidation and low physical activity, in stunted children, could participate in the development of overweight in countries undergoing nutrition transition[1,2]. Focused on preschool stunted-overweight children of Yaoundé (Cameroon), the present study aims at confirming these hypotheses in this population.

Methods: On the basis of a representative screening in all districts of Yaoundé, children of both sex, aged 24–72 months, were recruited according to their nutritional status category: stunted-overweight (n = 15), non-stunted non-overweight (n = 51), overweight (n = 31) and stunted (n = 28). To assess possible energy imbalance and reduced fat oxidation, physical activity, resting metabolic rate and respiratory quotient were measured using 24-hours dietary recall, 6 days accelerometry and indirect calorimetry. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Preliminary analysis of data shows no differences in basal metabolic rate and in fasting respiratory quotient after adjusting for body weight and age among the four groups and in the analysis between stunted children as a group (stunted plus stunted-overweight) and non stunted-non overweight plus overweight children as the other group.

Conclusions: Unlike previous studies, stunted and stunted-overweight preschool children of Yaoundé do not seem to present impaired fat oxidation. The analysis of energy balance data is actually in progress.

1. A.R. Frisancho. *Am. J. Hum. Bio.*, 15:522–532, 2003.
2. D.J. Hoffman *et al.*, *Am. J. Clin. Nutr.,* 72:702–7, 2000.

P-5A-190

Birth weight and chronic disease profiles in a high risk Australian Aboriginal community

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Objectives: Re-examine the relationship between birth weight (BW) and chronic disease profiles in a remote Aboriginal community. Aborigines have high rates of low BW. We previously described an inverse relationship of BW with both albuminuria and blood pressure in females in one community1,2. However, adults with recorded BW were relatively few (n = 245) and were relatively young (maximum age 38 yr). We now describe the relationship 10 years on.

Methods: Among 1,078 adults screened between 2004 and 2006, 523 had recorded BW (309 males and 214 females). Ages were 20–49 years. Blood pressure (BP), urine albumin creatinine ratio (ACR) and diabetes (history, medications, glycaemic indices) were assessed. High BP (HBP) was defined as $\geqslant$140/90 mmHg and albuminuria as $\geqslant$2.5 g/mol.

Results: Mean (SD) BWs were 2.80 kg (0.54) for males and 2.72 kg (0.50) for females, and 25.9% and 34.1% had BWs $<$2.50 kg.In males and females respectively, 13.6% and 7.9% had HBP, 35.7% and 53.9% had albuminuria and 6.5% and 17.3% had diabetes. With adjustment for age and current weight, BW was not associated with BP in males, but it was inversely correlated with ACR (log transformed), p = 0.01. In females, with adjustment, BW was inversely associated with systolic BP ($-$4.5 mmHg per kg BWT, p = 0.014). Females with BWs above the median had lower rates of HBP, OR 0.30(CI 0.09–0.97), p = 0.04 and lower rates of albuminuria, OR 0.54(CI 0.29–1.00), p = 0.05. BW was not associated with diabetes in males or females.

Conclusions: These data confirm a predisposing effect of low BWs to higher BPs and albuminuria in females, and now expose a similar effect on albuminuria in males. Efforts should continue to improve BW as well as the other environmental risk factors for chronic disease in this setting.

1. W.E. Hoy *et al., Kidney Int.*, 56:1072–1077, 1999.
2. G.R. Singh, W.E Hoy. *Med J Aust.*, 179:532–535, 2003.

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P-5A-191

Birth weight and adult cardiovascular risk in a developing southern chinese population: the Guangzhou Biobank Cohort Study

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Birth weight is negatively associated with cardiovascular diseases and diabetes, although the underlying mechanisms are unclear. Most evidence comes from long-term industrialized populations, potentially confounded by social patterning or by subsequent exposures, including growth and final size. In developing populations birth weight is typically lower and potentially an important contributor to the growing epidemic of non-communicable chronic diseases, but the associations are less well-established and birth weight is often unavailable.

Objectives: To clarify the association of birth weight with cardiovascular risk we used birth rank as an instrumental variable for birth weight in a large sample from Southern China.

Methods: We used published data on birth weight by birth rank from an appropriate population and baseline data from the Guangzhou Biobank Cohort Study phases 2 & 3 (2005–8) to examine the adjusted associations, using instrumental variable analysis, of birth weight with clinically measured cardiovascular risk factors in older (≥50 years) men (n = 5,051) and women (n = 13,907).

Results: Birth weight was associated with lower blood pressure (systolic −0.25 mm Hg 95% confidence interval (CI), −0.53 to 0.03 and diastolic −0.33, 95% CI −0.48 to −0.18 per standard deviation higher birth weight), but had little association with glucose, lipids, waist-hip ratio or body mass index, adjusted for relevant confounders (age, sex, early life environment and number of offspring).

Conclusions: Birth weight may impact blood pressure; however associations of birth weight with other cardiovascular risk factors may not be universal nor related to foetal exposures, with corresponding implications for prevention. Support: The University of Hong Kong (HKSAR), Guangzhou Public Health Bureau (China), Guangzhou Science and Technology Bureau (China), The University of Birmingham (UK).

P-5A-192

Birth weight, current body mass index and insulin resistance and secretion in early adult life in two Latin American populations

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Objectives: The aim of this study was to evaluate the associations between birth weight (BW) current body mass index (BMI) and weight gain from birth to adult age on insulin sensitivity (IS) and insulin secretion (ISc) among two populations of young adults from developing countries.

Methods: Data from two birth cohort studies, one from Ribeirao Preto (Brazil), with 1984 participants, aged 23 to 25 years old, and the other from Limache (Chile) with 965 subjects aged 22 to 28 years old were used. Weight was recorded at the time of birth and anthropometric (weight and height) and laboratory (fasting plasma glucose and insulin) measurements were taken at adult age. IS and ISc were estimated using the updated Homeostatic Model Assessment (HOMA2) index. Four multiple linear regression models were carried out to test the associations between BW and adult BMI on log IS and log ISc. Appropriate institutional ethics committed clearance and participants' informed consent were obtained.

Results: In the early model young adults in the first birth weight tertile had a log IS 0.10 standard deviation score (SDS) lower in Brazil and 0.13 lower in Chile. This effect was small and close to statistical significance. In the late model, those in the higher BMI tertile at adult age showed log IS 0.78 SDS lower in Brazil and 0.39 SDS lower in Chile. In both countries, higher weight gain from birth to adult age reduced insulin sensitivity and the combination of low BW tertile and high adult BMI tertile reduced IS even more only in Brazil. Among Brazilians, those in the low BW tertile presented higher ISc. Adults in the high BMI tertile showed log ISc 0.83 SDS higher in Brazil and 0.66 SDS higher in Chile. High weight gain from birth to adult age and the interaction between low BW tertile and high BMI tertile increased insulin secretion in Brazilians only.

Conclusions: Low birth weight tertile and weight gain from birth to adult age were associated with low IS in both countries and with high ISc in Brazil only. High BMI tertile was associated with low IS and high ISc in both countries. The interaction between low BW tertile and high BMI tertile reduced insulin sensitivity and increased insulin secretion among Brazilians only. In those populations of young adults high compensatory ISc was observed among those presenting low insulin sensitivity and high BMI. Support: CNPq, FAPESP and FAEPA (Brazil) and Fondecyt (Chile).

P-5A-193

Increased glucose production in fetal sheep with intrauterine growth restriction is not suppressed by insulin

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Objective: Intrauterine Growth Restriction (IUGR) increases the risk for metabolic diseases including diabetes in later life, yet little is known about the mechanisms in utero that may underlie these developments. Here we evaluated the effect of IUGR on insulin–mediated glucose metabolism in the developing fetus during late gestation and in isolated fetal hepatocytes.
Methods: Pregnant ewes were exposed to elevated ambient temperatures daily during mid gestation resulting in placental insufficiency IUGR. Hyperinsulinemic-euglycemic clamps (3 mU/min/kg insulin infusion) were performed in combination with glucose tracer infusions in late gestation control and IUGR fetuses (132 d gestation) to measure glucose metabolism. Fetal blood samples were analyzed at steady state during basal and clamp periods. Liver tissue was collected under hyperinsulinemic conditions and from fetuses that received saline infusions for molecular analyses and primary hepatocyte studies.
Results: Late gestation IUGR fetuses weighed 35% less and had lower circulating glucose, insulin, and oxygen concentrations. IUGR fetuses also had a 10-fold increase in endogenous glucose production rate during the basal period and strikingly increased PEPCK and G6 Pase gene expression (20-fold, $P < 0.05$) indicating increased gluconeogenesis. Hyperinsulinemia was unable to suppress glucose production in the IUGR fetuses, yet significantly increased glucose utilization and oxidation rates by 50% in control and by 2-fold ($P < 0.05$) in the IUGR fetuses indicating increased whole body insulin sensitivity, despite severe hepatic insulin resistance. Interestingly, insulin robustly stimulated AKT phosphorylation in control and IUGR livers but was unable to suppress PEPCK or G6 Pase expression in IUGR animals to levels in control fetuses, suggesting that insulin resistance is downstream from AKT in the fetal IUGR liver. Expression of PGC1-alpha, a transcriptional co-activator, was increased by 4-fold ($P < 0.05$) and expression of cytochrome C oxidase, a PGC1-alpha transcriptional target, was increased 2-fold ($P < 0.05$) in the IUGR liver, suggesting that PGC1-alpha may be a crucial early signal for increased hepatic glucose production in utero. Moreover, chromatin immunoprecipitation assay results indicate increased binding of HNF4-alpha, a key liver transcription factor, to the proximal PEPCK promoter. In culture, primary hepatocytes from IUGR fetuses show a robust increase in PEPCK (5-fold, $P < 0.05$) and G6Pase (20-fold, $P < 0.05$) mRNA expression after 24 h with hormone stimulation (dexamethasone and cAMP), whereas control hepatocytes remain refractive to PEPCK activation and had a reduced response to G6 Pase activation (11-fold increase). Importantly, IUGR fetal hepatocytes also have strikingly increased rates of basal (2-fold, $P < 0.05$) and hormone stimulated (5-fold, $P < 0.05$) glucose production after 24 h compared to hepatocytes from control fetuses. This suggests a mechanism whereby IUGR stimulates early chromatin remodeling events that enable certain transcription factors such as HNF4-alpha to bind and activate PEPCK gene transcription.
Conclusions: These data indicate that increased gluconeogenesis and hepatic insulin resistance are likely targets for fetal programming and suggest that these phenotypes persist in culture, consistent with the hypothesis that IUGR may result in epigenetic modifications leading to chronically increased glucose production and susceptibility to diabetes.

P-5A-194

Perinatal salt restriction: a new model to programming low body weight and beta-cell dysfunction in adult Wistar rats

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Several studies support the hypothesis that chronic diseases in adult life might be triggered by events occurring during fetus life. Dietary salt consumption represents one of the major environmental factors in the genesis of hypertension and type 2 diabetes. Previous data have shown that maternal salt intake during pregnancy and lactation has short and long-term consequences on body weight, blood pressure, insulin sensitivity and plasma lipid profile in adult Wistar rats[1].
Objective: to evaluate the influences of maternal salt restriction during the perinatal period on birth weight, neonatal plasma glucose and beta-cell function in adult offspring.
Methods: With this purpose, female Wistar rats were fed a low (LSD: 0.15% NaCl) and normal (NSD: 1.3%) salt diet since 8 weeks of age. At 12 weeks of age, they were matched with males that received only NSD. After birth, only 8 pups (4 males and 4 females) were kept with their mothers. After weaning, all offspring received NSD and only male rats were used in the following experiments. Weekly body weight was evaluated since birth until 12 weeks of age. Plasma glucose was measured within 24 hours of life. At 8, 10 and 12 weeks of age, the offspring were submitted to overnight fasting glucose and insulin measures. At 12 weeks of age, a pool of offspring pancreatic islets was extracted and incubated in 4 different glucose concentrations (5.6; 8.3; 11.1 and 16.7 mM) and insulin secretion was evaluated. Results (mean ± SEM, n = 8/group)*:* Birth weight and neonatal plasma glucose were lower ($p < 0.05$) in offspring from LSD dams when compared with control group. No differences were found in fasting plasma glucose at 8, 10 and 12 weeks of age. Higher ($p < 0.05$) fasting plasma insulin was found in

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offspring from LSD maternal group. Finally, no differences were found in insulin secretion from pancreatic islets on a basal glucose concentration (5.6 mM). Nevertheless, lower ($p < 0.05$) insulin secretion was found in offspring pancreatic islets from LSD dams on 8.3, 11.1 and 16.7 glucose concentrations.
Conclusions: perinatal salt restriction resulted in low birth weight and modifications in carbohydrate metabolism, with beta-cell dysfunction, in adult offspring. Support: FAPESP.

1. Vidonho *et al.*, *Pediatr Res,* 56:842–848, 2004.

P-5A-195

Insulin resistance syndrome in 12 year-old children: small at birth and big at 12 years - The Pune Maternal Nutrition Study

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Objective: DOHaD theory proposes that discordance between intrauterine and post-natal growth predisposes to adult disease[1,2]. We demonstrated in the Pune Children Study (PCS) that urban Indian children born small but grown big were insulin resistant at 8y of age[3]. Some of the western studies have failed to find such an association[4]. We investigated these relationships in 12 year-old rural children in the Pune Maternal Nutrition Study (PMNS) which is a community based prospective study with high participation and follow up rates.
Methods: Seven hundred and seventy normal singleton babies were delivered in PMNS. We studied 638 full term births (338 boys and 300 girls). Weight was measured within 72 h of birth. Children are followed up every 6 months for growth measurements, and every 6 years for detailed anthropometry, body composition (DXA), fasting plasma glucose, insulin, lipids and blood pressure. We calculated HOMA-R by the online program. We studied the association of diabetes and cardiovascular risk factors at 12 years of age with birth weight and current weight adjusting for age, gender and socio-economic status. We followed the system of Lucas *et al*[5] for assessing the contribution of early life and later life factors. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: 90% of pregnant mothers participated in the PMNS and over 95% of live children were studied at 12 years of age. Mean birth weight was 2.7 kg; mean weight at 12 was 29.3 kg.These children were short (−1.1 sd for boys and −1.2 for girls) and thin (BMI) (−1.7 for boys, −1.8 for girls) by WHO standards. In the 'early model' birth weight predicted higher fat mass (DXA), in the 'late model' weight at 12y predicted all components, while in the 'combined model' low birth weight and high 12y weight predicted higher insulin resistance (HOMA-R), triglycerides, blood pressure and fat mass. Interaction term (birth weight × 12 year weight) was significant for all risk factors except HOMA-R.
Conclusions: This is the first prospective study to test DOHaD predictions in an Indian rural community undergoing rapid transition. The results replicate our findings in an urban study. Our results provide further evidence to highlight the importance of the antenatal period in the aetiology of diabetes and CVD, and reiterates the need to evaluate the policy of 'normalising' the growth of low birth weight children. The study was funded by Wellcome Trust, London, and the Medical Research Council (UK).

1. A. Lucas. Programming by early nutrition in man. Bock GR, Whelan J. ed. *Chichester: Wiley*, 38–55, 1991.
2. D.J.P. Barker, ed. London *BMJ Publishing*, 1992.
3. A. Bavadekar *et al.*, *Diabetes*, 48:2422–29, 1999.
4. R. Valdez *et al.*, *Diabetologia*, 37:624–31, 1994.
5. A. Lucas *et al.*, *BMJ.*, 319:245–49, 1999.

P-5A-196

Hypoglycemia aggravates brain injury in preterm infants with acidosis: a clinical multi-factor analysis

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Objective: To explore the effect of perinatal hypoglycemia on brain injury in preterm infants with acidosis ($pH < 7.30$).
Methods: The medical histories of a total of 153 preterm infants with acidosis were analyzed retrospectively. Short term brain injury included death induced by severe hypoxic ischemic encephalopathy (HIE) and moderate to severe HIE with or without seizures. Hypoglycemia was defined as an initial blood glucose ≤47 mg/dL.
Results: One hundred and sixteen preterm infants (75.8%) developed brain injury of different degree, of whom, 10 (8.6%) died of severe HIE and 106 (87.1%) had moderate to severe HIE. Seventy percent of the preterm infants had hypoglycemia, of which 102 (95.3%) vs. 14 (30.4%) of euglycemia preterm infants developed brain injury ($OR = 46.6$, $P < 0.01$). Cutoffs of birth weight, arterial blood pH, 5-min Apgar score and lactic acid analyzed using Receiver Operating Characteristic (ROC) curve were 1775 g, 7.15, 6 and 6.15 mM, respectively. The multiple factor logistic regression analysis showed that the risk factors of brain injury were as follows: blood glucose ≤47 mg/dL ($OR = 10.1$), arterial blood pH ≤ 7.185 ($OR = 21.4$), 5-min Apgar score ≤6 ($OR = 8.5$), intubation ± cardiopulmonary resuscitation (CPR, $OR = 14.5$), birth weight ≤1775 g ($OR = 13.4$) and lactic acid ≥6.15 mM ($OR = 7.4$). The regression equation was as follows: Logistic (brain injury) = −38.763 + 2.315 X 1 + 3.061 X 2 + 2.139 X 3 + 2.676 X 4 + 2.598 X 5 + 2.005 X 6 ($x^2 = 32.461$, $P < 0.001$).

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Conclusion: Perinatal hypoglycemia is an important risk factor of brain injury in preterm infant, much as in those with concomitant neonatal asphyxia and acidosis requiring CPR.

P-5A-197

Vagal hypoactivity is one expression of maternal protein restriction-induced metabolic programming

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Objective: Lactation is one phase very important to brain development, while fetal and puberty time is also crucial to central nervous system (CNS) development. Any injury during milk-producing stage can alter central metabolism control of offspring, even when they turn to adult life. It has been suggested that maternal protein restriction during lactation provokes impairment of parasympathetic activity in adult rats, as an expression of CNS altered function. Present work using direct electrical recording observe whether protein malnutrition during lactation induces changes on vagus nerve activity from adult rats.
Methods: During the first 2/3 of lactation Wistar rat mothers received a 4% protein diet (LP). Control group received normal diet (23% protein) (NP). After protein restriction, all mothers and pups received normal diet. At 81-days-old all animals from both experimental groups were anesthetized to isolate the superior vagus nerve branch and sympathetic ganglion cervical superior nerve branch. Using a bipolar electrode connected to a device that amplified a filtered electrical signal, 8–80 MHz, nerves activity was registered. Nerve firing rates were expressed by spike numbers during 5 seconds.
Results: Vagus firing rate was decreased in LP rats, 7.1 ± 0.8 spikes/5 sec, when compared to vagal activity from NP rats, 12.3 ± 0.7 spikes/5 sec, $p < 0.001$. Whereas, presenting increased activity of sympathetic nerve from LP animals, 13.9 ± 2.0 spikes/5 sec, compared to NP rats, 10.5 ± 1.5 spikes/5 sec, it did not found any significant statistical difference.
Conclusion: Perinatal protein malnutrition decreases parasympathetic activity, which can be a candidate mechanism underlying metabolic changes observed on adult rats.
Support: CNPq/CAPES/Fundação Araucária.

P-5A-198

Gender specific regulation of Pdx-1 and insulin gene expression in adulthood after exposure to protein restriction in early life at different windows of pancreatic development

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Restriction of protein (LP) during fetal and neonatal life has been shown in our laboratory to alter the normal development of the pancreas and resulting in the predisposition to glucose intolerance and striking gender differences in key insulin signaling molecules in skeletal muscle and adipose tissue in young adulthood (130 days) (Chamson-Reig A, *et al*, *unpublished*). During embryonic development of the pancreas, the endocrine cell lineage is controlled by a specific expression sequence of transcription factors, one of the most important being pancreatic duodenal homeobox 1 (Pdx-1) that regulates pancreatic development, beta cell differentiation and function. Studies had shown that insulin secretion and islet area, measured at 28 d of life was reduced in the LP diet treated animals. Moreover, the levels of Pdx-1 protein were decreased in the LP offspring rats at this age, without a change in the steady-state levels of Pdx-1 mRNA. Given that gender specific differences in glucose intolerance have been observed in later life (d130), we hypothesized that the expression of Pdx-1 and downstream Pdx-1 target genes may altered by nutritional challenges pre- and post-weaning.
Objective: To examine whether decrease in dietary protein at different times of pancreatic development is involved in the overall gene expression of Pdx-1 and insulin in adulthood (d130) that lead to the observed glucose intolerance.
Methods: Wistar pregnant rats treated with C (20%) or LP (8%) protein diet were separated to four groups: Control (C) all life, LP-1 all life, LP-2 at weaning the diet was changed to C and LP-3 changed the diet to C at birth. Five to seven different litters were used per each group. Offspring from each mother, male and female, were sacrificed at d130, the pancreata dissected and RNA extracted and analyzed by quantitative real time PCR for the expression of PDX-1 and insulin. The data obtained was normalized to β-actin.
Results: In males, pancreata at d130, both PDX-1 and insulin expression was decreased in the offspring of LP-1 and LP-3 dietary groups. On the other hand, PDX-1 and insulin expression was unaltered in the LP-2 offspring. This is of great interest considering that our previous studies have demonstrated that these animals LP-2 were becoming insulin resistant in peripheral tissues. In the female offspring the expression of Pdx-1 or insulin was unaltered by any dietary regime.
Conclusions: Collectively, this data illustrates the importance to further understand the molecular basis of fetal programming between genders and the timing of nutritional challenges insults. Furthermore, it will give us new insights of the role of transcription factors involved in the maintenance and function of beta cells that will allows the use of new specific drugs to target prenatal prevention of adult diseases.

P-5A-199

The effect of stage of gestation on feeding high-protein on hepatic glucocorticoid sensitivity in the neonate and later metabolic homeostasis in the young adult offspring

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Objectives: Altering maternal nutrition affects fetal development, potentially predisposing offspring to later metabolic disease. Glucocorticoid (GC) excess has been linked to the clinical observations associated with the metabolic syndrome. Tissue sensitivity to cortisol is regulated, in part, by GR and 11βhsd1 and 2. Several studies have shown the effects of maternal nutrition on the programming of GC action in the offspring[1,2]. The hypothesis is that feeding a high protein diet during gestation alters development of GC sensitivity.

Methods: Pregnant sows were fed one of four isoenergetic diets (Table) that differed in composition and quantity fed during the first and final trimester of gestation. Sows fed on a low–high feeding scheme were fed 2.7 kg/d between day 0 and day 40 of gestation and 3.5 kg/d between day 70 and day 110 of gestation. The dietary regimes were reversed for sows fed on a high–low feeding scheme. At 7 days and 6 months, one median piglet per litter were selected, humanely euthanized, a blood sample taken and its liver sampled. GR and 11βhsd1 and 2 mRNA expression was quantified by real-time PCR (Values are presented as arbitrary units). Plasma concentrations of glucose and non-esterified fatty acids (NEFA) were assessed using enzymatic-colorimetric assays. Offspring that were not selected for tissue sampling were raised commercially and body composition was recorded at the slaughterhouse.

	C (n = 8)	HP(L-H) (n = 8)	C(H-L) (n = 8)	HP(H-L) (n = 8)
Diet description	High starch	High protein	High starch	High protein
Feeding scheme	Low-high	Low-high	High-low	High-low

Results: Piglets born to sows fed high protein had increased ($p < 0.05$) liver expression of GR and 11βhsd1 at 1 week. At 6 months of age these piglets born to sows with reduced feed intake in late gestation had decreased glucose and increased NEFA plasma concentrations and diameter of the longissimus dorsi muscle (table).

	C	HP(L-H)	C(H-L)	HP(H-L)
GR/18S (arbitrary units)	0.57 ± 0.11^{a}	1.05 ± 0.19^{b}	0.83 ± 0.18	1.70 ± 0.44^{b}
11βHSD-1/18S (arbitrary units)	2.47 ± 0.76^{a}	6.53 ± 1.44^{b}	4.74 ± 1.05^{a}	12.12 ± 2.20^{b}
Glucose (mmol/l)	4.71 ± 0.41^{a}	4.40 ± 0.61	4.55 ± 0.23	3.75 ± 0.16^{b}
NEFA (mmol/l)	0.93 ± 0.15^{a}	1.04 ± 0.19	1.17 ± 0.14	1.41 ± 0.07^{b}
Muscle diameter (mm)	56.35 ± 0.84^{a}	58.30 ± 0.89	58.99 ± 0.94^{b}	61.79 ± 1.15^{b}

All results are expressed as means ± SEM. Different letters denote significant differences ($P < 0.05$) between groups (Mann-Whitney U test).

Conclusions: Feeding a high-protein diet during pregnancy programs increased hepatic cortisol sensitivity in the newborn. Surprisingly this was not associated with any major long term adverse metabolic outcomes. Indeed, it appeared to improve glucose tolerance, an adaptation possibly mediated by increased muscle growth. Studies are currently in progress to further investigate the potential effects on insulin responsiveness together with liver and muscle metabolism.

1. M.G. Gnanaligham *et al.*, *Am J Phys.,* 289: 1407–1415, 2005.
2. C.B. Whorwood *et al.*, *Endocrinology*., 142: 2854–2864, 2001.

P-5A-200

Glucose tolerance near term and offspring birth weight; effect of high-fat feeding during pregnancy

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Diabetes during pregnancy in humans has been linked to adverse effects on the offspring such as fetal macrosomia, and impaired glucose tolerance and obesity in later life[1]. Previous studies in pigs have shown that high fat feeding during late gestation can reduce glucose tolerance at term[2], but the long term effects on the offspring are not known.

Objective: This study examines whether increased fat intake during pregnancy influences glucose tolerance near to term and offspring birth weight. The hypothesis is that increased fat intake will decrease glucose tolerance without effecting birth weight.

Methods: Pregnant sows were fed one of four isoenergetic diets (Table). Sows fed on a low–high feeding scheme were fed 2.7 kg/d between day 0 and day 40 of gestation and 3.5 kg/d between day 70 and day 110 of gestation. The dietary regimes were reversed for sows fed on a high–low feeding scheme. On day 108 of gestation glucose (0.5 g/kg; i.v.) was administered, via a catheter inserted into the sows ear, and regular blood samples were taken for 1 hour. Blood from each time point was tested using a glucometer, in order to determine the rate of glucose clearance.

	C (n = 8)	HF(L-H) (n = 8)	C(H-L) (n = 8)	HF(H-L) (n = 8)
Diet description	High starch	High fat	High starch	High fat
Feeding scheme	Low-high	Low-high	High-low	High-low

Results: Gestational diet had no effect on basal glucose concentrations, but resulted in glucose intolerance in mothers, irrespective of the timing of the additional fat. Birth weight was unaffected by gestational diet or sow glucose tolerance.

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	C	HF(L-H)	C(H-L)	HF(H-L)
Basal glucose conc. (mmol/l)	4.14 ± 0.17	4.6 ± 0.09	4.13 ± 0.12	4.06 ± 0.13
Glucose AUC (mmol min^{-1})	112.75 ± 6.53^{a}	140.80 ± 3.65^{b}	134.57 ± 10.83^{a}	165.88 ± 11.47^{b}
Mean birth weight of offspring (kg)	1.20 ± 0.06	1.28 ± 0.06	1.23 ± 0.09	1.21 ± 0.06

All results are expressed as means ± SEM. Different letters denote values that are significantly different from each other (Mann-Whitney U test).

Conclusions: Feeding a high fat diet throughout gestation impairs glucose tolerance without any immediate effects on birth weight. The prospective findings from this ongoing study will inform further on the long-term effects that impaired maternal glucose tolerance may have on her offspring.

1. D. Dabelea *et al.*, *J Matern Fetal Med.,* 9:83–88, 2000.
2. C. M. C. van der Peet-Schwering., *J Anim Sci.,* 82: 2964–2971, 2004.

P-5A-201

The waist-to-height ratio as a predictor of cardio metabolic risk in children

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Objective: The increasing prevalence of the metabolic syndrome (MS) in children and adolescents is worrisome. Studies of anthropometric markers which may simply and accurately predict MS in children and adolescents are needed. We planned to compare body mass index (BMI) and central obesity, as determined by waist to height ratio (WHR), as predictors of MS in Chilean children.
Method: A prevalence study of 618 schoolchildren from middle and low social economic strata, all from the urban area of Santiago, was organized including more obese children than the general population. We determined weight, height, waist circumference, systolic and diastolic blood pressure and fasting lipids and glycaemia. Diagnosis of MS was based on the presence of ⩾ 3 Cook criteria[1] (waist ⩾ p90; arterial hypertension ⩾ p90[2]; Col HDL ⩽ 40 mg/dl; Triglycerides ⩾ 110 mg/dl, and Glycaemia ⩾100 mg/dl). MS was modelled using WHR and z score BMI, through logistic regression. ROC curves were used to compare zBMI and WHR as predictors of MS.
Results: This selected sample had a mean age of 11 ± 2 years, 52% were females, and nutritional status as determined by BMI had the following distribution: 190 eutrophic, 174 overweight, and 254 obese. The prevalence of MS in the sample was 15%. Mean zBMI was +1.22 ± 0.90 and for WHR 0.52 ± 0.07, indicating an over-representation of obese individuals in this study. The ROC curves determined that the cut-off points and their diagnostic abilities were the following:

	Cut-off point SM	Sensitivity (%)	Specificity (%)	Correctly classified (%)	LR +
WHR	0.55	71.6	70.2	72	2.5
zBMI	1.76	71	74	73	2.8

LR: Likelihood ratio

Conclusions: Both WHR and zBMI similarly predicted cardio metabolic risk expressed as MS in children and adolescents from Santiago. The greater facility for registering and calculating its value makes WHR a preferable tool for defining cardio metabolic risk in this group. Similar recent studies performed in adults have shown that indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI[3]. The prognostic ability of the measurements used with children and adolescents may be further assessed using more accurate estimations of cardio metabolic risk, such as the carotid intima-media thickness[4].

1. S. Cook *et al.* 1988–1994. *Arch Pediatr Adolesc Med.,* 157: 821–7, 2003.
2. J. Fernández *et al. J Pediatr.,* 145:439–44, 2004.
3. C.M. Ying Lee *et al. J Clin Epidemiol.,* 61:646–653, 2008.
4. N. Mattsson *et al. Ann Med.*, 40:542–52, 2008.

P-5A-202

Low protein diet during gestation alters long chain polyunsaturated fatty acid synthesis in rat mammary gland

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Objective: Long chain polyunsaturated fatty acid (LCPUFA) synthesis essential for normal neonatal development of neonate are synthesized in the lactating mammary gland by desaturase (Δ5D and Δ6D) and elongase (Elvol 5) enzymes. Docosahexaenoic (DHA) and Arachidonic (AA) acids are important in neonatal brain and retinal development. We evaluated effects of low maternal protein intake on expression of these enzymes in the mammary gland.
Methods: Pregnant rats were assigned to control (C - 20% casein; n = 6) or a restricted (R −10% casein; n = 6) isocaloric diet in pregnancy (P). At 19 days of P, mothers were weighed, the mammary gland removed and immediately frozen in liquid nitrogen. Following extraction (Folch procedure), DHA and AA were measured by gas chromatography. mRNA was

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measured by RT real time-PCR Expressed relative to control group. Data are Mean ± SEM and analysis was by Student's T-test with the Bonferroni correction. Significance set at $P < 0.05$.

Results: Maternal body weight was not different between groups. Total mammary gland fat ($C = 75.8 \pm 1.4$ vs $R = 69.1 \pm 2.2^*$ g.100 g^{-1}, $p < 0.05$) and relative expression of Δ5D, Δ6D and Elovl 5 mRNA and DHA and AA (Fig 1) were decreased in R vs C.

Conclusion: The mammary gland is the major site of synthesis of milk LCPUFA. Low dietary protein decreases mammary gland fat production. Decreased LCPUFA will adversely affect brain and retinal development with potential consequences for cognitive and visual function in later life.

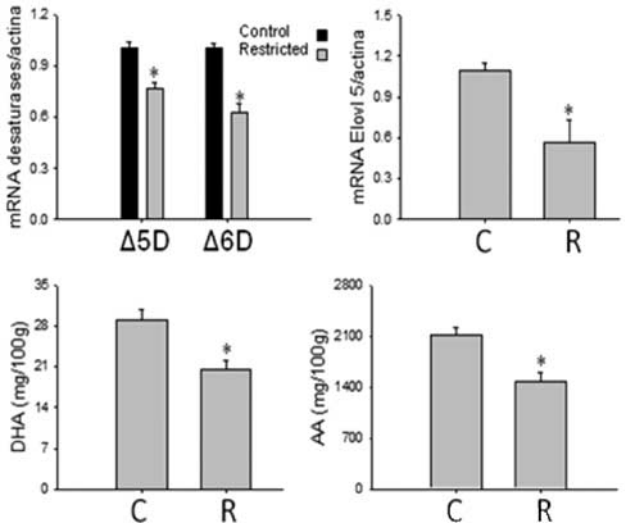

Fig. 1 Mammary gland mRNA Δ5D and Δ6D and Elovl 5/mRNA actina normalized ratio and DHA and AA concentration at 19 day of P. Mean ± SEM, $p < 0.05$, $n = 5$. * vs C, T-test.

P-5A-203

Effects of fish oil intake in the diet in mice subjected to perinatal protein restriction

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Objective: This study aimed to verify the effects of the experimental diets (with or without fish oil) in offspring that received perinatal protein restriction on quantitative and morphological parameters of the liver and adipose tissue.

Methods: Swiss female mice were divided into two groups: normal protein (NP-19% protein) and low protein (LP-5% protein). Both diets followed AIN93-G dietary allowances[1]. At 21 days, male offspring were divided into 4 groups, according to the post-weaning diet (SC – standard chow or FO – fish oil rich diet): a) NPSC; b) NPFO; c) LPSC; d) LPFO. Body mass was verified weekly until 4 mo-old, when euthanasia occurred. Fat deposits (retroperitoneal fat and epididymal fat masses) were removed and weighed. Liver was also removed and had its volume measured by Scherle's method[2]. For statistical analysis, ANOVA and post-hoc test of Tukey were used ($p < 0.05$). Appropriate Institutional Ethics Committee clearance was obtained.

Results: LP diet yielded low birth weight in the LP group in comparison with NP group (−17%, $p < 0.0001$). At the end of the experiment, NPFO and LPFO groups showed lower body mass compared to their respective controls NPSC and LPSC (−25%, $p < 0.001$; −25%, $p < 0.001$). Considering the amount of epididymal and retroperitoneal fat pads, the LPSC group showed a grater values when compared to NPSC group (+31%, +97%; respectively, $p < 0.05$). Furthermore, the fish oil rich diet was capable of reducing significantly the amount of fat pads in LPFO group compared to LPSC group (−54%, $p < 0.001$). The stereological analysis revealed that LPSC group showed more macro and microvesicular steatosis in the liver compared to NPSC group that had a well-organized liver structure (+80%, $p < 0.05$). On the order hand, restricted animals that received fish oil diet showed reduction of volume density steatosis in comparison with LPSC group (−50%, $p < 0.05$). Besides, fish oil diet lowered hepatocyte binucleation in LPFO group compared to LPSC group (−41%, $p < 0.0001$). As for adipose tissue alterations, maternal protein restriction yielded bigger adipocytes in LPSC than NPSC group (+17%, $p < 0.001$). On the other hand, both NPFO and LPFO groups had a smaller adipocytes compared to their respective controls groups (−19%, $p < 0.001$; −19% $p < 0.0001$, respectively).

Conclusions: This study demonstrates that perinatal protein restriction yields hepatic and adipose tissue structural alterations. However, fish oil rich diet was able to lower hepatocyte binucleation and hepatic steatosis as well as sectional area of adipocytes. Support: Embrapa Caprinos.

1. P.G. Reeves, *et al. J.Nutr,* 123:1939–1951, 1993.
2. W. Scherle. *Mikroskopie*, 26:57–60, 1970.

P-5A-204

Gastrointestinal changes in adult rats provoked by dam food restriction during gestation through lactation

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Many organs and functions have been studied in relation to maternal programming, but there are few studies on digestive function. However, changes in gastrointestinal function may contribute, for example, to the programmed obesity observed in adult offspring that are food-restricted in uterine[1]

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or food-restricted in post-natal life[2]. Our hypothesis is that the gastrointestinal tract of adult offspring can develop morphophysiological alterations as a consequence of undernutrition during early development. Since rats are altricial species whose developmental period extends after birth[2] we decided to include the nursing period in our investigation.

Objective: Our objective was to investigate the effects of dams 30% food restriction during pregnancy and lactation on intestinal dissacharidases, food efficiency ratio and weight gain in adult offspring.

Methods: Rat pups were divided in two groups (n = 6 in each group) according to maternal diet during gestation through lactation: C = pups born to ad libitum dams and R = pups born to 30% restricted dams. After weaning and until 60 days of age, both groups were fed ad libitum diet. Lactase, sucrase and maltase activities, weight gain and food efficiency ratio were determined at 21 and at 63 days of age. Disaccharidase activity was determined according to the DAHLQVIST method[3]. The food efficiency ratio in first and last post-weaning weeks was calculated with the following formula: body weight gain [grams]/food intake [grams]) $\times 10^2$. The results were expressed as mean $\pm$ standard deviation of the mean. The comparison among groups was achieved using the Student t-test for two independent samples. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: On Day 1 after birth, the body weight of the R group was lower ($P < 0.001$) than that of the C group (5.11 ± 0.3 vs. 6.24 ± 0.8 g, respectively). The difference between groups was larger at weaning, when the weight averages were 28.78 ± 5.6 g and 52.14 ± 8.6 g, respectively. The weight gain between weaning to 63 days of age was higher ($P < 0.05$) for pups born to restricted dams. Rats born to food-restricted mothers showed higher food efficiency ($P < 0.05$) than rats born to ad libitum mothers at both early post-weaning (from 21 to 28 days) and adult (from 56 to 63 days) ages. At 21 days of age, the R group had higher ($P < 0.05$) intestinal lactase activity than the C group. There were no differences among weaned groups for sucrase and maltase activities ($P > 0.05$). At 63 days of age, the R group had higher maltase activity ($P < 0.05$) than the C group. Sucrase activity was not altered in either group ($P > 0.05$).

Conclusion: Maternal undernutrition during pregnancy and through lactation affects the gastrointestinal function of adult offspring, indicating programming of the gastrointestinal tract in the early stages of development.

1. H. Desai *et al. Reprod Sci,*15:785–796, 2008.
2. M.G. Ross, M. Desai. *Am J Physiol,* 288:R25–R33, 2005.
3. A. Dalquist. *Scand J Clin Lab Invest*, 44:69–72, 1964.

P-5A-205

The impact of omega-3 fatty acid supplementation on C57BL/6J male mice subjected to antenatal diet restriction

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An adverse antenatal environment is associated with fetal growth restriction and the subsequent development of the metabolic syndrome (characterized by obesity, atherogenic dyslipidemia, hypertension, and insulin resistance). We have previously shown that imposing an antenatal diet restriction on C57BL/6J mice leads to the development of symptoms of the metabolic syndrome in male offspring.

Objective: To determine whether a postnatal diet rich in omega-3 fatty acids will reduce the likelihood of the development of glucose intolerance, insulin resistance and obesity in C57BL/6J (B6) male mice subjected to antenatal dietary restriction.

Methods: Pregnant female mice were either treated as controls (C) and fed ad libitum for the duration of pregnancy, or subjected to a 30% caloric restriction (R) from day 6.5 to 18.5 of gestation. Post-weaning, male pups were fed either the control diet ($\sim$ 1% of dietary fat content omega-3 fatty acids) or an enriched omega-3 diet (n3, $\sim$ 35% of dietary fat content omega-3 fatty acids). Glucose tolerance testing was conducted at 3 and 12 months of age; serum insulin was measured using a commercially available kit. At 12 months of age, body composition was measured by Dual-energy X-ray absorptiometry (DEXA).

Results: In response to the postnatal n3 diet, at 3 months of age, B6R/n3 mice had significantly improved insulin resistance compared to B6R/C (Insulin AUC −43.9%, $p = 0.0065$); however, at 12 months of age this improvement was no longer present (Insulin AUC −20.8%, $p = 0.0871$). At 3 months of age, B6C/n3 had unchanged insulin resistance compared with B6C/C (Insulin AUC −7.3%, $p = 0.4295$); whereas at 12 months of age insulin resistance was significantly improved (B6C/n3 vs. B6C/C: Glucose AUC −18.9%, $p = 0.0254$; Insulin AUC −13.8%, $p = 0.0369$). DEXA revealed the B6R/n3 mice to have a lower percentage of body fat compared to B6R/C, while there was no difference between B6C/n3 and B6C/C (B6R/n3 vs. B6R/C: −36.9%, $p = 0.0006$; B6C/n3 vs. B6C/C: −3.5%, $p = 0.70$). B6 mice fed n3 diet had significantly lower body weights than their C counterparts (B6C/n3 vs. B6C/C: AUC −25.4%, $p = 0.0001$; B6R/n3 vs. B6R/C: AUC −13.7%, $p = 0.0155$). Although B6C/n3 mice had improved tolerance compared to control mice (B6C/C) they had a lower growth trajectory.

Conclusions: A postnatal diet enriched in n3 diet reduces the likelihood of development of symptoms associated with the metabolic syndrome in B6 male mice subjected to antenatal dietary restriction; however, it also appears to impair postnatal growth in offspring exposed to a normal intrauterine environment. Further studies are required to determine the risks and benefits of n-3 supplementation including the optimum timing and dose of supplementation.

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P-5A-206

Endocrine pancreas malfunction caused by early protein restriction may be related to changes on beta cell muscarinic receptors

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Objective: Maternal protein restriction is a nutritional disorder that cause pancreatic malfunction and it allow hypoinsulinemia in adult life, as shown in animal models such as in rats. Fetal and lactation phase are very fragile, any perturbation like nutritional restriction may lead changes on central nervous system development (CNS). Central control of insulin secretion process is altered in adult rats that were fed by protein malnourished mothers. Pancreatic beta cells respond poorly to parasympathetic stimulation. Acetylcholine release by neural ends is bound to muscarinic receptors (mAChR) which potentiate glucose-induced insulin secretion; however, it has been shown that mAChR has a subfamily with 5 subtypes. It has some evidence that mAChRM odd numbers are insulinotropic while even ones inhibited insulin secretion. Present work was designed to test the hypothesis that mAChR2 from beta cell has function to inhibit insulin secretion.
Methods: Milk-giving Wistar rats received a poor protein diet (4%) during initial 2/3 of lactation (LP group), while, control animals (NP) received normal protein diet (23%). Mothers and pups, from both groups, ate normal protein diet at the end of lactation until young rats turn to 81-days-old. Animals were submitted to intravenous glucose tolerance test (ivGTT) receiving a glucose load of 1 g/Kg body weight (bw). Another batch of rats, from both groups before ivGTT were pre-treated with acetylcholine, dose of 27 nM/Kg bw or with a mAChR2 agonist, metoctramine, dose of 0.002 nM/Kg bw.
Results: Fasting hypoinsulinemia was showed by LP rats (0.102 ± 0.015 ng/ml) when compared with NP rats, 0.170 ± 0.016 ng/ml, $p < 0.05$. Calculated the area under the curve of ivGTT shows that LP rats are glucose intolerants (8337 mg/dl/60 min) when compared to NP rats, 7082 ± 203 mg/dl/60 min, $p < 0.01$. Acetylcholine pretreatment caused 16.4% glycemic reduction in NP rats; whereas, same treatment on LP animals provoked 33.8% glycemic decrease, $p < 0.01$. As it expected metoctramine induced decline of plasma glucose concentration during ivGTT in both animal groups; however, the decrease was deeper to LP rats (24.5%) than NP ones (16.1%), $p < 0.001$.
Conclusions: Perinatal protein restriction causes changes on activity and/or number of muscarinic receptors subfamily of pancreatic beta cell from adult rats, including a strong inhibition of mAChR2, which may justify the weak cholinergic insulinotropic response. Support: CNPq/CAPES/Fundação Araucária.

P-5A-207

Presence of early risk markers of metabolic syndrome in prepubertal children with a history of intrauterine growth retardation

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Studies on people with low weight at birth found metabolic syndrome associated with intrauterine growth retardation (IUGR).
Objective: To study the presence of early risk markers of metabolic syndrome in a prepubertal population with IUGR.
Methods: We studied 45 prepubertal children with a history of IUGR, without apparent disease, and 47 children control group. BMI, weight, height and BMI Z score, and body fat mass were calculated. Basal glycemia, insulin, proinsulin, cortisol, serum lipids and uric acid levels were analyzed. Insulin sensitivity was calculated by QUICKI and insulin resistance by HOMAIR.
Results: Basal insulin levels were higher in the IUGR group compared with the controls (6.6 μU/ml vs. 4.4 μU/ml, p 0.008). Similar results were found for the basal cortisol levels (18.8 ug/dl vs. 13.1 ug/dl, p 0.006) and uric acid (4.2 mg/dl vs. 2.7 mg/dl, p 0.0008). QUICKI index was lower in the IUGR group (2.06 vs. 2.86, p 0.001). The IUGR children who developed obesity presented higher levels of proinsulin (fig 1)(26.04 ug/dl vs. 13.3 ug/dl, p 0.05), insulin (11 μU/ml vs. 5.5 μU/ml, p 0.005), and HOMA (2.06 vs. 0.9, p 0.004), and lower QUICKI (1.71 vs. 2.16, p 0.01) than in the case of the IUGR children with appropriate weight; these differences weren't observed among the control group.
Conclusions: Children with IUGR, without apparent disease, showed metabolic changes that were expressed through risk markers of metabolic syndrome in childhood. In this population, the subgroup of obese children presented higher levels of insulin and proinsulin.

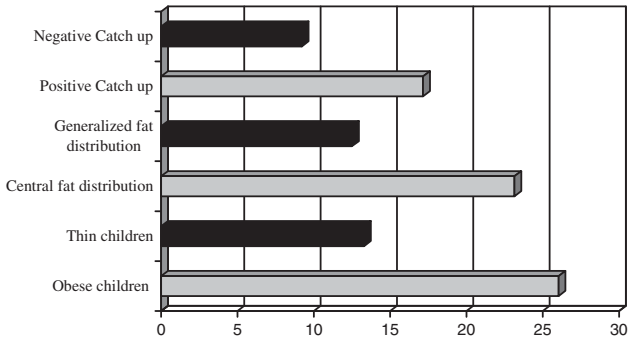

Fig. 1. Proinsulin in IUGR by obesity, fat distribution and catch up.

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P-5A-208

Metabolic imprinting induced by perinatal protein restriction impaired cholinergic insulinotropic, while did not alter adrenergic response on pancreatic beta cell from adult rat

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Objective: Nutritional injuries impose during perinatal phase provokes changes on glycemic controls which allows metabolic diseases onset in adult life. It has been suggested that autonomic nervous system imbalance is evolved on altered glucose metabolism of adult rat that was protein malnourished during lactation. The aim of present work is to know how is the cholinergic and adrenergic effect on glucose-induced insulin secretion of pancreatic islets isolated from adult rats that were protein malnourished during perinatal life.

Methods: Wistar rats received a protein restricted diet (4%) during initial 2/3 of lactation, LP group; while, control animals (NP) ate normal protein diet (23%). At the end of lactation, mothers and pups, from both experimental groups received normal protein diet. Until sacrificed NP and LP rats, aged 81-days-old ate normal diet. Islets were isolated from rat pancreas using collagenase technique. Islets were perifused with a solutions containing 8.3 mM glucose in the presence or in the absence of 1.0 mM carbamylcholine. To test the adrenergic response, islets were perifused with 16.7 mM in the presence or in the absence of 0.1 μM adrenaline.

Results: Calculating the area under the perifusion curve, LP islets show 60% insulin secretion reduction, 21.3 ± 1.8 ng/ml, when compared to NP islets secretion stimulated by 8.3 mM glucose, 53.9 ± 2.5 ng/ml, 0.001. Carbamylcholine potentiated secretion in both islet types; however, islets from LP rats secreted less, 182.6 ± 27.4 ng/ml, than islets from NP ones, 693.2 ± 121.0 ng/ml, $p < 0.01$. Glucose 16.7 also better stimulated more insulin secretion in perifused islets from NP rats, 220.8 ± 11.3 ng/ml, than islets from LP rats, 122.7 ± 8.5 ng/ml, $p < 0.01$. Adding adrenaline to the perifuse solution, it caused drastic reduction of the same magnitude in insulin secretion from both islet origin: 81.3 ± 8.8 ng/ml to NP and 58.4 ± 3.7 ng/ml to LP rats, $p > 0.05$.

Conclusions: Maternal protein malnourishment provokes metabolic imprinting in adult rats, including low insulin secretion, which may due to an impairment of parasympathetic activity on pancreatic beta cells. Support: CNPq/CAPES/Fundação Araucária.

P-5A-209

Altered adipocyte size regulation without obesity in juvenile microswine offspring following maternal protein restriction

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Objectives: Perinatal maternal protein restriction (MPR) in microswine results in low birth weight and accelerated growth during prepubertal development, together with increased appetite and feed utilization efficiency, and increased fat deposition rate without overt obesity (as % body fat). We hypothesized 1) that the increase in fat deposition would be associated with adipocyte hypertrophy, which has been previously linked with cardiovascular and metabolic disease; and 2) that preventing accelerated growth by feed limitation to normal levels would prevent adipocyte hypertrophy.

Methods: Time-bred microswine sows were randomized to either normal- (14%) or low- (1%) protein diets for the last quarter of gestation plus 2 wks postnatally. Upon weaning at 4wks of age, Normal Protein Offspring (NPO $n = 14–26$) and Low Protein Offspring (LPO $n = 21–22$) were randomized to either ad libitum (AL) or feed limitation (FL) groups. FL piglets were limited to g feed/kg body weight equivalent to the average consumed spontaneously by NPO AL piglets. Body composition was measured by dual energy X-ray absorptiometry (DEXA) scanning at 6 and 11 wks of age. Adipose tissue (subcutaneous, SAT; and intra-abdominal, IAT) was collected from juvenile offspring (12–20 wks) in an age-matched manner for analysis of adipocyte size. Adipocyte size was measured in formalin fixed tissue sections stained with hematoxylin using image analysis of 4 micrographs/slide, 2 slides/block, 2 blocks/fat depot/animal, and reported as cross-sectional area in um^2. In IAT, age was used as a covariate in analysis.

Results: Feed limitation restored normal growth rates in LPO-FL, such that LPO piglets born small remained relatively small throughout prepubertal development. Excess accrual rates of total body fat ($p < .005$) and lean mass ($p = .02$) in LPO-AL were normalized by FL. Adipocyte size was directly age-dependent in IAT, but not in SAT. In all pigs, females had 37% larger adipocytes than males in both depots ($p = .0005$). Adipocyte hypertrophy was observed in SAT of female LPO (4029 ± 240 um^2 in LPO vs 2994 ± 236 um^2 in female NPO, $p < .02$), but not in males. No adipocyte hypertrophy was observed in IAT.

Conclusions: SAT adipocyte hypertrophy was observed in juvenile female microswine offspring after MPR and was not modified by feed limitation to normalize growth rate. This suggests that SAT adipocyte hypertrophy may be programmed during perinatal development (late prenatal/early postnatal), and occurs independently of accelerated postnatal growth. In contrast, there was no IAT adipocyte hypertrophy. Our data show that accelerated

postnatal growth may occur without obesity; perinatal (vs early gestational) timing of the insult may account for differences in development of obesity. However, even without obesity, adipose tissue may be programmed in sex- and depot-specific ways that contribute to metabolic or cardiovascular disease.

P-5A-210

Gestational protein restriction programs the mammalian amino acid response pathway in male offspring rats

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Objective: The mammalian amino acid response (AAR) pathway senses and reacts to protein or amino acid limitation. This signaling cascade is initiated by the GCN2 kinase detection of reduction of essential amino acids. Subsequently the AAR pathway is activated by phosphorylation of the eukaryotic translation initiation factor 2 alpha subunit (eIF2alpha) and translational induction of the transcription factor ATF4 protein. The complete activation of the AAR pathway induces up-regulation of downstream target genes containing the amino acid response element (AARE) sequences. Activation of the mitogen-activated protein kinase (MAPK/ERK) pathway is also required in the complete activation of the AAR pathway for the up-regulation of downstream target genes. The present study is designed to investigate the effects of gestational and lifelong protein restriction on activation of the AAR pathway in male rat offspring.
Methods: Sprague-Dawley (SD) offspring rats were exposed to a low protein diet (LP, 90 g/kg casein compare to 180 g/kg in control) either through gestation only or throughout life. Rats in control group were fed control rat chow throughout life. The livers of offspring rats were collected and used to isolate RNA, protein and chromatin. Quantitative real-time PCR, Western blot and chromatin immunoprecipitation were performed for the analysis of the AAR and MAPK pathways.
Results: The present study compared the effects of low protein diet during gestation only or lifelong on the AAR and MAPK/ERK pathways in offspring rat liver. Our results demonstrated that the low protein diet fed either during gestation only or lifelong was able to induce phosphorylation of eIF2alpha and ATF4 protein synthesis in the liver of male offspring rats. However, in rats fed only gestational low protein diet, the MAPK/ERK pathway was not activated, as revealed by no detectable changes on ERK phosphorylation. Consequently, there were no changes in mRNA expression of the downstream target genes in the AAR pathway, such as ASNS (asparagine synthetase), CHOP (C/EBP homology protein) and SNAT2 (sodium-coupled neutral amino acid transporter 2). In rats fed low protein diet throughout their life, the target genes of AAR pathway were up-regulated and phosphorylation of ERK was enhanced. Analysis of ASNS also demonstrated that, correlated to the changes, chromatin immunoprecipitation showed increased binding of ATF4 at the promoter region of the above-mentioned AAR genes. Histones H3 and H4 were also specifically modified at the promoter region.
Conclusions: Maternal protein restriction programmed the activation of the AAR pathway, inducing phosphorylation of eIF2alpha and ATF4 protein synthesis in the liver of male offspring rats. On the other hand, the complete activation of target-genes downstream of the AAR pathway depended upon the further activation of the MAPK/ERK pathway.

P-5A-211

Glucocorticoid programming: prenatal or perinatal?

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Objective: Previously we have shown high sensitivity of neonatal rat pituitary cells and fetal rat hepatocytes in primary cultures to glucocorticoids and other bioregulators, as evaluated by the production of growth hormone and serum albumin, as well as the biosynthesis of macromolecules (DNA, total RNA and protein). Later we have observed irreversible or partially reversible body and organ growth inhibition by glucocorticoids administered to rats in neonatal period[1–4]. Recently, we suggested that such effects could be related to glucocorticoid programming/imprinting phenomena[5]. In the present work, literature data were analysed, in order to establish the correspondence of experimental models in rats to human patients.
Methods: Computer-assisted search was performed in several databases in English, using the key words ''glucocorticoid'', ''programming'', ''growth'', ''development'', ''aging' and some others, during the period of approximately 30–40 years. This procedure was completed by manual search in reference lists of the articles localized.
Results: Although the group of Seckl *et al.* concerns almost exclusively prenatal glucocorticoid programming, the commonly expressed opinion establishes a correspondence of neonatal period in rats to third trimester in human fetus. Therefore, employing neonatal rats in experimental models results in preferable use of a term ''perinatal'', rather than ''prenatal'' for the period of glucocorticoid treatment. Moreover, considering the difference in degree of tissue maturation, neonatal rats correspond probably to preterm human newborns.
Conclusion: In fact, synthetic glucocorticoids like dexamethasone are actively transported by P-glycoprotein out of adult rat brain, however in neonatal period such mechanism is not fully operative. Therefore, special care should be taken when administering glucocorticoids to preterm human newborns. On the other hand, it seems to us that rats are better suited for developmental modelling, whereas mice would be preferably employed in geriatric models, principally due to

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convenience and cost. Finally, sequential use of *in vivo* and *in vitro* approaches may be highly advantageous for detailed understanding of tissue-specific mechanisms of programming/imprinting phenomena in experimental models.

1. V.I. Goudochnikow, *et al.*, *Neuroendocrinology*, 60:59, 1994.
2. V.I. Goudochnikow, *et al.*, *Medicina* (Buenos Aires), 56:613–614, 1996.
3. V.I. Goudochnikow,. *NewsLab* (São Paulo), 22:90–100, 1997.
4. V. Cassol, *et al. Eur. J. Pharm. Sci.*, 13: S76, 2001.
5. V.I. Goudochnikow, *Adv. Gerontol.* (St. Petersburg), 20:35–36, 2007.

P-5A-212

Prenatal high-fat diets induce hepatic steatosis in rat offspring, which is fully reversible and do not induce liver ceramide accumulation

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Objective: The objectives of present study were: 1) To determine if hepatic steatosis in rat-pups, induced by high-fat feeding of the dams during pregnancy and lactation leads to increased accumulation of ceramide in the young rat-liver and if the steatotic state is reversible after transfer to low-fat diets after weaning.
Methods: Female Sprague-Dawley rats were fed ad libitum one of two experimental diets, for 10 days before pregnancy, during pregnancy and during suckling. The diets were chow containing 5% fat (w/w) (C) or chow with added palm oil (total fat content 23% (w/w)) (HF diet). At 48 hours postpartum, litters were reduced to 8 pups. Twenty days *post partum,* one male pup from each litter was sacrificed. The remaining male pups from each litter were split into 2 groups after weaning (21days *post partum*). Half of the offspring from dams fed the control diet continued on this diet (C→C) while the other half were fed the HF diet (C→HF). The offspring from the dams fed the HF diet was splitted in the same way (HF→C and HF→HF). After 70 days *post partum*, one male offspring from each litter were again sacrificed. Food intake and weight gain were analyzed daily and lipids were analyzed with standard methods.
Results: Total energy intake was identical between the groups, both in the dams pre-weaning and in the pups post-weaning. At 20 days of age, pups from the HF mother suffered from severe hepatic steatosis with TAG concentration of 43 ± 11 mg/g compared to 12 ± 5.2 mg/g in the C-group ($p < 0.0001$). Despite the very high liver TAG levels in the HF-group, there was no difference in the ceramide content of the livers; average ceramide levels being approximately 90 nmol/g. However, at 70 days of age (after 50 days on the post-weaning diets), the pups from the HF mothers that were given the control diet post-weaning had as low TAG levels as the C→C pups (6.6 ± 1.5 and 6.2 ± 1.3 mg/g, respectively). On the other hand, pups raised by dams on the control diet, that had been switched to the high fat diet (C→HF) had increased TAG levels as had the HF→HF pups (21 ± 8.5 and 24 ± 6.9 mg/g, respectively). Interestingly, at the age of 70 days, hepatic ceramide concentration was significantly higher in the two groups that had been given HF-diets, compared to the post-weaning control groups (C→HF 106 ± 19 & HF→HF 102 ± 15 nmol/g vs. C→C 82 ± 4.4 & HF→C 84 ± 12).
Conclusion: Although energy intake was identical in C and HF dams, pups from the HF dams developed hepatic steatosis. However, in the lactating pup, this did not lead to increased hepatic ceramide levels, whereas high-fat feeding after weaning caused increased ceramide accumulation in the liver. High-fat feeding of the dams during pregnancy and lactation did not have any programming effect, since no effect of the pre-weaning diets was observed in either liver TAG or ceramide at 70 days of age.

P-5A-213

In utero exposure to insulin resistance results in accelerated weight gain and hyperinsulinemia in offspring

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Objective: Intrauterine exposure to gestational diabetes and maternal obesity are each associated with risk of childhood obesity. In clinical and epidemiologic studies, it can be difficult to dissect the relative contribution of maternal genetics, diet, and the metabolic environment (e.g. glucose, insulin) to offspring phenotypes. Thus, we asked whether in utero exposure to an insulin resistant environment *in the absence of hyperglycemia or obesity* affects offspring growth or glucose metabolism.
Methods: Mice with heterozygous disruption of the insulin receptor substrate 1 gene (IRS1 het) have reduced insulin signaling. To assess the metabolic effects of prenatal exposure to genetically-determined insulin resistance, we bred wild type C57BL6 males with (a) wild type (WT) or (b) IRS1 het females, and assessed metabolic phenotypes in WT offspring mice (C = WT offspring of WT dams; IR-exp = WT offspring of IRS1 het dams). Offspring were weaned to a high-fat, high sucrose diet.
Results: During pregnancy, IRS1 het dams are insulin resistant, as evidenced by significantly higher insulin levels and a trend to higher fatty acid levels, but remain normoglycemic and have similar weight gain. Despite similar offspring birth weight in C and IR-exp groups, body weight of male IR-exp mice exceeded

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controls at 1 week of life (IR-exp: 4.8 + 0.2 g; C: 4.1 + 0.2 g, $p = 0.007$), a pattern which was maintained to 18 weeks of age (IR-exp: 46.1 + 1.1 g; C: 42.5 + 0.9 g, $p = 0.03$). In females, body weight was elevated in IR-exp mice at 1 week, but indistinguishable from controls by 4 weeks. Analysis of plasma metabolites in early postnatal life revealed significant elevations in several fatty acid species in IR-exp offspring. IR-exp males had similar fed and fasted glucose levels as controls, but higher fed insulin levels at 1 and 4 months (1 month C: 0.51 ± 0.10, IR-exp: 1.07 ± 0.15 ng/ml, $p = 0.02$; 4 months C: 2.81 ± 0.56, IR-exp: 6.59 ± 1.04 ng/ml, $p = 0.01$). At 2 months, male IR-exp mice were more insulin resistant (AUC glucose during intraperitoneal insulin tolerance testing: IR-exp: 5134 ± 277, C: 4442 ± 100, $p = 0.04$).

Conclusions: Intrauterine exposure to genetic insulin resistance, even in the absence of maternal hyperglycemia or obesity, may promote rapid postnatal growth and alter glucose and fatty acid metabolism during adult life.

P-5A-214

Periconceptional undernutrition affects the relationship between early growth and later glucose tolerance in lambs

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Objective: The aim of this experiment was to determine whether relationships between early postnatal growth, later weight and glucose tolerance are affected by maternal periconceptional undernutrition.

Methods: Singleton lambs of normally nourished (N, n = 30; male (M) n = 10, female (F) n = 20) or peri-conceptionally undernourished (UN, n = 29; M n = 14, F n = 15) ewes were weighed weekly to weaning at 12 weeks, then at 4 and 10 months of age. Exponential growth velocity for weight was calculated from birth to 6 weeks of age (postnatal GV). At 4 and 10 months of age, intravenous glucose tolerance tests were performed on a subset of lambs and area under the curve (AUC) for both glucose and insulin was calculated using a triangulation method. Data were analysed using ANOVA with Fisher post hoc test and groups compared using regression. Results are expressed as mean ± SEM.

Results: Offspring of N ewes had a lower birth weight than those of UN ewes ($p = 0.02$), and males were heavier than females in both nutritional groups ($p = 0.005$) (N M 5.8 ± 0.3, F 5.2 ± 0.2 kg; UN M 6.4 ± 0.2 kg, F 5.7 ± 0.2 kg). Postnatal GV was greater in offspring of N than UN ewes (N 28 ± 1; UN 25 ± 1 $g.kg^{-1}.d^{-1}$, $p = 0.008$) and tended to be greater in females than males ($p = 0.05$), so that by 6 weeks of age there was no longer any weight difference between lambs of either nutrition group or sex (N M 18 ± 1, F 17 ± 1 kg; UN M 17 ± 1, F 18 ± 1 kg). This remained the case at 12 weeks (weaning) and at 4 months (juvenile). At 10 months (post puberty), offspring of N ewes were lighter than those of UN ewes ($p = 0.04$), and males were heavier than females in both nutritional groups ($p = 0.0007$) (N M 50 ± 4, F 39 ± 1 kg; UN M 57 ± 3, F 44 ± 2 kg). Birth weight was inversely related to postnatal GV in all lambs ($R^2 = 0.4$, $p < 0.0001$). Postnatal GV was positively associated with 4 month weight in offspring of N, but not UN, ewes (N $R^2 = 0.7$, $p = 0.004$; UN $R^2 = 0.1$, $p = 0.3$). In lambs of N, but not UN ewes, postnatal GV was positively associated with glucose and insulin AUC at 4 months in lambs of both sexes, significant only for insulin (N $R^2 = 0.9$, $p = 0.01$; UN $R^2 = 0.4$, $p = 0.1$). There was no association between postnatal GV and either glucose or insulin AUC at 10 months in either nutritional group. There was no association between weight at 4 and 10 months in lambs of either sex or nutritional group, and no association between weight change between 4 and 10 months and glucose or insulin AUC at 10 months.

Conclusion: Maternal undernutrition around conception results in altered regulation of the relationships between postnatal growth and carbohydrate metabolism. Intrauterine events may contribute to the effects of early postnatal growth on later health, which may in turn be modified by pubertal changes in growth and metabolism.

P-5A-215

No correlation of a family history of obesity and type 2 diabetes with the body mass index of nutrition freshmen in México

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Objective: To examine whether the association between Body Mass Index-based (BMI) overweight/obesity classification and type 2 diabetes among Nutrition freshmen differs according to the presence of a biological parental or grandparental history of obesity and diabetes.

Methods: A cross-sectional study was conducted with 108 students, which represented 100% of sampling frame of the cohort class of 2008 at Veracruz-campus Universidad Veracruzana School of Nutrition, in México. Weight (by scale) and standing height (by stadiometer) were measured. BMI was defined as weight divided by height squared (kg/m^2). BMI was grouped into 4 categories with the corresponding adolescent percentiles as recommended by the Centers for Disease Control and American Academy of Pediatrics or with the corresponding adult cut-off values as recommended by the WHO. Freshmen students provided information on first-degree family history (biological parental/siblings or grandparental) of obesity and type 2 diabetes

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at time of interview and was used as a surrogate for hereditary factors.[1,2,3] The family history was defined as negative if the interviewed freshmen reported the absence of a specific condition among first-degree relatives, and as missing if she/he reported not knowing whether any relatives had ever been told that they were affected. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Eighty six freshmen were females (80%) and 22 males (20%). Most of them (98%) aged between 18-22 years (mean = 19). BMI-based nutritional classification showed an overall prevalence rate of 8% underweight, 79% normal, 9% overweight and 4% obesity among freshmen. The overall prevalence rate of self-reported diabetes was 0% among freshmen. A significant number of freshmen reported a family history of obesity (55%) and diabetes (60%), with 77% of freshmen reporting any of these chronic diseases. More than three-quarters of and more than half of those with a higher BMI had a family history of obesity and diabetes respectively. Overall, there was no association between family history of obesity ($r = 0.18$, $p > .06$) and diabetes ($r = -0.14$, $p > 0.15$) and BMI overweight/obesity classification of Nutrition freshmen.

Conclusions: First-degree family history of obesity or diabetes seems not to be the most powerful risk factor for these young freshmen for becoming obese or diabetic, perhaps suggesting the presence of genetic susceptibility but not shared environmental influences (dietary restraint and physical activity).[4] Thus, there is no guarantee that these freshmen will continue to be so categorized in adulthood. This study was sponsored by Dr. Francisco Jiménez-Guerra and performed in facilities which are owned by Universidad Veracruzana.

1. Barsh GS *et al. Nature*., 404:644–651, 2000.
2. Stunkard AJ *et al. N Engl J Med*., 322:1483–1487, 1990.
3. Undurti ND, Allam AR. *Lipids in Health and Disease*, 6:35, 2007.
4. Hill JA, Peters JC. *Science*., 280:1371–1374, 1998.

P-5A-216

Physiological significance of the intake of protein-to-digest during the suckling period for the development of pancreatic digestive functions during and after the suckling period

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Objective: Protein matter in breast milk consists of large molecule protein for the most part. It has been often thought that protein hydrolysate might be nutritionally equal to original large protein for infants, but several recent reports suggest large molecule protein may be nutritionally superior as a dietary amino acid source to protein hydrolysate or free amino acids for infants. Some scientists and pediatricians therefore have described concerns about nutritional adequacy of protein hydrolysate infant formulas and also about their long-term effects. Nevertheless, there is still little evidence especially about physiological significance of the intake of large protein to digest for infants. We, thus, evaluated the impact of protein matter fed during the suckling period on the development of pancreatic digestive functions during and after the suckling period, using a rat pup model and a piglet model.

Methods: Rats were artificially reared on a rat formula based on cow's milk protein or a rat formula based on cow's milk protein hydrolysate (molecular weight: <3,500) from 7 days of age, weaned at 21 days of age, and fed a standard solid diet until 42 days of age. Pancreas weights and pancreatic amylase and trypsin concentrations were measured at 14, 21, 28, and 42 days of age. At 28 days of age, a pancreatic cannula was inserted into the pancreatic duct to collect the secreted bile-pancreatic juice. The rats were intraduodenally administered soybean protein to evaluate the pancreatic secretory ability in response to dietary protein. Some other artificially reared rats were intravenously injected cholecystokinin to examine whether pancreatic responsiveness to circulating cholecystokinin can be affected by protein fed during the suckling period. Piglets were raised on a protein formula designed for piglets or a protein hydrolysate formula for piglets from 7 to 21 days of age. Pancreatic digestive enzymes were measured at 21 days of age.

Results: Pancreas weights and pancreatic amylase and trypsin concentrations in hydrolysate formula-fed rats were significantly lower than those in protein formula-fed rats during and also after the suckling period, even 3 weeks after weaning. Pancreatic enzyme secretion in response to dietary protein in rats weaned from the hydrolysate formula was significantly weaker than that in rats weaned from the protein formula. There was no difference in pancreatic secretory response to cholecystokinin between two groups, suggesting that necessity to digest dietary protein might affect the development of intestinal ability to release cholecystokinin in response to dietary protein. All measurements of standard formula-fed rats were equivalent to those of dam-fed rats. Similar results were obtained from the piglet study.

Conclusions: Our recent results suggest that the presence of large protein to digest in milk may be significant for the proper development of pancreatic digestive functions and that insufficient digestive functions due to hydrolysate-feeding could remain in the later life. Further studies on physiological and nutritional significance of protein intake in infancy, i.e. effects of hydrolysate formula-feeding are necessary for proper designing and proper choice of infant milk formulas.

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P-5A-217

Maternal dietary exposure during pregnancy induces specific changes in the hepatic transcriptome of the adult offspring

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Objectives: Feeding pregnant rats a protein-restricted (PR) diet induces an altered phenotype in the adult offspring which have been reported to be prevented by supplementation of the PR diet with folic acid (PRF diet). Because the effects of maternal diet on the offspring have only been reported for candidate genes, we investigated the effect of maternal PR and PRF during pregnancy on the expression of the whole liver transcriptome in their adult male offspring.
Methods: Rats were fed either a control (18% (w/w) casein), PR (9% (w/w) casein) or PRF (9% (w/w) casein supplementated with Folic acid (5 mg/kg) diet from conception to delivery, then standard chow (AIN-76A) during lactation (Langley & Jackson, 1994). Male offspring weaned onto a semi-synthetic diet and killed on d 84. Liver transcription was analysed using an expression microarray (6 livers per maternal dietary group), followed by post hoc analysis of relative mRNA levels and of gene ontology. The results of microarray analysis were confirmed for specific genes by real time RTPCR.
Results: 312 genes differed significantly ($\geqslant$1.5-fold change, $P < 0.05$) between offspring of PR and control dams (222 increased, 90 decreased), while 191 genes differed significantly between offspring of PRF and control dams (45 increased, 146 decreased). Gene ontology analysis showed that maternal PR induced significant changes (Z score >2) in the gene ontology pathways - developmental process, ion transport, and response to oxidative stress. In PRF offspring, developmental process and ion tranport pathways remained altered however alterations in fatty acid and steroid hormone metabolic process were also observed. There was no effect of maternal PR on mRNA expression of imprinted genes, although Ins1 and Phlda2 were significantly altered in PRF compared to PR offspring.
Conclusions: Our findings show that the effects of altered maternal diet on the expression of the transcriptome are highly specific and differ between dietary exposures, We also show that although folate supplementation of the maternal PR diet prevented some of the effects on the transcriptome induced by the PR diet alone, it either did not affect others or changed the expression of genes in pathways notably involved in fat metabolism. Support: MAH and GCB are supported by the British Heart Foundation. PDG is funded by the National Research Centre for Growth and Development (New Zealand). The University of Southampton and by the UK Medical Research Council.

P-5A-218

Offspring of rats subject to maternal protein restriction in pregnancy and suckling show improved whole body insulin sensitivity when catch-up growth does not occur

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Objective: Metabolic syndrome and type 2 diabetes may arise following disparity in prenatal and postnatal nutrition or growth. Intrauterine growth restriction (IUGR) resulting from a sub-optimal environment is associated with altered organogenesis which may also contribute to long term disease risk. The central tenant to this process is that IUGR offspring are most susceptible to disease when they exhibit catch-up growth postnatally. There is evidence that maternal protein restriction or IUGR leads to an increased risk of type 2 diabetes however reports in animal studies are equivocal. We aimed to examine the effect of maternal protein restriction on whole body insulin sensitivity in young adult rats in a model that does not display catch-up growth.
Methods: Female Wistar Kyoto rats were fed either a control protein (20% protein as casein) or a low protein diet (LPD; 8.7% protein) for 2 weeks prior to mating, during pregnancy and lactation. At 3 weeks of age offspring were weaned to a control diet ad libitum. Whole body insulin sensitivity (ISI: steady-state glucose infusion in $mg^{-1}kg^{-1}$.min/plasma insulin concentration in $\mu U.ml^{-1} \times 100$) was assessed in 32 week old offspring of control and LPD fed rats ($n = 7$ males and females per group) by euglycemic hyperinsulinemic clamp. Plasma insulin and pancreatic insulin concentration were assessed by radioimmunoassay. Data were analysed by ANOVA or RM ANOVA with maternal diet (P_D) and offspring gender (P_G) as factors. Appropriate institutional ethics committee clearance was obtained.
Results: LPD offspring were born of lower birth weight than controls (males: control 5.77 ± 0.17g vs LPD 4.44 ± 0.13g, $P < 0.0001$ and females: 5.90 ± 0.13g vs LPD 4.28 ± 0.14g, $P < 0.0001$) and remained significantly lighter throughout the experimental period (RM ANOVA, $P_D = 0.0002$, $P_G < 0.0001$). There was a significant effect of maternal diet (LPD > control, $P_D < 0.001$) and offspring gender (Female > Male, $P_G = 0.004$) on insulin sensitivity. LPD offspring demonstrated greater insulin sensitivity than sex matched control offspring (ISI of males: LPD 5.03 ± 0.22 vs control 2.75 ± 0.15, $P < 0.0001$ and females: LPD 6.70 ± 0.82 vs control 4.04 ± 0.25, $P = 0.02$). This finding was consistent with a reduction in the concentration of circulating insulin in LPD offspring when compared with controls (Male: control 7.678 ± 1.22 ng/ml vs. LPD

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5.64 ± 1.20 ng/ml, $P = 0.02$. Female: control 7.40 ± 0.21 ng/ml vs LPD 4.39 ± 0.99 ng/ml, $P = 0.02$). Pancreatic insulin content did not differ between the groups (Male control 0.119 ± 0.013 mg/g vs LPD 0.086 ± 0.01 mg/g, $P = 0.375$. Female control 0.096 ± 0.023 mg/g vs LPD 0.083 ± 0.014 mg/g, $P = 0.375$).

Conclusion: Maternal protein restriction and/or IUGR does not necessarily lead to programmed disease. In fact, in this model the maternal challenge leads to the programming of improved postnatal insulin sensitivity and normal pancreatic insulin concentrations, most likely because postnatal growth trajectories are appropriate to that seen *in utero*. The findings further highlight the importance of maintaining a similar growth trajectory after birth to that *in utero*. Support: KL Australian NHMRC Postgraduate Training Award (519410), Australian Heart Foundation Fellowship (PF-06M-2766) to JAA.

P-5A-219

Low salt intake during pregnancy and lactation causes low birth weight and changes in energy metabolism in adult Wistar rats female offspring

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Objective: To study the energy metabolism in adult offspring of dams low salt diet during pregnancy.

Methods: Female Wistar rats were fed low (LSD: 0.15%) or normal (NSD 1.3% NaCl) salt diet since 8 weeks of age. At 12 weeks of age, they were mated. All offspring groups were fed NSD from weaning. At 12 weeks of age, the animals were kept on 4°C (4°) or room temperature (RT) environment during 24 hours to measure plasma nor epinephrine (NOR), epinephrine (EPI), triacylglycerols (TG), cholesterol (CHO), and leptin (LEP). RT-PCR for mRNA uncoupling protein-1 (UCP1), coenzyme Q1 (CoQ1) and coenzyme Q2 (CoQ2) of brown adipose tissue were performed.

Results: (mean±SEM, $p < 0.05$, n = 5–8/group): Birth weight (g) was lower in LSD (5.1 ± 0.2) than in NSD (5.9 ± 0.1) offspring. NOR (pg/dL) was higher on 4° (471 ± 42) than on RT (206 ± 28) in NSD but not in LSD group. EPI (pg/dL) was lower on 4° (224 ± 5.5) than on RT (738 ± 18) in LSD but not in NSD group. On LSD, EPI was higher (738 ± 18) than on NSD (393 ± 68) at RT but not at 4°. TG (mg/dL) were lower on LSD (25 ± 2) and NSD at 4° (29 ± 6) than at RT (LSD - 94 ± 14, NSD - 72 ± 10). CHO (mg/dL) also decreased in response to cold in both groups. LEP (mg/dL) was lower in LSD (1.6 ± 0.2) than NSD (2.5 ± 0.2) at RT but not at 4°. UCP1, CoQ1 and CoQ2 were lower in LSD (UCP1-1.5 ± 0.1, CoQ1- 0.7 ± 0.1, Coq2-1.0 ± 0.1) than in NSD (UCP1- 2.0 ± 0.1, Coq1 1.1 ± 0.1, CoQ2-1.3 ± 0.1) at RT.

Conclusions: Low salt diet during pregnancy and lactation causes low birth weight and an imbalance on energy metabolism in adult female offspring. Supported by FAPESP.

P-5A-220

Evaluation of the association between birth weight and risk factors of non transmissible chronic diseases in a youth adult group

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During the fetal development and the first years of life could take place adverse or unfavorable situations that affect the tissues growth and its regulation increasing the diseases risk in the adult state. Studies in animal model and European population had showed that the low height and weight at birth are associated with the risk of development of non transmissible chronic diseases. At the same time during the gestational period. The intensity and duration of the exposure to factors that cause low height and weight in the newborn, could have a ''programming'' effect on some functions in the persons, like, gene expression, protein activity and enzyme regulation.

Objective: The goal in this study is to evaluate the association between the newborn anthropometry and non transmissible chronic diseases (NTCD) in a group of adults between 18 to 25 years old.

Methods: longitudinal descriptive study, retrospective type. During 2004 and 2005 all the 6th semester medicine students of Universidad del Valle were invited to participate; 148 subjects was recruited, then blood samples, anthropometric measures, blood pressure, diet and physical activity survey and weight at birth were taken. Total cholesterol(TC), C-HDL, triglycerides(TGC), glucose were measured and apolipoprotein E genotype was determined. Appropriate institutional ethics committee clearance and participants' informed consent were obtained'. For the analyses the group had divided in the intercuartiles, and the comparation was accomplished between the subjects in the lowest cuartil and the subjects in the highest cuartil. Table 1 shows the definition of the NTCD risk factors.

Results: The average age was 20.7 years. The Odds Ratios OR) were calculated between the NTCD risk factors and the birth weight.

Conclusions: The subjects with low birth weight and with apoE 4 had bigger risk to have high cLDL values (Table 2). The women with lower birth weight had a bigger risk to have overweight. The subjects with higher birth weight and with apo E 4 had a bigger risk to have low cHDL values and it was independent of the overweight. The women with higher birth weight had bigger risk of overweight and high systolic blood pressure.

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Table 1. Classification of Non transmissible chronic diseases risk factors.

MEASURES		HIGH LEVEL
Lipids	TC	>200 mg/dL
	cLDL	>100 mg/dL
	cHDL	<40 mg/dL
	TGC	>150 mg/dL
Abdominal obesity - Waist circumference	Men	>90 cm
	Women	>80 cm
Blood pressure	Sytolic	120–139 mm Hg
	Diastolic	80–89 mm Hg

Table 2. Odds Ratios between non-transmissible chronic diseases risk factors and birth weight.

Characteristic Risk	OR(IC)	p-value
Subjects with low birth weight		
With apo ε4 alele cLDL elevated	25(1,01–1309,68)	Fischer p = 0,039
Women Overweight	9(1,0–21,8)	Fischer p = 0,034
Subjects >birth weight/high		
Overweight	3,82(1,0–15,2)	Fischer p = 0,035
Subjects with high birth weight		
Low cHDL	3,13(1,24–7,97)	p = 0,0071
Without overweight Low cHDL	3,48(1,23–9,97)	p = 0,0078
Without overweight High sistolyc blood pressure	2,43(1,0–6,40)	p = 0,044
Women Overweight	12,0(1,0–34,0)	Fischer p = 0,04

P-5A-221

Maternal age at first conception affects developmental programming of male offspring growth, triglycerides and leptin in the rat

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Objective: Several maternal protein restriction (MPR) rat models exist to study effects on offspring (OFF) growth and development. However, we know of no systematic study of influences of maternal age. We have observed that post-natal growth of females in our strain of rat continues until approx 120 days. We therefore evaluated effects of maternal age at first conception on OFF function in a well established MPR model. We hypothesize that differences in OFF outcomes can be related to maternal growth rate at the age of conception. ***Methods:*** We reared and bred Wistar rats fed rodent diet 5001 from weaning (18CMS-5001) whose own mothers had received rodent diet 5001 during pregnancy and lactation. These females were bred at two different ages: 70 and 150 postnatal days (PND). When pregnant they were fed either a control (C – 20% protein) or restricted (R – 10% protein) isocaloric diet. We studied two groups: C during pregnancy and lactation (CC) and R in pregnancy and C in lactation (RC) with litters adjusted to 12 pups per dam. Male pups ate 18CMS-5001 from PND 21 until PND 220 days when body weight, serum leptin (RIA) and triglycerides (TG) were measured. Data M ± S.E.M. Statistical analysis was by two-way ANOVA.

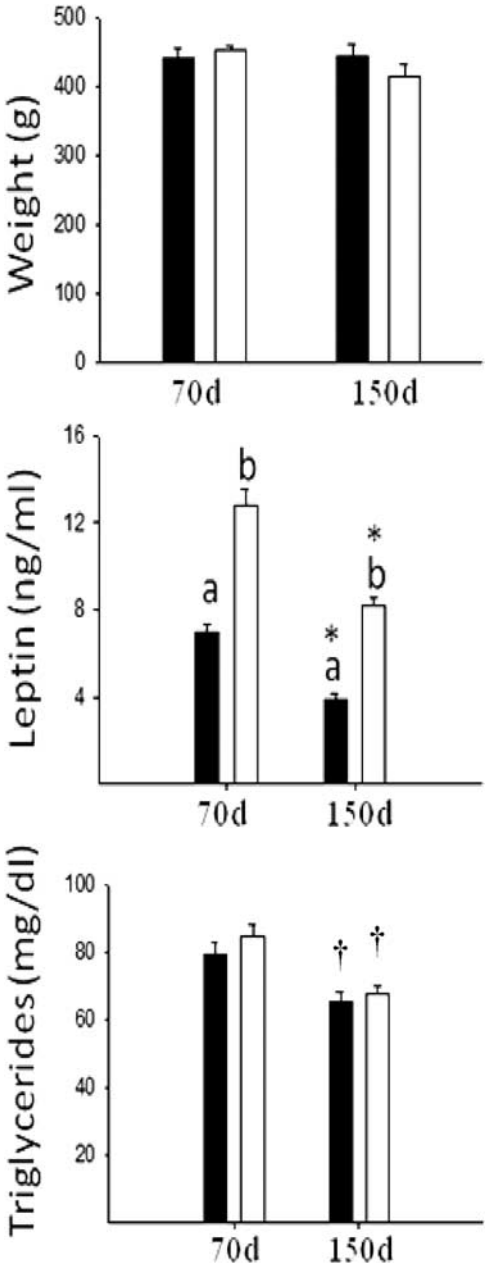

Fig. 1. Male offspring at 220 PND. CC-OFF■, RC-OFF□, $p < 0.05$ Data not sharing a letter differ from same age different group. * $p < 0.001$, † $p < 0.01$ different from 70d-OFF in same experimental group, n = 5–6.

Results: OFF body weight did not differ by treatment or maternal age. OFF serum leptin levels were higher in RC vs CC in both maternal groups and values for both CC and RC were higher in the pups of the younger mothers. TG were not

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affected by maternal diet but were lower in pups of the older mothers (Fig. 1).

Conclusion: These findings demonstrate that baseline OFF leptin and TG levels are affected by maternal age at conception and that leptin outcomes following MNR differ according to maternal age. These data demonstrate the need to control for maternal age effects in developmental programming.

P-5A-222

First trimester adiponectin and subsequent development of preeclampsia or fetal growth restriction

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Objective: The purpose of this study is assessing the utility of determining the maternal serum concentration of adiponectin (ApN) as a marker for insulin-resistence in the prediction of Preeclampsia (PE) and Fetal Growth Restriction (FGR) during the first trimester.

Methods: A prospective, case-control study was conducted in which 1094 pregnant women who received the 11–14 wk ultrasound screening and delivered their babies at University of Chile's Clinical Hospital's Fetal Medicine unit were enrolled. Informed consent and blood samples were obtained and kept at −80°C (−112°F) for future analysis. Among this population, we recruited 10 women who developed PE, 10 who developed IUGR and a control group of 40 healthy women. ApN concentrations in maternal serum were determined using a commercial ELISA kit . PE was defined as recommended the International Society for the Study of Hypertension in Pregnancy[3]. FGR was defined as growth at the 10th or less percentile for weight of all fetuses at that gestational age using a growth curve representative of Chilean population[4]. We studied the relationship between maternal serum concentration of ApN and variables like newborn weight and maternal BMI. Institutional ethics committee clearance and participants' informed consent were obtained.

Results: There were no significant differences between studied groups regarding age, parity, gestational age and tobacco consumption (Table 1) There were no significant differences among first-trimester ApN serum levels in the groups. Average concentration was 8, 6.8 and 10.8 ng/ml for controls, PE and FGR groups, respectively. However, there was a significant difference between the groups alter adjusting for BMI ($p < 0.046$). There was also a significant inverse relationship between ApN levels and maternal BMI ($r = -0.36$; $p = 0.005$) except for the control group (Figure 1). A significant negative relationship ($r = -0.37$; $p = 0.002$) was found when correlating maternal ApN levels with weight percentiles at birth for the whole group (60 patients). Similar results were obtained when analyzing both variables in the control group ($r = -0.36$; $p = 0.002$).

Conclusions: In our study, maternal serum ApN levels were not useful in predicting development of PE and FGR. However, maternal serum ApN concentration adjusted by BMI was significantly higher during the first trimester in women who developed FGR. It is also of interest the negative relationship between ApN levels and weight at birth. Support source: University of Chile: 033/03.

1. Myles Wolf. *et al*. 87(4): 1563–1568.
2. D'Anna R. *et al*. Bjog 2006;113:1264–69.
3. Brown MA. *et al*. Hypertens Pregnancy 2001;20:ix–xiv.
4. Juez G, *et al*. Rev Chil Pediatr. 1989:60;198–202.

Table 1. Maternal and neonatal demographic characteristics.

	IUGR (10)	PIH (10)	Controls (35)	p
Age, years				
Mean	31.6 (6.7)	31 (8.6)	28.6 (6.6)	0, 60
Range	20–40	17–45	16–39	
Primigravida n (%)	6 (60, 0)	6 (60, 0)	17 (48, 5)	0, 77
Tobacco n (%)	0 (0, 0)	0 (0, 0)	3 (8, 5)	
Alcohol n (%)	0 (0, 0)	0 (0, 0)	1 (2, 8)	
BaW (Mean)	2358.1 (256.3)	2584.5 (1127.0)	3327.1 (655.1)	0, 0001*0,017**
BMI	23.6 (3.4)	26.2 (3.0)	25.3 (3.6) 0,84	

Maternal and neonatal demographic characteristics and results for cases and controls recruited in this study.

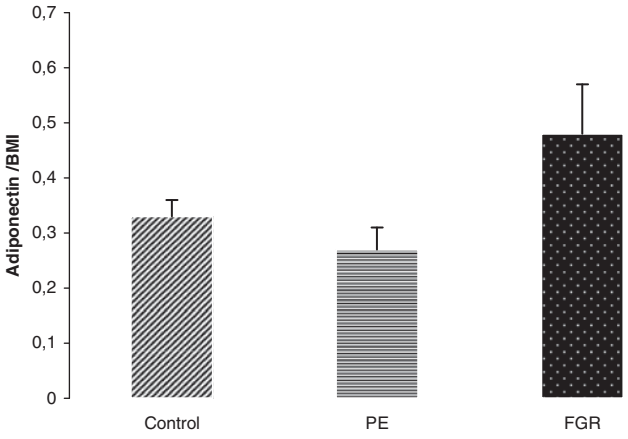

Figure 1. There is a significant difference in serum ApN levels between the groups after adjusting for BMI.

P-5A-222B

Exercise training early in life prevents pancreatic β-cell mass deficits in growth restricted male rats

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Objective: Fetal growth restriction is associated with reduced pancreatic β-cell mass that contributes to adult-onset diabetes. Exercise training enhances pancreatic morphology in diabetic animal models and has recently been shown to have long-lasting metabolic effects following exercise cessation. We therefore studied the effects of brief juvenile exercise training during a period of significant β-cell regenerative capacity in growth restricted male rats.
Methods: Uteroplacental insufficiency and fetal growth restriction was induced on day 18 of pregnancy and resulted in *Restricted* litters compared to sham-operated *Controls*. To control for reduction in litter size in *Restricted*, sham-operated *Reduced litter* offspring were included, where litter size was reduced at birth, previously reported to alter postnatal lactation. Male offspring remained sedentary or underwent Early (5–9 weeks) or Late (20–24 weeks) exercise training. Exercise training involved treadmill running 5 days/week, 60 minutes/day. We examined offspring growth, islet and β-cell parameters (immunohistochemistry) and pancreatic gene expression (real-time qPCR) at 9 and 24 weeks ($n = 4$–11).
Results: *Restricted* pups were born smaller ($P < 0.001$) than *Controls* and remained lighter ($P < 0.01$) until 20 weeks. *Reduced litter* were heavier ($P < 0.001$) than *Controls* from 20 weeks. Early exercise training in *Restricted* partially normalised the 68% reduction in β-cell mass at 9 weeks, with full restoration at 24 weeks despite no further training. This reprogramming was not due to altered pancreatic Pdx-1, Vegf or Insr mRNA expression, despite being down regulated ($P < 0.05$) as a result of uteroplacental insufficiency. Late exercise training tended to restore β-cell mass in *Restricted* but to a lesser extent. Early exercise training had beneficial effects on β-cell mass in *Controls,* but short-lived increases in *Reduced litter*.
Conclusions: In conclusion, early life exercise training in growth restricted rats reprograms the pancreas by preventing β-cell mass deficits in adulthood. This has implications for the promotion of physical activity in low birth weight humans.

P-5C-223

Maternal smoking, blood pressure and placental haemodynamics and risk of preeclampsia. The Generation R Study

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Objective: Maternal smoking during pregnancy is an important modifiable risk factor for various adverse birth outcomes but lowers the risk of preeclampsia. The mechanisms underlying these opposite effects are yet unknown. We examined the effects of maternal smoking on repeatedly measured blood pressure and placental haemodynamics and the subsequent risks of preeclampsia and pregnancy-induced hypertension in a low risk population-based cohort among 7,106 women.
Methods: This study was embedded in a population-based prospective cohort study from early pregnancy onwards. Maternal smoking, systolic and diastolic blood pressure and placental haemodynamics (uterine and umbilical artery resistance) were assessed by questionnaires, physical examinations, and Doppler measurements in each trimester of pregnancy. Information on preeclampsia and pregnancy-induced hypertension was obtained from medical records. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

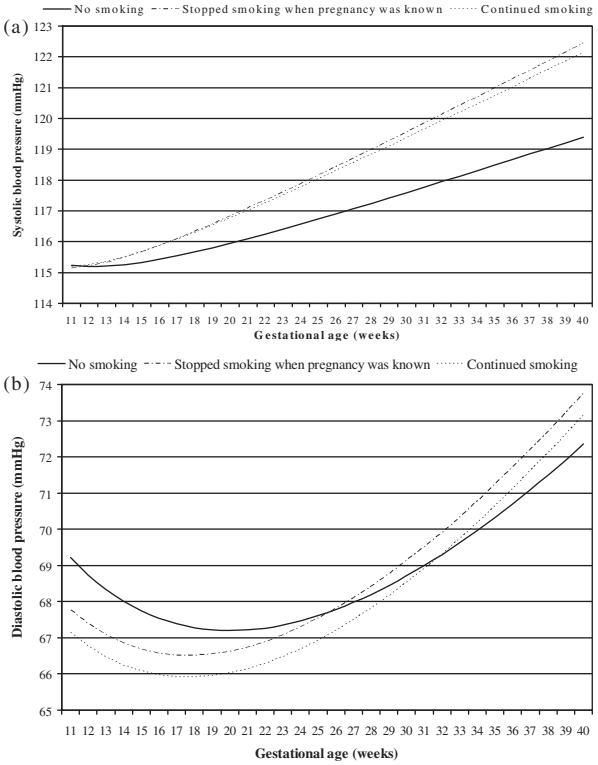

Figure 1a and 1b: Systolic and diastolic blood pressure patterns during pregnancy in different smoking categories.

Results: Compared to non-smoking mothers, both first trimester only and continued smoking mother were associated with the largest increase for systolic blood pressure and the lowest mid-pregnancy level and largest increase thereafter for diastolic blood pressure. Among smoking mothers, second and third trimester umbilical artery but not uterine artery resistance were increased ($p < 0.01$). Tendencies towards increased and decreased risks of preeclampsia were observed for first trimester only smoking (odds ratio of 1.25 (95% CI: 0.72, 2.17) and continued smoking (odds ratio of 0.81 (95% CI: 0.49, 1.34), respectively.
Conclusions: Our results suggest that both first trimester only and continued smoking are associated with persistent

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maternal cardiovascular adaptations during pregnancy. Strategies for prevention of smoking during pregnancy should be focused on the preconception period. The differential effects of early and late pregnancy smoking for the risks of preeclampsia should be further explored.

P-5C-224

Increased placental apoptosis in maternal food restricted gestations: Role of the Fas pathway

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Objective: The placenta has a pivotal role in fetal development, shuttling nutrients and exchanging gases and metabolites. Maternal under-nutrition (MUN) during pregnancy results in intrauterine growth restricted (IUGR) fetuses, a consequence attributed primarily to restricted maternal nutrient supply. However, MUN may further compromise fetal growth as a result of decreased placental growth or increased placental apoptosis. Apoptosis occurs mainly via mitochondrial or Fas pathways. Specifically the Fas system (Fas, FasL, caspase-8, capsase-3 and tBID) is responsible for the placental villous trophoblast turnover, promoting the deletion of potentially harmful cells. Alterations in this system during pregnancy may result in gestational complications as is the case in IUGR pregnancies. We sought to determine the role of the Fas pathway in MUN stimulated placental apoptosis, examining the two morphologically and functionally distinct zones (basal and labyrinth).
Methods: Pregnant rat dams were fed an ad libitum diet (Control; $n = 6$) or were 50% undernourished (MUN, $n = 6$) beginning at E10 of gestation. At E20, Control and MUN dams were weighed, euthanized, uterus operatively delivered and the gestational sacs removed. All fetuses and placentas were labeled according to their position in each uterine horn and weighed. The right horn placentas were fixed in 4% paraformaldehyde and used for terminal dUTP nick-end labeling (TUNEL) assay and immunostaining for activated caspase-3. The corresponding left horn placentas were separated into basal and labyrinth zones and used for Fas pathway protein expression by Western blotting.
Results: MUN significantly reduced maternal, fetal and placental (basal and labyrinth) weights. Parallel to zone-specific weight reduction in MUN placentas, both basal and labyrinth zones had significantly higher levels of apoptotic indices from both proximal and mid-horn, as compared to Controls. Notably, MUN mid-horn placentas had a greater degree of apoptosis than proximal placentas, as demonstrated by enhanced activated caspase-3 immunostaining. Despite this, only the labyrinth zone of mid-horn MUN placenta showed increased expression of the entire Fas pathway related proteins. The basal zone only showed increased expression of tBID protein, suggesting that the alternate intrinsic apoptotic pathway may be operative. In contrast, proximal MUN placentas showed upregulated Fas pathway in both basal and labyrinth zones as compared to Control placentas.
Conclusions: Increased placental apoptosis in MUN pregnancies is mediated, in part, via the Fas pathway. Reduced fetal growth in response to maternal under-nutrition is a consequence of both deficient nutrient supply and impaired placental development.

P-5C-225

Peroxisome proliferator-activated receptor (PPARγ) mediated mechanism for placental apoptosis and fetal growth restriction

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Objective: Maternal undernutrition (MUN) during pregnancy leads to intrauterine growth restricted (IUGR) fetuses that have an increased risk of adult obesity, hypertension and diabetes. IUGR may occur secondary to insufficient maternal nutrient supply and/or reduced placental nutrient/oxygen transfer. Notably, increased placental apoptosis has been associated with IUGR fetuses. We hypothesized that increased apoptosis results from down regulation of peroxisome proliferator-activated receptor gamma (PPARγ), an anti-apoptotic transcription factor that also mediates cellular proliferation and differentiation. We focused our study on the two placental positions (proximal and mid-horn) with the extremes of nutrient/oxygen supply, and two distinct placental zones (basal, site of hormone production; and labyrinth, site of feto-maternal exchange).
Methods: Pregnant rat dams were fed an ad libitum diet (AdLib) or were 50% maternal undernourished (MUN) beginning at E10 of gestation. At E20, fetal and placenta zone weights were recorded. Six placentas from left proximal and mid-horns were used for terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) and immunodetection of activated caspase-3. The corresponding right mid- and proximal horn placentas were separated into basal and labyrinth zones and analyzed for zone and region specific expression of PPARγ using Western blot analysis. All results are presented as mean ± SE and considered significant at $P < 0.05$.
Results: At E20, maternal weight (272.4 ± 4.3 *vs.* 343.5 ± 7.3 g), and proximal and mid-horn fetal weights were significantly decreased in the MUN as compared to AdLib. Similarly, placental basal and labyrinth zones from both proximal and mid-horn placentas weighed significantly less in MUN as compared to their respective AdLib placentas. MUN proximal placentas had markedly increased levels of apoptosis in basal (3.3 ± 1.0 *vs.* $0.3 \pm 0.1\%$) and labyrinth (8.6 ± 1.4 *vs.* $0.6 \pm 0.3\%$) zones as compared to their respective AdLib placentas. Similarly, MUN mid-horn

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placentas showed greater degree of apoptosis in basal (5.5 ± 0.2 *vs.* 0.7 ± 0.1%) and labyrinth (10.1 ± 1.1 *vs.* 0.13 ± 0.07%) zones, as evident by both TUNEL and activated caspase-3 immunostaining. PPARγ protein expression was significantly downregulated in the MUN proximal placentas in basal (2.3 fold) and labyrinth (2.1 fold) zones. A further decrease in PPARγ protein expression was observed in MUN mid-horn placentas in basal (3.1 fold) and labyrinth (3.4 fold) zones.

Conclusions: Maternal undernutrition during pregnancy results in reduced fetal and placental (basal and labyrinth zones) growth. Parallel to zone specific weight reduction in MUN placentas, both placental regions showed significantly higher levels of apoptosis with concomitant decreased PPARγ protein expression. These results suggest that reduced expression of the anti-apoptotic factor PPARγ contributes to increased placental apoptosis and reduced placental and fetal growth.

P-5C-226

Abnormal placental vascular development in early pregnancy contributes to fetal growth restriction in smokers

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Small for gestational age (SGA) infants are a serious complication of cigarette smoking with potential life long consequences. The mechanisms of this effect of cigarette smoking are multifactorial, but are still not well understood.

Objectives: To compare umbilical and uterine artery Doppler waveforms and fetal ultrasound measurements at 20 weeks gestation between smokers and non-smokers.

Methods: The study population comprised nulliparous women in the Screening for Pregnancy Endpoints (SCOPE) study in Auckland, New Zealand and Adelaide, Australia. Self-reported smoking status was determined at 15 ± 1 weeks' gestation. At the 20 ± 1 weeks uterine and umbilical artery Doppler resistance indices (RI) and fetal measurements were compared between smokers and non-smokers. The main outcome measures were umbilical and mean uterine artery Doppler RI values, abnormal umbilical and uterine Doppler (RI > 90th centile) and fetal biometry. SGA was defined as birthweight <10th customised birthweight centile, adjusted for fetal sex, maternal weight, height, parity and ethnicity.[1] Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Amongst the 2459 nulliparous participants, 248 (10%) were smokers. Smokers had higher umbilical RI [0.75 (SD 0.06) vs 0.73 (0.06), $p < 0.0001$] and mean uterine RI [0.59 (0.09) vs 0.56 (0.10), $p < 0.0001$]. They were twice as likely to have an abnormal umbilical Doppler at 20 weeks compared with non-smokers [n = 35 (14.6%) vs n = 156 (7.2%), OR 2.21 95% CI 1.49–3.27]. This effect remained significant after adjusting for age, ethnicity, marital status, employment and BMI (adjusted OR 1.62, 95% CI 1.03–2.54). Smokers were more likely to have an abnormal mean uterine RI [n = 33 (13.7%) vs n=198 (9.2%), OR 1.57, 95% CI 1.06–2.33], but this association was not significant after adjusting for confounders. Fetuses of women who smoked had a small reduction in femur length and estimated weight at 20 weeks gestation compared with non-smokers. Amongst women who had SGA babies (n = 255), smokers (n = 41) had babies with even lower birthweights [2380 (691)g vs 2615 (524)g, $p = 0.04$] and customised birthweight centiles [3.2 (2.9) vs 4.9 (2.9), $p = 0.0005$] than non-smokers. Smokers with SGA babies were also three times more likely to have an abnormal umbilical Doppler RI compared with the non-smokers with SGA babies [11/40 (27.5%) vs 20/210 (9.5%), $p = 0.002$].

Conclusions: At 20 weeks' gestation, women who smoked had higher umbilical artery RI values which are a surrogate measure for an abnormal placental villous vascular tree. This may be one mechanism contributing to fetal growth restriction among smokers. SGA babies born to women who smoked also had more severe growth restriction and evidence of more severe placental vascular disease compared to SGA babies born to non-smokers.

1. L.M.E. McCowan *et al. Aust NZ J Obstet Gynaecol.* 44: 428–31, 2004.

P-5C-227

Low birth weight and the latitude of country of birth

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Objective: Low birth weight is a major cause of infant mortality in the world. There are numerous articles hypothesizing the relationship of heat content of the environment and the incidence of low birth weight (LBW) in humans. There also now some animal studies relating the heat stress in pregnant sheep to retarded placental and fetal growth. The purpose of this study is to determine the relationship of LBW and latitude of the country of birth.?

Methods: The percent LBW in each country was obtained from the United Nations Children's Fund and World Health Organization, *Low Birthweight: Country, regional and global estimates.* UNICEF, New York, 2004. The mean latitude and GDP per capita (GDP/C) data for each country was obtained from CIA Fact Book, from the CIA website. Percent LBW was plotted against latitude in degrees in the north and south of the equator. Data for the LBW percentages and the GDP/C were

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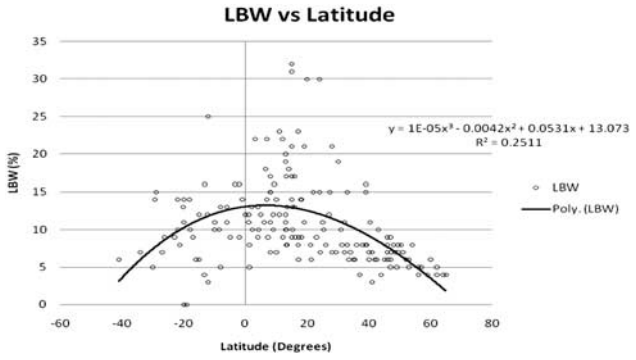
compared with the corresponding mean latitude of the country. Data was analyzed and graphs drawn on the Excel *2007.*

Results: There were a total of 213 countries listed in the WHO data. Usable data was available for only 184 countries. Mean latitude data was available for 179 countries. So only the data for the 179 countries was used in the analysis. Percent LBW (%LBW) was highest close to the equator and decreased moving away from the equator in either direction. GDP/C did not correlate with %LBW.



Conclusions: 1. This is the first study relating the incidence of LBW with latitude. 2. Higher mean temperature during pregnancy could be an additional cause of intrauterine growth retardation (IUGR) as hypothesized previously in human and animal studies unrelated to GDP/C. 3. Higher %LBW is directly related to countries closer to the equator which have relatively higher mean temperature and humidity. 4. Higher incidence of adult onset diseases can be expected in babies of populations residing for a number of generations in countries closer to the equator. 5. Alteration of the nutrition and/or lifestyle due to migration or behavior change could potentially impact the adult health adversely. 6. How would global warming impact the overall incidence of LBW in the world?. Acknowledgements: Dr Kwang Sun Lee of University of Chicago and Mercy Hospital and Medical Center for support.

1. Wells JC. *J Theor Biol.* 214:413–25, 2002.
2. Wells JC *et al. Am J Phys Anth.* 119:276–282, 2002.
3. Wallace JM *et al., J Phyisol* 565:19–26, 2005.

P-5C-228

Placental weight relative to birth weight and long term cardiovascular mortality

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Objective: There is evidence that fetal adaption to challenges in the intrauterine environment may adversely affect long term cardiovascular health1. Placenta is in a key position to mediate such effects since adequate placental function is necessary for the delivery of nutrients, oxygen and hormones to the fetus. We have studied whether placental weight relative to birth weight is associated with the risk of cardiovascular death in adulthood.

Methods: In this prospective population study based on data from standardised hospital birth charts, we have studied 31,307 Norwegian men and women born between 1934 and 1959, who could be followed from 1961 through 2005 with regard to cause specific death. Appropriate institutional ethics committee clearance was obtained.

Results: During 45 years of follow-up, 382 people died from cardiovascular causes (median age 51.3 years). The placenta to birth weight ratio was positively associated with death from cardiovascular diseases and specifically with death from ischemic heart disease and stroke.

Conclusions: A disproportionately large placenta relative to birth weight was associated with increased risk of death from cardiovascular diseases in adulthood. This finding suggests that placental function is important for the association of intrauterine factors with cardiovascular disease later in life. Support: KRR was financed by the Regional Health Authority (RHA) and NTNU.

1. P.D. Gluckman *et al. NEJM* 359: 61–73, 2008.

Table Hazard Ratios (HR) of Death from Cardiovascular Disease.

Birth size measure (in thirds[a])	Deaths	Person years	Adjusted[c]HR	95% CI	*P*-trend
Placenta to birth weight ratio[b]					
Lowest (median, 16%)	103	451,867	1.00	Reference	–
Middle (median, 19%)	128	450,808	1.19	0.92, 1.54	–
Highest (median, 22%)	151	448,911	1.38	1.07, 1.77	0.01
Placental weight					
Lowest (median, 560 g)	135	469,533	1.00	Reference	–
Middle (median, 680 g)	115	481,660	0.97	0.75, 1.26	
Highest (median, 800 g)	132	401,392	1.35	1.02, 1.78	0.04

[a]According to sex and birth cohort (<1945, ⩾1945).[b]Placental weight (g)/ birth weight (g) × 100%. [c]Adjusted for sex, birth cohort (<1945, ⩾1945), with 95% confidence intervals; and placental weight also adjusted for birth weight (thirds).[d]P-value from trend test where the categories where treated as an ordinal variable in the Cox regression model.

P-5C-229

Heavier babies are born in the north

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The relationship of heavier babies being born to moms exposed to light for higher percentage of pregnancy is well agreed upon in the neonatal and the perinatal literature. One

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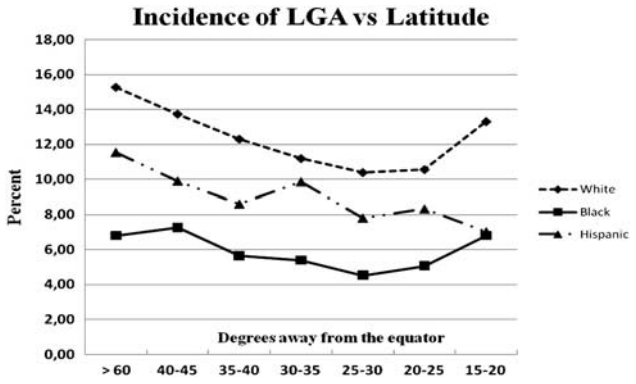
would conclude then heavier babies would be delivered to mothers in areas closer to the equator. There are few studies associating environmental temperature with low placental weight and lower newborn birth weight. Could this phenomenon explain a higher low birth weight and prematurity incidence in the southern most states in the US. Would births in a relatively lower environmental temperature and heat be associated with a higher mean birth weight due to a greater placental weight.

Objective: To determine whether babies born in the southern states are heavier than those born in the northern states of the USA.

Methods: The linked birth and infant death certificate files compiled by the National Center for Health Statistics for the CDC for the years 1995–2002 were used for the analysis. The states were divided according to latitude in to seven categories as follows: Categories above 60, 40–45, 35–40, 30–35, 25–30, 20–25, and 15–20 degrees north of the equator. Babies weighing greater than or equal to 4000 grams (GTE4000 or LGA) were determined for each latitude categories. Incidence LGA babies were determined for non-hispanic white (White), non-hispanic black (Black), and hispanic races and controlled for maternal education, maternal age, maternal tobacco usage, maternal alcohol usage, maternal history of hypertension (pregnancy induced or chronic hypertension), diabetes. Also were analyzed was the place of birth of mother (US or outside US). Compare the mean birth weight with the respective average temperature in each state over the 1995–2002 period, Analysis was performed using Excel 2007 and Stata 9.0.



Results: There were over 31 million births in the US from 1995–2002.Overall incidence of LGA was 9.88%. Incidence of LGA was significantly higher in all babies born in the northern latitudes in the USA irrespective of race, maternal education, maternal age, maternal usage of alcohol or tobacco, maternal history of diabetes, hypertension (pregnancy induced or chronic), maternal place of birth, and marital status. Mean birth weight of babies born in the northern latitudes was also comparatively higher. The effect of latitude was highest in non-hispanic white and lowest in the non-hispanic black.

Conclusions: 1. This is the first report of relationship of latitude and birth weight. 2. Heavier babies are born farthest away from the equator irrespective of ethnicity or other factors. 3. The reason for this phenomenon is unclear and should be investigated further. 4. How will global warming have an impact on birth weight in the future?

P-5C-230

The effects of famine on the placental programming of hypertension

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Objective: People who were small at birth are at increased risk of hypertension. This is thought to reflect programming whereby fetal undernutrition permanently changes the structure and function of the body. Undernourished fetuses generally have small placentas but in some circumstances they may expand the placental surface to extract more nutrients from the mother. Consequently, nutritional status of the mother may impact this process. A Finnish study has shown that both small and large placental surface area predict later hypertension. We examined the association between size of the baby and placenta at birth and the later risk of hypertension among men and women who had and had not been exposed to famine during pregnancy.

Methods: We examined 860 subjects born around the time of the Dutch famine at age 58. Their birth records included two diameters of the placental surface, a maximal one and a lesser one at right angles to it. These diameters were used to calculate the placental surface area. At the time of the examination, 216 persons were taking anti-hypertensive medication. Logistic regression analysis was used to examine associations between birth weight, placental size and hypertension. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Exposure to famine during gestation was associated with reduced birth weight and with reduction in the size of the placental surface. The reduction in placental area was greater in boys than in girls. *385 male subjects.* Among men not exposed to famine, a 1 cm increase in the lesser placental diameter was associated with a decreased risk of hypertension (OR 0.82, $p = 0.003$). In men exposed to famine, a 1 cm increase in the lesser diameter was associated with an increased risk of hypertension (OR 1.26, $p = 0.032$). The

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difference in these trends was statistically significant (p for interaction = 0.001). *475 female subjects.* Among women not exposed to famine, a 1 kg increase in birth weight was associated with a decreased risk of hypertension (OR 0.52, $p = 0.042$). Among women exposed to famine a small placental surface was associated with a higher risk of hypertension, though the trends were not statistically significant. The associations between placental size and hypertension in men and women exposed to famine were significantly different (p for interaction = 0.012 for the lesser diameter, 0.155 for the maximal diameter, 0.020 for placental area).

Conclusions: Our findings suggest that the programming of hypertension may be mediated through placental responses to suboptimal intrauterine conditions in boys, whereas it is mediated though reduced fetal growth in girls.

P-5C-231

Early prenatal glucocorticoid exposure on placental anthropometry in sheep differs from late gestation exposure

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Objective: Previously we have shown that maternal injections of synthetic glucocorticoids (GC) late in gestation reduced total placenta weight, reduced numbers of B and C-type placentomes and increased the number of A-type placentomes[1]. We hypothesized that a two day exposure to GC early in pregnancy, during the main phase of placental development, would also alter placental development.

Method: Pregnant ewes carrying singleton fetuses (total n = 119) of known gestational age were randomized to control (saline) or dexamethasone (DEX) treatment (0.14 mg/kg ewe body weight) consisting of four intramuscular injections at 12-hourly intervals over 48 hours on days of gestation (dG) 40–41. Animals were euthanized at 50, 100, 125, 140dG. Major fetal organs were removed, weighed and collected for use in other studies. The uteri were hysterectomised and placentomes were dissected from the uterus; classified according to their gross morphology into A, B, C and D-subtypes[2] and weighed.

Results: DEX-exposed fetuses had increased fetal-to-placental weight ratios at 50dG, and reduced body weights at 100* and 120dG. Total placentome number and total placentome weight significantly increased between 50 and 140dG and was unaffected by DEX treatment. In both groups, A-type placentome weights and numbers (as percentage of total numbers) were lowest at 125dG*, whereas B- and C-types showed highest weight and number at 125dG*. In males, DEX exposure reduced A-type weights at 100dG* and increased C-type weights and numbers at 125dG* compared to controls. There were higher numbers and weights of B- and C-type placentomes in females than in males at 125dG* (* indicates $p < 0.05$).

Conclusions: Early exposure to DEX resulted in a decrease in fetal weight and altered placental development. These changes were sex-dependent, opposite to late gestation glucocorticoid exposure effects and did not persist until term. Fetal growth potential, reflected by body weight, was preserved despite the modulating effects of 'the environment' (early DEX) along the way. Further studies are required to investigate whether these intermittent changes in placentome numbers and weights after early DEX exposure impairs placental function and, if so, whether they differ from the late glucocorticoid exposure.

1. D.M. Sloboda *et al. J Soc Bynecol Investig,* 12: 332A, 2005.
2. Vatnick I. *et al. J Dev Physiol,* 15: 351–356, 1991.

P-5C-232

Maternal dietary protein intake influences conceptus biosynthesis before implantation

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Objective: Minor changes in maternal diet can induce developmental adaptations in fetal growth and metabolism leading to increased risk of adult onset diseases. Key metabolic processes involved in growth regulation of the developing fetus mediated by sensing energy status and adapting biosynthesis levels (eg mTOR or AMPK) are altered by prevailing nutrient conditions within the mother[1,2]. How and during which developmental window this occurs remains elusive. We have previously shown that postnatal phenotypic changes can be induced during the preimplantation period, becoming detectable by the blastocyst stage[3,4] when cell lineage divergence into inner cell mass (ICM, giving rise to the embryo proper) and trophectoderm (TE, giving rise to placental lineages) emerges, coinciding with major metabolic transitions. Gene expression, cell numbers and lineage allocation within the blastocyst and mTOR signaling (Watkins *et al.*, this meeting) are altered, suggesting mechanisms whereby energy status and metabolic signalling in response to different maternal protein intake affect

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anabolic activity and growth potential of the conceptus. Here, we compare the overall protein biosynthesis activity and energy/stress status in blastocysts derived from mothers fed a control, restricted or elevated protein diet.

Methods: Naturally mated female MF1 mice (7 1/2–8 1/2 weeks of age) were randomly allocated to either a low protein diet (9% casein, LPD), high protein diet (30% casein, HPD) or control diet (18% casein, control) on day of plug (d 0.5). On day 3.5, mothers were sacrificed and overall protein synthesis over either 4 hrs or 12 hrs of blastocysts flushed from the uterus was measured using the CLICKit AHA and TAMRA detection technology (Invitrogen). Alternatively, embryos were fixed and examined by immunofluorescence and confocal microscopy for localisation of AMPKβ1.

Results: Blastocysts from HPD mothers synthesised significantly more protein compared to controls (50% upregulation) and LPD ($p < 0.001$) after 4 hrs or 12 hrs. Protein restriction, in contrast, caused a mild initial reduction in protein biosynthesis compared to controls (15%) which diminished to 5% after 12 hrs ($p > 0.1$). Distribution of AMPKβ1 as indicator of cellular stress, although very variable between embryos and cell lineages, also responded to maternal diet. AMPKβ1 was increasingly found in nuclei of the TE in response to either protein challenge compared to controls. However, whilst HPD induced a diffuse distribution across nucleus and cytoplasm in more than 50% of blastocysts, LPD blastocysts showed a more localised pattern.

Conclusions: We show that maternal protein intake, either over- or mild undernutrition, influences embryonic biosynthesis levels suggesting that maternal-embryonic crosstalk regulates conceptus growth potential even before implantation. Possible mechanisms include AMPK signalling which proved to be highly versatile in the early embryo. Increased AMPK distribution within TE nuclei after both dietary challenges may suggest a general stress response in embryonic lineages directly exposed to adverse intrauterine environments yet treatment-specific distributions may suggest different responses dependent upon the challenge. Taken together, we show that the early embryo can adapt its anabolic metabolism before implantation, likely involving a number of energy sensing mechanisms including AMPK signalling. Support by the BBSRC and Wellbeing of Women is gratefully acknowledged.

1. Philp LK *et al. Am J Physiol.* 295:R1982–R1990, 2008.
2. Zhu MJ*et al*., *Biol Reprod*. 71:1968–1973, 2004.
3. Kwong *et al*., *Development* 127:4195–4202, 2000.
4. Watkins AJ *et al*., *Biol Reprod*. 78:299–306, 2008.

P-5C-233

Could the trophoblast influence the intrauterine environment and the extrauterine life?

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Objective: The kallikrein-kinin system (KKS) has been implicated in protecting against cardiovascular disease and in the antihypertensive effect of potassium supplementation; its deficit is associated to higher prevalence of essential hypertension, preeclampsia and sodium sensitivity. On the other hand we have demonstrated that extravillous and intravascular trophoblasts express the type 2 bradykinin receptor (B2R) in humans and guinea-pigs. Since trophoblast migration is crucial to the transformation of uterine arteries into high flow non-reactive vessels, and bradykinin (BK) promotes migration in astrocytes and leucocytes, we tested the effect of BK on migration of immortalized extravillous trophoblasts with the hypothesis that a defective KKS might impair trophoblast invasion and thus the fetal environment.

Methods: The HTR-8/SVneo cell line (kindly donated by Dr.C.H. Graham) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 1 mg/ml gentamicine and maintained at 37°C, 5% CO_2 in air. These cells showed expression of kallikrein and B2R by ICC. Cell migration was studied using the wound healing assay under the effect of bradykinin and the B1R and B2R antagonists alone and combined. Migration images were obtained with a Nikon inverted microscope connected with a Nikon CoolPix 4500 camera and quantified at different times using the ImageJ v.1.34 software; migration index was defined as cells migrating in response to BK, divided by cells migrating in response to medium alone. The effect of bradykinin and antagonists of the B1 and B2R on the expression of the B2R was also evaluated by western blotting and immunofluorescence. The effect of BK on cell proliferation was evaluated with the cell viability MTS assay at the same time periods.

Results: At 18 hours the migration index of HTR8/SVneo treated with BK 10–5 M increased 3-fold (3.39 ± 0.27, $p < 0.001$) as compared to that of control cells and to cells preincubated with the antagonists of the B1 and B2R, either alone (B1 antagonist: 1.24 ± 0.052; B2 antagonist: 0.859 ± 0.39) or in combination (0.608 ± 0.016) ($n = 4$; $p < 0.001$). BK had no effect on cell proliferation at the different study periods under these conditions.

Conclusion: BK stimulates the migration of HTR-8/SVneo extravillous trophoblasts, effect that support its participation in extravillous trophoblast invasion and the generation of a favorable intrauterine environment. We postulate that a deficient KKS may associate with an unfavorable fetal environment and in the long term with cardiovascular risks.

P-5C-234

Arginase activity participates in intrauterine growth restriction-induced early vascular dysfunction: evidences from isolated human umbilical vessels

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Endothelial cells derived from intrauterine growth restriction (IUGR) placentae present altered nitric oxide (NO) synthesis which persist even under normal culture conditions[1]. Arginase II is expressed in placenta[2] and has been implicated in adult vascular dysfunction through a counteracting activity with the NO synthases (NOS)[3]. However, it is not known if arginase activity has a role in vascular function in normal placenta vessels and, if it is participating in the early vascular dysfunction evidenced in IUGR derived endothelium.

Objective: We studied and compared the vascular effects of arginase activity in normal and IUGR umbilical arteries and veins.

Methods: Arteries and veins from umbilical cords derived from normal and IUGR pregnancies were dissected, and vessel rings were mounted in a wire-myograph. Isometric force in response to increasing concentrations of insulin (10^{-12}–10^{-8} M) without (control) and with the NOS inhibitor L-nitroarginine (L-NA 100 μM) and the arginase inhibitor S-2-boronoethyl-L-Cysteine-HCl (BEC 10 μM) were determined in vessels pre-contracted with KCl (37.5 mM). Responses were expressed as a percentage of relaxation relative to maximal effects of KCl (%Kmax) and adjusted to concentration response curves.

Results: Umbilical arteries (UA) and veins (UV) from normal pregnancies ($n = 4$) relaxed to insulin in a concentration dependent manner with a maximal effect of 18.1 ± 2.2 (% Kmax) and 37.6 ± 3.8 for UA and UV respectively. UA from IUGR placentae showed a lower response to insulin (6.1 ± 2.7) (figure A) compared with normal arteries, whilst in UV this response was absent (figure B). Preincubation of vessels with BEC increased the maximal relaxation to insulin in normal UA (40.0 ± 7.0), UV (57.5 ± 3.1) and IUGR UA (15.0 ± 2.6). Additionally, BEC partially recovered the insulin response in IUGR UV (12.0 ± 3.3). In all the experiments the response to insulin with and without BEC were abolished by the presence of L-NA. On the other hand, in IUGR vessels insulin showed a decreased potency (pD_2, UA 8.6 ± 0.7; UV > 8) compared with normal vessels (~ 10.4) which were restored to normal values by BEC (~ 10.1).

Conclusions: IUGR-derived vessels present reduced insulin-dependent NO-mediated relaxation. In this context, arginase exerts a counteracting activity that further reduces NO synthesis and increases vascular dysfunction. Support: FONDECYT 1080534, 1070865. BKL holds a CONICYT PhD fellowship.

1. P. Casanello *et al.*, *Placenta*, 30:625–633, 2009.
2. M. Noris *et al.*, *Hypertension*, 43:614–622, 2004.
3. L. Santhanam *et al.*, *J. Appl. Physiol.*, 105:1632–1642, 2008.

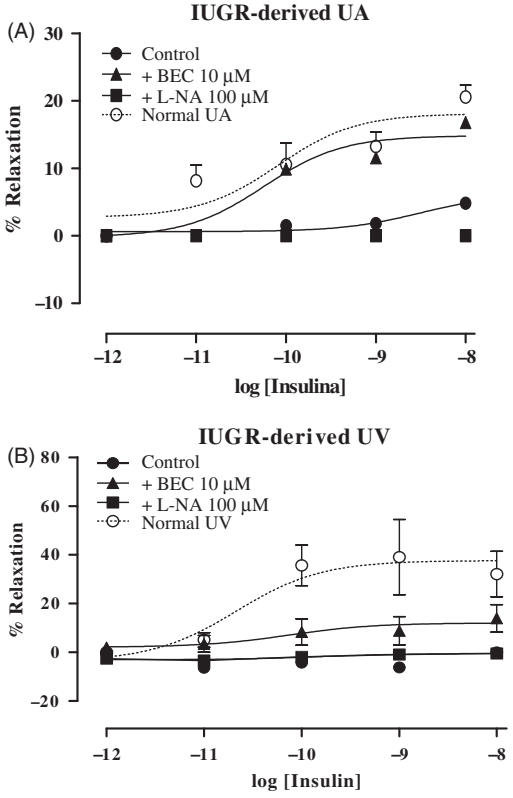

Figure. Insulin-induced vasodilation in IUGR umbilical arteries (UA) and veins (UV).

P-5C-235

Downregulation of eNOS activity in hypoxia is regulated by arginase II in human umbilical vein endothelial cells

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The human fetoplacental vascular bed lacks autonomic innervations, therefore adequate blood flow, fetal nutrition and oxygenation depends on circulating and locally released vasoactive factors. In endothelial cells, nitric oxide (NO), a potent vasodilator that regulates vascular tone, is synthesized by endothelial nitric oxide synthase (NOS), whose substrate is the amino acid L-arginine[1]. Accumulated evidence strongly suggests that many of the features of endothelial dysfunction are intimately linked to the altered expression and function of the L-arginine/NO pathway. In human umbilical vein endothelial cells (HUVEC) exposed to hypoxia, L-arginine uptake and NOS activity are down-regulated[2]. This phenomenon could involve L-arginine metabolism preferentially by

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arginases I (ARGI) and II (ARGII) leading to a functional competition with eNOS for their common substrate[3].

Objective: We studied the role of arginase on eNOS activity and expression under hypoxia in HUVEC.

Methods: Appropriate institutional ethics committee clearance and participants' informed consent were obtained. HUVEC from full term normal pregnancies were isolated by collagenase digestion (0.2 mg/ml) and cultured (37°C, 5% CO_2) in medium 199 containing 10% fetal bovine serum. Cells were serum-starved twelve hours prior to the experiment. Confluent cells were exposed to normoxia (5% O_2) or hypoxia (2% O_2) for 0–24 hours in a sealed hypoxic chamber. Cells were exposed to *S*-(2-boronoethyl)-L-cysteine (BEC, ARG inhibitor, 100 μM) or N^{ω}-nitro-L-arginine-methyl ester (L-NAME, NOS inhibitor, 100 μM). Arginase activity (60 min, 50 mM L-arginine) was determined by measuring urea production by spectrophotometry (DO_{540}). ARG I, ARG II, eNOS and phosphorylated eNOS at ser1177 (p-eNOS, activated) were determined by Western blot. Immunocytochemistry was assayed for ARGI, ARGII, eNOS. A mitochondrial probe (Mitotracker CMXRos) was used to study cellular localization of ARG and DAPI was used to stain nuclei.

Results: Hypoxia increased ARG II protein abundance (12–24 h), and its localization was in the mitochondria as well as in the cytosol. This expression was strongly induced in the presence of the ARG inhibitor, BEC. ARG I protein abundance was extremely low in HUVEC and was not modified under hypoxia. No significant changes were observed in total eNOS protein levels, but significant lower p-eNOS was observed in HUVEC exposed to hypoxia (24 h), an effect that was partially recovered at short periods of incubation (3 h) with BEC. eNOS was located preferentially at the cell membrane and colocalized in some regions with ARGII. The increase in ARG activity in hypoxia was paralleled by a low eNOS activity and was recovered to control values when exposed to the ARG inhibitor.

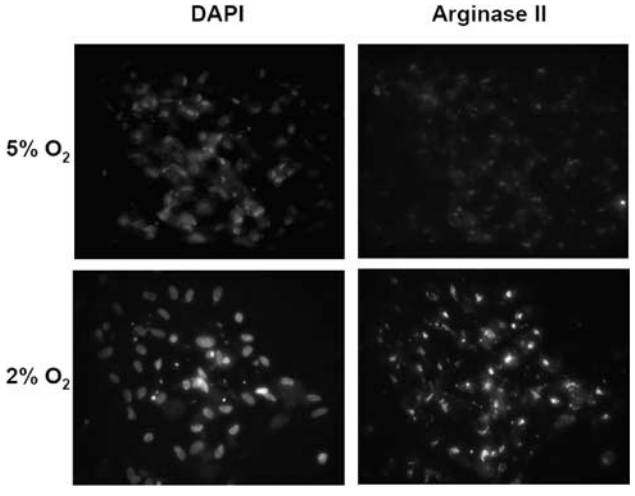

Figure 1: Expression of Arginase II in HUVEC under hypoxia. Original magnification x60.

Conclusions: In hypoxia there is a functional competition between ARGII and eNOS, which show cellular colocalization in normoxia and further more in hypoxia. ARGII overexpression could explain, at least in part, the lower NO synthesis observed in HUVEC exposed to hypoxia. Arginase inhibition could partially recover this endothelial dysfunction. Support: FONDECYT 1080534, 1070865. C Prieto and B Krause hold CONICYT PhD fellowships.

1. H. Lim *et al. Am J Physiol*, 293: H3317–H3324, 2007.
2. P. Casanello *et al. Placenta*., 30: 625–633, 2009.
3. G. Topal *et al. J Pharmacol Exp Ther*, 318: 1368–1374, 2006.

P-5C-236

Human chorionic gonadotropin and reproductive failure: genetic variation and disease-specific gene expression

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Human chorionic gonadotropin (hCG) is one of the first proteins synthesized by fetus. Besides its luteotropic function, hCG regulates implantation and immunomodulation at the maternal-fetal interface. Low level of hCG is related to miscarriage and extrauterine pregnancy whereas high hormone level may indicate a trophoblastic disease. HCG hormone consists of alpha- and beta-subunit. Critical for hCG function is the beta-subunit coded by *hCG beta* genes (*CGB, CGB5, CGB7, CGB8*) that share a common gene cluster with highly homologous luteinizing hormone beta gene and two *beta*-subunit non-coding genes (*CGB1, CGB2).* Aberrations in the expression profile and genetic variation of the hCG beta coding genes may lead to the reproductive failure.

Objectives: The aim of the study was to (i) determine the expressional profile of *CGB* genes during the normal and complicated pregnancy; and (ii) find variants in *hCG beta* coding genes that are related to recurrent miscarriage (RM, ⩾3 consecutive pregnancy losses).

Methods: The expression of *CGB* genes in trophoblastic tissues from normal and complicated pregnancies (RM, ectopic pregnancy-EP, molar pregnancy) was determined by real-time PCR. *CGB5* and *CGB8* genes that contribute the most to hCG hormone production were fully resequenced in Estonian and Finnish RM patients (n = 184) and fertile women (n = 195). Study was approved by Ethics Committee of the University of Tartu (Estonia) and Helsinki University

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Central Hospital (Finland); an informed consent was obtained from every patient.

Results: In the cases of RM, the expression level of *CGB* genes is low and correlates with reduced hCG hormone level indicating their possible involvement in the pathogenesis of miscarriage. In EP, *CGB* genes were highly expressed but the hormone concentration in serum was significantly reduced. In molar pregnancies both hCG level in serum and expression of *CGB* genes were at the upper range of the distribution compared to normal first trimester pregnancies. As a result of the resequencing study, six polymorphisms (SNPs) showed significant protective effect towards RM in *CGB5* and *CGB8* genes ($p = 0.042–0.007$) decreasing the risk to miscarry up to1.8-fold. The minor allele frequency of these SNPs was 12.05%–14.36% in fertile females compared to 7.10%–8.15% in RM group. Three non-synonymous amino acid changes in *CGB5* and *CGB8* and a rare promoter polymorphism located within transcription initiator element of *CGB8* were identified only in RM cases as possible risk variants for RM.

Conclusions: Comparison of expression levels of the *CGB* genes and hCG hormone levels in serum indicate a different contribution of *CGB* genes in the miscarriage, ectopic and molar pregnancies Changes in the expression levels of *CGB* genes may be caused by genetic variants as demonstrated in case of *CGB5* and *CGB8* with variants that either increase or decrease the risk of miscarriage. The results provide evidence that genetic factor contributes to the pathogenesis of RM. Support: HHMI, Wellcome Trust, Estonian Ministry of Education and Science, Estonian Science Foundation, Sigrid Juselius Foundation & Finnish State Fund.

P-5C-237

Vascular endothelial growth factor in placentas from diabetic pregnant rat

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Objectives: The vascular endothelial growth factor (VEGF) is a potent angiogenic factor that acts by inducting endothelial cell proliferation and increasing vascular permeability[1]. VEGF gene deletion studies show embryonic lethal phenotype associated with a failure of placental vascular development[2]. In normal pregnancy, placental VEGF expression increases near term in the rodent, in association with increased placental vascularization[3,4]. Previous results showed that diabetes promotes morphological changes in the organization of the spongiotrophoblast layer of placenta, which is related to accumulation of cystic vesicles in this region. Moreover, increased placental size has been observed[5], probably due to an increase in cell proliferation in several regions of diabetic placenta[6]. Ample data on the regulation of VEGF has demonstrated that this growth factor levels are exquisitely sensitive to multiple ischemic agents, as glucose, affecting several tissues, such as retina and renal glomerulus[7]. However, it is unknown whether diabetical condition promotes changes in the expression of placental VEGF.

Methods: To accomplish this, single injections of alloxan (40 mg kg i.v.) were used to induce diabetes on day 2 of pregnancy in Wistar rats. Placentas were collected on days 14, 17 and 20 postcoitum. We used immunoperoxidase technique to identify VEGF-A in placental sections, which was visualized and captured with a CX81 microscope and DP71 digital camera (Olympus).

Results: VEGF was immunodetected in labyrinth, spongiotrophoblast and giant trophoblast regions in placentas from 14 and 20 days of pregnancy. However, qualitative evaluation showed that in diabetical condition, the immunoreaction was stronger in the labyrinth regions.

Conclusions: We conclude that in placentas from the diabetic rat the strongest immunoreaction of VEGF in the labyrinth region, may be correlated with the increase of the vascular tree and blood vessel size observed in these diabetic placentas. Support: DIPUV (Universidad de Valparaiso, Valparaiso, Chile; grant no. 12/2006).

1. Ferrara N. *Endocr Rev,* 25:581–611, 2004.
2. Carmeliet P, *et al. Nature,* 380:435–439, 1996.
3. Ni Y, *et al. Am J Physiol*, 273:H938–H944, 1997.
4. Coan PM, *et al. Biol Reprod*, 70:1806–1813, 2004.
5. Giachini F, *et al. J Anat*. 212:31–41, 2008.
6. Zuñiga *et al. Placenta,* 29:81, 2008.
7. Benjamín L. *Am J Pathol,* 158:4, 2001.

P-5C-238

Intrauterine growth alteration and their relationship with the distribution of somatotrophic factors in the human placenta

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The placenta is a temporary organ that supplies the fetus with oxygen and food, and allows fetal waste to be disposed of via the maternal kidneys. The IGF-I and its receptor (IGF-IR) participate in both pre and postnatal growth. The human placenta expresses the mRNA and the protein for IGF-I and IGF-IR.

Objective: Study the IGF-I, IGF-II and IGF-IR mRNA and protein expression in placentas from small (SGA), appropriate (AGA) and large (LGA) for gestational age placentas. Correlate these expressions with birth and placental weight.

Methods: We collected full term (37–41 weeks) placentas from 12 SGA (birth weight (BW) = −1.99 ± 0.17 SDS), 12 AGA (BW = 0.08 ± 0.17 SDS) and 11 LGA (BW = 2.75 ± 0.22 SDS) newborns. We determined in the chorionic (CP) and basal (BP) plates of the placentas the mRNA expression by RT-PCR and the protein expression by immunohistochemistry (IHC) using the HScore signal intensity where 0 is negative staining 1: low intensity; 2: mid-intensity; and 3: high intensity.

Results: are shown in the table as mean ± SEM: The differences were studied by ANOVA and the correlations studies were studied by Spearman test. We observed an inverse correlation between birth weight with IGF-I mRNA in CP ($r = -0.456$, $p = 0.017$) and BP ($r = -0.542$, $p = 0.004$) and with IGF-IR mRNA in CP (r = − = .416; $p = 0.031$). These inverse correlations were also observed for IGF-I (IHC) in both plates of the placenta and a direct correlation in basal plate for IGF-IR ($r = 0.431$, $p = 0.011$).

Conclusions: These results suggest that the modifications in the mRNA and protein expression to IGF-I and IGF-IR observed in placentas according birth weight, may be influencing fetal growth. Support: Fondecyt 106-1082 and Dipuv 12/2006 (Universidad de Valparaíso, Chile).

mRNA		SGA (12)	AGA(12)	LGA(11)
IGF-I	CP	0.21 ± 0.02*	0.15 ± 0.02	0.11 ± 0.03
	BP	0.22 ± 0.02*	0.16 ± 0.02	0.10 ± 0.02
IGF-II	CP	0.37 ± 0.03	0.33 ± 0.05	0.30 ± 0.15
	BP	0.37 ± 0.05	0.29 ± 0.05	0.16 ± 0.09
IGF-IR	CP	0.27 ± 0.02	0.22 ± 0.04	0.08 ± 0.05[&]
	BP	0.28 ± 0.03	0.21 ± 0.02	0.06 ± 0.02[&]
Protein (IHC)				
IGF-I	CP	1.6 ± 0.3	1.5 ± 0.2	0.6 ± 0.2[&]
	BP	1.5 ± 0.4	1.4 ± 0.3	0.6 ± 0.2[&]
IGF-II	CP	1.3 ± 0.2**	2.3 ± 0.3	1.8 ± 0.4
	BP	1.9 ± 0.3	2.6 ± 0.3	1.6 ± 0.4
IGF-IR	CP	0.8 ± 0.2	1.1 ± 0.2	1.7 ± 0.3[&]
	BP	0.8 ± 0.2	1.2 ± 0.1	1.9 ± 0.3[&]

$p < 0.05$ SGA vs LGA; **$p < 0.05$ SGA vs AGA; [&] $p < 0.05$ SGA and AGA vs LGA.

P-5C-239

First trimester adiponectin and subsequent development of preeclampsia or fetal growth restriction

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Objective: The purpose of this study is assessing the utility of determining the maternal serum concentration of adiponectin (ApN) as a marker for insulin-resistence in the prediction of Preeclampsia (PE) and Fetal Growth Restriction (FGR) during the first trimester.

Methods: A prospective, case-control study was conducted in which 1094 pregnant women who received the 11–14 wk ultrasound screening and delivered their babies at University of Chile's Clinical Hospital's Fetal Medicine unit were enrolled. Informed consent and blood samples were obtained and kept at −80°C (−112°F) for future analysis. Among this population, we recruited 10 women who developed PE, 10 who developed IUGR and a control group of 40 healthy women. ApN concentrations in maternal serum were determined using a commercial ELISA kit. PE was defined as recommended the International Society for the Study of Hypertension in Pregnancy[1]. FGR was defined as growth at the 10th or less percentile for weight of all fetuses at that gestational age using a growth curve representative of Chilean population[2]. We studied the relationship between maternal serum concentration of ApN and variables like newborn weight and maternal BMI. Institutional ethics committee clearance and participants' informed consent were obtained.

Table 1. Maternal and neonatal demographic characteristics.

	IUGR (10)	PIH (10)	Controls (35)	p
Age, years				
Mean	31.6 (6.7)	31 (8.6)	28.6 (6.6)	0.60
Range	20–40	17–45	16–39	
Primigravida n (%)	6 (60)	6 (60)	17 (48.5)	0.77
Tobacco n (%)	0 (0)	0 (0)	3 (8.5)	
Alcohol n (%)	0 (0)	0 (0)	1 (2.8)	
BaW (Mean)	2358.1 (256.3)	2584.5 (1127.0)	3327.1 (655.1)	0.017
BMI	23.6 (3.4)	26.2 (3.0)	25.3 (3.6)	0.84

Maternal and neonatal demographic characteristics and results for cases and controls recruited in this study.

Results: There were no significant differences between studied groups regarding age, parity, gestational age and tobacco consumption (Table). There were no significant differences among first-trimester ApN serum levels in the groups. Average concentration was 8, 6.8 and 10.8 ng/ml for controls, PE and FGR groups, respectively. However, there was a significant difference between the groups alter adjusting for BMI ($p < 0.046$). There was also a significant inverse relationship between ApN levels and maternal BMI ($r = -0.36$; $p = 0.005$) except for the control group (Figure). A significant negative relationship ($r = -0.37$; $p = 0.002$) was found when correlating maternal ApN levels with weight percentiles at birth for the whole group (60 patients). Similar results were obtained when analyzing both variables in the control group ($r = -0.36$; $p = 0.002$).

Conclusions: In our study, maternal serum ApN levels were not useful in predicting development of PE and FGR.

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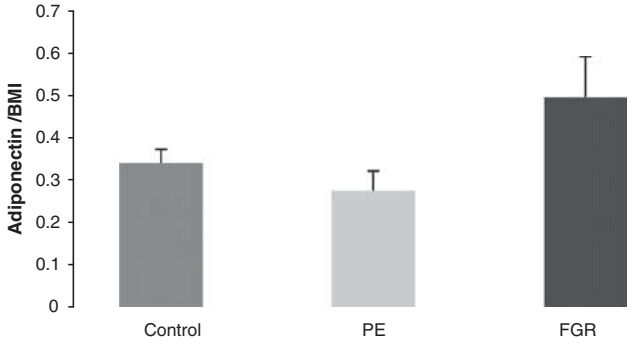
However, maternal serum ApN concentration adjusted by BMI was significantly higher during the first trimester in women who developed FGR. It is also of interest the negative relationship between ApN levels and weight at birth. Support source: University of Chile: 033/03.



1. Brown MA *et al. Hypertens Pregnancy*. 20:9–14, 2001.
2. Juez G *et al. Rev Chil Pediatr*. 60:198–202, 1989.

P-6A-240

The effect of neonatal hyperglycaemia in preterm lambs on prepubertal pancreatic function

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Objective: Exposure of the late gestation fetus to high blood glucose concentrations (BGC) results in decreased insulin secretion in later life. Preterm babies who become hyperglycaemic, a common problem, are exposed to high BGC at an equivalent stage of pancreatic maturation, and it is known that preterm babies have decreased insulin sensitivity in later life. We hypothesised that exposure of the preterm newborn lamb to prolonged neonatal hyperglycaemia would result in altered insulin secretion and glucose tolerance post-weaning. ***Methods:*** Singleton preterm lambs (137d gestation; term = 148d) were born following antenatal glucocorticoids. Vascular catheters were inserted the following day and lambs randomised to a 12-day intravenous infusion of 50% dextrose (hyperglycaemic (H)), saline (controls (C)) or 50% Dextrose + insulin (insulin (I)). Term controls (T) were not exposed to glucocorticoids and received an infusion of saline. 50% dextrose and insulin infusions were titrated to maintain BGC at 10–12 mM (H) or 4–6 mM (I) respectively. At 4 months of age, after an overnight fast, the lambs underwent an intravenous glucose tolerance test (GTT) and several days later a 2 hour hyperglycaemic clamp (HGC) followed by an arginine challenge (AC). *Statistics:* Area under the curve (AUC) was calculated from baseline. Parametric data were analysed by ANOVA. Non parametric data were log transformed and analysed by ANOVA. Insulin sensitivity (S_I) was calculated from the HGC during the last hour of steady state. Data were analysed using univariate and multiple regression techniques taking into account sex, birth-weight, weight at challenge, and glucocorticoid exposure. Data are presented as mean(SD).
Results: 48 lambs underwent GTT (12C, 12H, 12I, 12T), 29 underwent HGC (7C, 8H, 7I, 7T), and 33 underwent AC (9C, 8H, 8I, 8T). There was no difference in baseline glucose concentration (C 3.4(0.4), H 3.6(0.5), I 3.7(0.5), T 3.8(0.4) mM, $p = 0.23$), baseline insulin concentration (C 0.09(0.13), H 0.05(0.04), I 0.07(0.07), T 0.06(0.03) ng/ml, $p = 0.62$), or baseline insulin: glucose ratio (C 0.02(0.03), H 0.02(0.01), I 0.02(0.02), T 0.02(0.01), $p = 0.70$) amongst the groups. Neither GTT glucose AUC (C 680(200), H 585(156), I 643(195), T 701(147) mM/min, $p = 0.40$), nor GTT insulin AUC (C 120(49), H 154(74), I 123(29), T 189(80) ng/ml.min, $p = 0.25$) were different amongst the groups. There was no difference in the S_I between the groups (C 22(13), 16(9), I 13(6), T 11(3) $\mu mol\,kg^{-1}\,min^{-1}\,ng^{-1}\,ml^{-1}$, $p = 0.16$). There was no difference in mean plasma insulin concentration during the HGC steady state (C 1.8(0.7), H 2.9(1.4), I 3.2(1.6), T 2.8(0.7) ng/ml, $p = 0.16$) nor insulin secretion AUC after AC between the groups (C 106(57), H 118(72), I 119(52), T 140(55) ng/ml.min, $p = 0.70$). Adjustment for sex, birth-weight, weight at challenge, and glucocorticoid exposure did not alter these results.
Conclusions: Neither exposure to high blood glucose concentrations nor intravenous insulin in preterm lambs altered insulin secretion or insulin resistance at 4 months of age. Insulin resistance can change from pre-puberty to adulthood and it will be important to re-challenge these animals again in adulthood.

P-6A-241

Developmental body changes and AMPK activity in adult obese rats exposed to α-gluco-oligosaccharide BioEcolians® supplementation in early life

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Objective: Obesity is a worldwide health problem with an estimated 500 million overweight adult humans and 250 million obese adults. This epidemic of obesity is leading to worldwide increases in the prevalence of obesity related disorders, such as diabetes, hypertension as well as cardiac pathology and fatty liver disease. The early postnatal nutritional is recognized as a factor contributing to the development of metabolic diseases in adulthood. A change in the quality of calories-specifically, increased carbohydrate

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intake in the immediate postnatal period, results in chronic hyperinsulinaemia and adult-onset obesity. The aim of this study was to show that a α-gluco-oligosaccharide supplementation at early life could protect against obesity developed after consuming high fat diet.
Methods: Thirty Wister male rats were randomly divided in 4 groups and received during 16 weeks either a normal diet, a high fat diet (HFD) or BioEcolians® supplemented HFD.
Results: Remarkably, at 15 weeks of age, BioEcolians® HFD rats took less weight and presented a lowest total and abdominal adiposity as compared to the HFD animals. At this stage, a significant ($P < 0.01$) decrease of food intake was observed in the BioEcolians® supplemented diet with a diminution of leptin and triglycerides levels. As compared to HFD group, BioEcolians® supplementation reversed the HFD-induced decreases in the AMP-activated protein kinase (AMPK) expression and phosphorylation at protein level in the liver.
Conclusions: These results demonstrate that a non digestible oligosaccharide BioEcolians® supplementation during the early postnatal life could protect against overweight and obesity by stimulating the tissular energetic consumption and inhibiting fat storage during growth. It is established that regular consumption of a non digestible gluco-oligosaccharide during growth is able to modulate energy balance and energy expenditure which underscores the importance of considering the microbiota as a metabolic organ.

P-6A-242

Preterm birth in lambs increases insulin sensitivity at 4 months of age, but increased growth velocity in preterm lambs is associated with insulin resistance

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Objective: Decreased growth *in utero* followed by increased postnatal growth increases insulin resistance. Increased growth in children born preterm has been associated with increased pre-pubertal insulin resistance. We hypothesised that preterm birth, and postnatal growth velocity, would affect glucose tolerance and insulin sensitivity at 4 months of age.
Methods: Singleton preterm (P) lambs (137d gestation; term = 148d) were born following antenatal glucocorticoids. Term controls (T) were not exposed to glucocorticoids. Lambs were weighed twice weekly for 2 weeks and then monthly. Vascular catheters were inserted the day after birth and lambs randomised to a 12-day intravenous infusion of 50% dextrose, saline or 50% dextrose + insulin as part of an experiment investigating the effect of neonatal hyperglycaemia. At 4 months of age, after an overnight fast, the lambs underwent an intravenous glucose tolerance test (GTT) and subsequently a 2 hour hyperglycaemic clamp (HGC). *Statistics:* Growth velocity (GV) was calculated from birth to term corrected age (GV pre-TCA) and from TCA to 4 months (GV post-TCA). Insulin sensitivity (S_I) was calculated from steady state during the HGC. Continuous variables were correlated by bivariate analysis. Data were analysed using univariate and multiple regression techniques adjusting for sex, birth-weight, insulin and dextrose exposure. Data are presented as mean(SD).
Results: 48 lambs underwent GTT (36P, 12T) and 29 underwent HGC (22P, 7T). Preterm lambs were heavier at TCA than term lambs at birth (7.4(1.1) vs 6.1(1.2) kg, $p < 0.001$); term lambs grew faster from TCA to 4 months (43(13) vs 28(9) g/kg.d, $p < 0.01$), and were heavier at 4 months (37.3(5.0) vs 31.1(6.3) kg, $p < 0.005$). Preterm lambs had decreased GTT insulin AUC (114(54) vs 171(78) ng/ml.min, $p = 0.03$), but not glucose AUC (636(184) vs 701(147), $p = 0.23$), compared with term lambs. S_I was not significantly different on univariate analysis (P; 17(10), T; 11(3) $\mu mol\,kg^{-1}min^{-1}ng^{-1}ml^{-1}$, $p = 0.07$), but was increased in preterm lambs on multivariate analysis ($p < 0.05$). GV pre-TCA in preterm lambs was not associated with glucose tolerance on univariate analysis, but on multivariate analysis decreased GV was associated with an increased GTT insulin AUC ($p = 0.02$) and insulin: glucose AUC ($p = 0.01$), but not with S_I ($p = 0.2$) or GTT glucose AUC ($p = 0.4$). Increased GV post-TCA in preterm lambs was associated with an increased GTT insulin AUC ($r^2 = 0.37$, $p = 0.0001$) and a decreased S_I ($r^2 = 0.25$, $p = 0.02$). GV from birth to 4 months of age in term lambs was not significantly associated with GTT insulin AUC ($r^2 = 21$, $p = 0.15$), but an increased GV tended to be associated with a decrease in S_I ($r^2 = 0.54$, $p = 0.06$).
Conclusions: Preterm lambs had increased insulin sensitivity at 4 months of age. Preterm lambs had an increased growth rate *ex utero* compared with lambs that remained *in utero* until TCA, but a decreased growth rate thereafter. Impaired insulin sensitivity was associated with decreased growth pre term-corrected-age and increased growth post term-corrected-age. Insulin sensitivity in preterm lambs may therefore depend on both prematurity and subsequent growth trajectory.

P-6A-243

Maladaptive cardiac remodelling as a result of preterm birth: Implications for future cardiovascular disease

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Objective: Preterm birth is the major cause of infant death and morbidity, affecting 10–12% of all pregnancies.

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Preterm birth occurs when the myocardium is still developing. Our hypothesis was that in response to preterm birth, maladaptive structural remodelling occurs within the myocardium, which enables the immature heart to adapt to the hemodynamic transition at birth but permanently alters myocardial structure. Our objective was to determine how preterm birth alters the final structure of the myocardium.

Methods: Using sheep, preterm birth was induced at 0.9 of term; hearts were examined at 9 weeks post term-equivalent age, when cardiomyocyte maturation has ceased. As the right ventricle (RV) and left ventricle with septum (LV + S) have different roles *in utero* and *ex utero*, they were examined separately. A multi-modality approach was used to examine the structure of the heart and cardiomyocytes. Cardiomyocyte number was determined using optical disector/fractionator stereology. Cardiomyocyte nuclearity (the number of nuclei within the cell, a marker of cardiomyocyte maturity) and cardiomyocyte nuclei ploidy (number of genome copies per nucleus) were examined using confocal microscopy. Immunohistochemistry (Ki-67) was used to assess cell proliferation. All experimental procedures were approved by the Monash University Animal Ethics Committee.

Results: Preterm lambs were significantly lighter at birth than term controls (3.37 ± 0.24 kg and 4.39 ± 0.17 kg respectively; $p < 0.05$), but weights were not different at necropsy (preterm, 17.10 ± 0.59 kg vs. term, 17.14 ± 0.91 kg). At necropsy, heart weights relative to body weight were not different between groups (preterm, 6.32 ± 0.52 g/kg vs, term 6.62 ± 0.61 g/kg). There were no differences in cardiomyocyte number in the RV or LV + S between preterm lambs and controls. There was, however, evidence of altered cardiomyocyte maturation in preterm lambs. There was a greater proportion of mononucleated cardiomyocytes in both ventricles of preterm hearts compared to controls (in the RV: $4.7\% \pm 0.7\%$ in preterm lambs and $1.4\% \pm 0.2\%$ in controls; $p < 0.01$ and in the LV + S: $8.9\% \pm 0.9\%$ in preterm lambs and $2.0\% \pm 0.3\%$ in controls; $p < 0.01$). Importantly there was a marked induction of polyploidy in preterm cardiomyocytes; this is normally associated with irreversible stress-related changes in DNA. In preterm lambs we also found a 6–7-fold increase in collagen deposition in both the RV (preterm = $2.90 \pm 0.52\%$, term = $0.44 \pm 0.11\%$; $p < 0.01$) and LV + S (preterm = $3.44 \pm 0.79\%$, term = $0.71 \pm 1.32\%$; $p < 0.01$) and this was usually accompanied by lymphocytic/mast cell infiltration.

Conclusion: We conclude that preterm birth *per se* leads to maladaptive remodelling of the heart in the early postnatal period. Of particular concern are the derangements of cardiomyocyte maturation, induction of polyploidy and increased extra-cellular matrix deposition. These changes are likely to program for long term cardiac vulnerability to later disease.

P-6A-244

Prolactin inhibition during lactation programs for metabolic syndrome parameters in adult rats

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Objective: Maternal undernutrition during lactation reduces milk production and serum prolactin (PRL)[1] as well as programs for overweight in adulthood[2]. Previously, we observed that the inhibition of lactation by dam's treatment with bromocriptine (BRO – a PRL inhibitor) reduces milk production[3] and programs for overweight, hyperleptinaemia, leptin resistance[4] and hypothyroidism in adult rat offspring[5]. The aim of our study was to analyze the parameters associated with metabolic syndrome (blood pressure, insulin sensitivity and lipid profile) in adult rats whose dams received BRO at the end of lactation (a precocious weaning model).

Methods: We used the pups from lactating wistar rats treated with BRO (1 mg/2x a day) or saline in the last 3 days of lactation (days 19, 20 and 21). Body weight and food intake were followed from weaning until adult life. Blood pressure was determined by a non-invasive method putting a cuff in rat tail. At 180 days-old, offspring were sacrificed, blood was collected, visceral fat was weighed and carcass was frozen for total body fat mass determination. Serum glucose, triglycerides, total cholesterol and high density lipoprotein (HDL-c) were quantified by colorimetric assays. Insulin and adiponectin were measured by radioimmunoassay. HOMA index was calculated. Results were significantly different when $p < 0.05$.

Results: When adult, BRO offspring had higher body weight (+10%), visceral fat (+2x), total fat mass (+41%), glycemia (+16%), HOMA index (+29%), total cholesterol (+30%) and triglycerides (+49%); with lower HDL-c (−28%) and adiponectin (−47%). There was no change in food intake, serum insulin and blood pressure.

Conclusions: BRO animals presented important alterations (higher central adiposity, hyperglycemia, insulin resistance, hypertrygliceridemia and lower HDL-c) related to metabolic syndrome development. This experimental model reproduces the shortening of lactation period, reinforcing the idea that neonatal malnutrition is an important for metabolic programming of future diseases. Support: FAPERJ, CAPES, CNPq.

1. P.C. Lisboa *et al.*, *Horm Metab Res.*, 38:295–299, 2006.
2. M.C.F. Passos *et al.*, *Nutr Res.*, 20:1603–1612, 2000.
3. I.T. Bonomo *et al.*, *Horm Metabol Res.*, 37:220–225, 2005.

4. I.T. Bonomo *et al.*, *J Endocrinol.*, 192:339–344, 2007.
5. I.T. Bonomo *et al.*, *J Endocrinol.*, 198:331–337, 2008.

P-6A-245

Body mass index and height growth from birth to 4 y and body composition at 5 y in Chilean children with normal birthweight

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Objective: To assess the association between body mass index (BMI) and height growth in different periods from 0 to 4 years and adiposity, body fat distribution, and lean body mass at 5 years of age, in children with normal birthweight.
Methods: We studied a subsample of 142 girls and 164 boys of a retrospective cohort study conducted in low-income Chilean children with a birth weight >2500 g. We obtained weight and height from medical records at: birth, 6, and 24m. At 48 and 60m we measured weight, height, and waist circumference (WC). At 60m, we additionally determined body composition [percentage body fat (%FM) and lean body mass (LBM)] using bioimpedance analyses (Tanita 418-B). We analyzed the associations of 3 periods of postnatal growth (0 to 6m, 6 to 24m and 24 to 60 months) with WC, %BF, and LBM, adjusting for age and sex. We used standardized coefficients to assess the relative importance of each period on the outcomes. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Mean birthweight was 3400 ± 400 g. and mean length, 49.8 ± 1.7 cm.Compared to the WHO 2006 reference population, children became increasingly fatter with age (obesity (BAZ > 2) at 5y = 16.4%) while height remained close to the mean (HAZ at 5y = 0.01 ± 1.1). At 5y mean WC was 56.3 ± 5.8 cm, %BF 23.3 ± 4.1, and LBM 17.1 ± 2.4 kg. Increasing BMI after 6m was positively associated with WC, %BF, and LBM at 5y ($p < 0.05$). Increased linear growth after 24 m was associated with higher WC, %BF, and LBM ($p < 0.05$). BMI gains 6-24 m were similarly related to WC, %BF, and LBM, while BMI gains after 24 m were more related to AC and %BF than LBM (Figure 1). The pattern and magnitude of the associations of BMI gains 6–24 m and body composition at 5y were similar to those of linear growth 24–48 m (Figure 2).
Conclusions: In order to decrease obesity and central obesity in preschool children excessive BMI gains should be avoided after 6 months. Associations between linear growth after 2y and both overall obesity and central obesity at 5y may reflect earlier maturation of obese children. Support: Fondecyt # 1090252 and Training Fellowship Wellcome Trust.

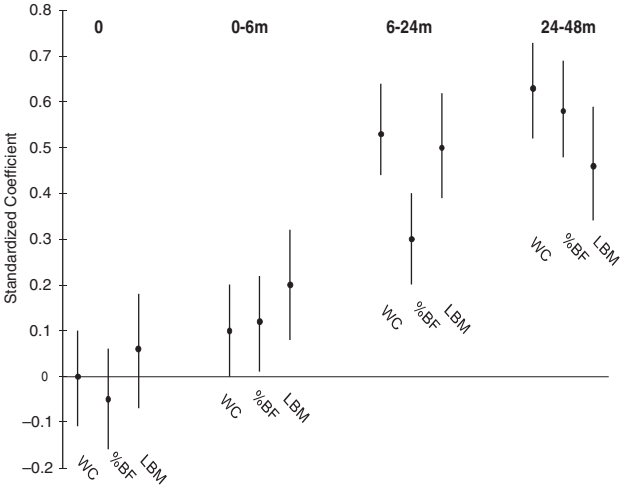

Figure 1: BMI changes 0–4y and body composition outcomes at 5y.

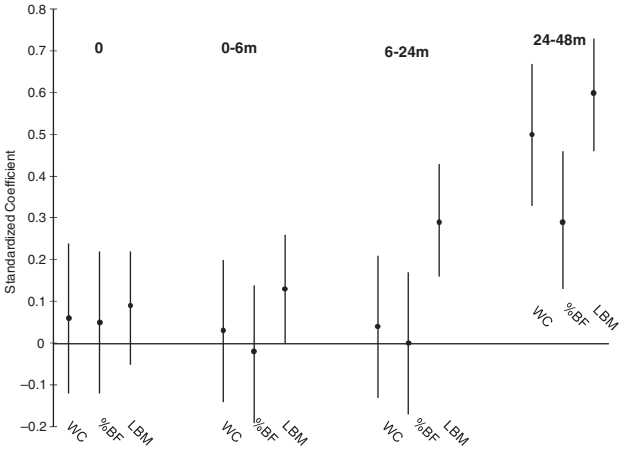

Figure 2: Height changes 0–4y and body composition outcomes at 5y.

P-6A-246

Association between catch-up in the first years of life and overweight/obesity at 10 years of age, in a low-income community in São Paulo, Brazil

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In the last years it has been shown that weight gain velocity is a determinant of future obesity. These findings are mainly from developed nations, and few studies from developing countries showed this association[1,2].

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Objective: To test if the weight gain velocity during different periods of age (0–6 m, 6–12 m, 12–24 m, 2–5y and 5–10y) is associated with overweight/obesity at 10 years of age.
Method: A historic cohort was made, based on clinical records from the Social Project of Einstein Hospital at the Paraisopolis Slum. All children, who were enrolled in the health service between October 1998 and August 1999 (845) were selected to participate in the study. Exclusion criteria were having some base health condition (neurological or endocrinological diseases), being malnourished and not having complete records. A final sample of 378 eutrophic and overweight/obese children was analysed. Weight gain velocity was categorized according to the quartile of its distribution. A multivariate logistic analysis looked for the independent effect of weight gain in each age period. The outcome was having a BMI at age 10 greater than 1.5 z-score. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The mean birth weight was 3.15 kg (1.33–4.89); 95% of the children were term at birth and 66% had a vaginal delivery. The median length of breastfeeding was 4 months and of exclusive breastfeeding was 2 months. The mean z-score of BMI at age 10y was 0.3 (−1.25–+3.74). After adjusting for birth weight and for being small-for-gestational-age, the effect on the BMI at age 10y of passing to a higher quartile of weight gain in every age period was OR 4.51 (1.97–10.32) at 0–6 m; 2.20 (1.19–4.06) at 6–12 m; 1.30 (0.78–2.19) at 1–2y; 4.23 (2.24–7.98) at age 2–5y and 1.96 (1.14–3.37) at age 5-10y (reciprocally adjusted).
Conclusions: Weight gain during the first 6 months of life was the main determinant of overweight/obesity at age 10y in children from a low income community in São Paulo, Brazil.

1. J.C.K. Wells *et al. Int J Obes*, 29:1192–1198, 2005.
2. E.M. Taveras *et al. Pediatrics*, 123:1177–1183, 2009.

P-6A-247

Assessment of cardiac function with trans-thoracic echocardiography in adult vitamin D deficient rats

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There is an increasing prevalence of vitamin D deficiency in many populations world-wide, resulting from both inadequate exposure to ultraviolet light and diet intake. It is well know that vitamin D deficiency is associated with heart disease. We have recently demonstrated in the rat heart that vitamin D deficiency leads to cardiac hypertrophy and vulnerability to ischemia later in life; female offspring appear to be the most vulnerable.

Objective: The aim of the present study was to investigate the effect of vitamin D deficiency on cardiac function, using echocardiography, in 14 week old adult rats.
Methods: Four week old Sprague-Dawley female rats were fed either a vitamin D deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. At weaning the offspring remained on their respective diets until adulthood. At 14 weeks of age non-invasive trans-thoracic echocardiography was performed in female offspring ($n = 10$ control and $n = 9$ vitamin D deficient). M-mode echocardiography images were obtained in the parasternal long and short axis views of the left ventricle. Anterior, posterior end-diastolic and end-systolic wall thickness, left ventricle internal dimensions and inter-ventricular septum were measured.
Results: Body weight was not different in control and vitamin D deficient offspring at 14 weeks of age. Left ventricular weight and left ventricular weight to body weight ratio was significantly increased ($p < 0.001$) in the vitamin D deficient offspring. This was accompanied by a significant decrease in diastolic volume ($p = 0.0002$) but no difference in systolic volume. In addition, stroke volume and cardiac output in the vitamin D deficient offspring was significantly reduced ($p = 0.001$). Fractional shorting and ejection fraction was unaltered in both control and vitamin D deficient female offspring.
Conclusion: Vitamin D deficiency leads to cardiac hypertrophy and impaired cardiac function in female vitamin D deficient rats. The findings are in agreement with clinical data where vitamin D deficiency is linked to heart disease.

P-6A-248

Early life growth patterns influence gene expression and DNA methylation in childhood

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Objective: Nutrient-mediated molecular changes that occur during early life may influence gene expression and produce functional changes which persist throughout life. Evidence suggests that epigenetic mechanisms are involved in this nutritional programming. We postulate that early nutritional events in postnatal life cause aberrant epigenetic marking and precipitate altered expression of specific genes that in turn result in changes in body composition and metabolic health in childhood.
Methods: Gene expression analysis was carried out using NuGO whole genome microarrays on RNA extracted from

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peripheral blood in 12 low thrive and 12 high thrive children, the 2 groups divided evenly by sex. 'Thrive' was determined by difference in z-score for weight between term and term plus 12 weeks of age. Children were drawn from 2 nutritional intervention studies of preterm infants carried out in early postnatal life (n = 136). Anthropometric, biochemical and nutrient markers in early life and at 10–12 years of age were collected. Blood and saliva samples were collected at age 10–12 years and DNA and RNA analysis carried out on whole blood. Up-regulation of gene expression in low thrive versus high thrive children was assessed. Sex specific analysis was undertaken due to the widely reported gender dichotomy observed in developmental programming. The level of methylation of *TACSTD2* was analysed by Pyrosequencing following bisulphite modification. 7 CpG sites, −467 to −427 from the transcription start site, within the CpG island of the predicted promoter region were analysed. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: 245 loci were upregulated in low thrive females versus high thrive females and 352 in low thrive males versus high thrive males with 28 of these loci being common to both sexes. Of these genes *TACSTD2* was selected for further investigation. *TACSTD2* was up-regulated 2.56-fold in males and 4.07-fold in females in children aged 10–12 years who were low thrive compared to those who were high thrive in infancy ($p < 0.0001$ for low thrive vs high thrive). Pyrosequencer analysis showed differential methylation of the *TACSTD2* promoter in blood from the cohort of children aged 10–12 years; DNA methylation was lower in low thrive individuals compared to high thrive (mean methylation across 7 CpG sites versus 'thrive'; Spearman correlation coefficient 0.335, $p = 0.015$).

Conclusions: Preterm infants who grew differently in early post natal life display considerable differences in gene expression levels and gene-specific DNA methylation levels at age 10–12 years. These data provide proof of principle in a human cohort of programmed changes in gene expression which are associated with early life exposures and the data further suggest that such programmed effects may be mediated by epigenetic mechanisms. *TACSTD2* encodes a cell surface calcium signalling protein which has been implicated in cellular proliferation. Further work is underway to relate the observed differential expression and methylation to childhood phenotypic traits and subsequent metabolic health. Support: BBSRC (BBF0079811).

P-6A-249

Gender differences in child health: Evidence from India

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Objective: The WHO Global Database on Child Growth and Malnutrition proves that gender disparity in child malnutrition in India is higher compared to other developing countries. Most of previous research has not taken birth order of children and mothers' autonomy in households into account when studying gender differences in child nutrition and anthropometry. In particular, it does not have to be the case that all girl children are discriminated. Hence, the aim of this study is to add information about birth order, infant feeding practices and autonomy to explain gender differences in child health measured in terms of height.

Methods: India's National Family Health Surveys (NFHS-2 and NFHS-3) conducted between 1998 (n = 24,600 children) and 2006 (n = 46,605 children) provides in-depth information about HAZ, WHZ and WAZ scores along with the sex and age of the child. Using data of these children born in the three years preceding both surveys, gender inequality in anthropometry was analysed. Logistic regression analysis was used to predict the probability of stunting. Along with socio-economic variables, sex and birth order variables were added as one of the predictors of stunting. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: There is no association between mothers' autonomy and child stunting. However, birth order of the child has a significant impact on the probability of stunting. Based on the NFHS-2 data, the odds of stunting is lower for the first female child compared with the second female child. The odds ratio of stunting increases significantly when mothers discard their colostrums. However, male and female heights are not significantly different based on the children included in the NFHS-3 survey.

Conclusion: Birth order of the child was one of the important determinants of childhood stunting even after controlling for breast feeding, occupational and income related characteristics. Based on the analysis, we can argue that first female child is not discriminated. However, subsequent female children are likely to suffer from stunting.

P-6A-250

Anthropometric evidence of gender inequality in India during the nineteenth and twentieth centuries

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Objective: Recently, declining gender equality in India and South Asia has received considerable attention. Indicators such as excess female mortality, decline in the sex ratio, child nutrition, educational gap, and access to resources document a strong gender bias against females in modern India. However, there is no evidence to support the existence of

gender inequality in historical periods due to the lack of appropriate data. The aim of the research is to explore gender inequality in India using anthropometric data from the nineteenth and twentieth centuries.

Methods: During the indentured system, hundreds and thousands of Indian labourers migrated to Jamaica, Fiji, South Africa and Mauritius for sugarcane plantation. The ship record data of more than 60,000 men and women that migrated between 1842 and 1916 to these four countries are provided by Lance Brennan, John McDonald, and Ralph Shlomowitz. The data were stored in the National Archives of the respective countries. For the post 1960, National Family Health Survey-3 (NFHS-3) data were used. The NFHS-3 is nationwide survey conducted with a representative sample of households throughout the country between 2005 and 2006. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Height can be used as a measure of net nutrition and living standards. The mean height of the men and women were organized by their birth cohorts. The indentured record data show that the South Indian women, born between 1840 and 1890, were doing better than the North Indian women. The mean Indian gender dimorphism in height increased for those born between 1955 and 1984 (n (males aged 15–54, 74,369), (females aged 15–49, 123,385)). However, there is a strong regional variation. The height of women from Kerala has increased by 3 centimetres from 1955 to 1984. Tamil Nadu state also showed a significant improvement in the heights of males (2.8 cm) and females (nearly 2 cm) from 1955 to 1984. However, one has to consider 'female robustness' hypothesis while using gender dimorphism in height as an indicator for gender inequality.

Conclusion: South Indian states had a lower gender dimorphism compared with the North Indian states even in the historical periods. Height can be successfully used as an indicator not only to understand secular trends but also gender inequality.

P-6A-251

Weight gain in childhood and blood lipids in early adulthood

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Objective: To assess the effect of weight gain in childhood on HDL cholesterol and triglyceride levels in early adulthood.

Methods: A population-based birth cohort carried out in Pelotas, southern Brazil. All newborns in the city's hospitals were enrolled in 1982. The subjects were followed-up on several occasions during childhood. In 2004–5 (mean age 23 years), we attempted to trace the whole cohort and obtain blood samples. Conditional growth modelling was used to assess the association between lipids (HDL cholesterol and triglycerides) and weight gain from birth to 20, and from 20 to 42 months. Adjusted analyses controlled for household assets index, family income, maternal schooling at birth, maternal smoking during pregnancy, and breastfeeding duration.

Results: In 2004–5, we interviewed 4297 subjects, with a follow-up rate of 77.4% of the original cohort, and 3911 blood samples were available. Mean HDL and triglycerides levels were 56.7 mg/dl and 106.5 mg/dl, respectively. Birthweight for gestational age and weight gain in the first 20 months were not associated with triglycerides in early adulthood. On the other hand, subjects whose weight gain from 20 to 42 months of age was faster than that predicted from birthweight and weight-for-age z-score at mean age of 20 months had higher triglycerides [regression coefficient 3.83 (95% confidence interval: 1.46; 6.21)]. HDL cholesterol was more strongly related to weight gain from birth to 20 months [regression coefficient for conditional growth: 1.81(95% confidence interval: 1.31; 2.31)] than to weight gain from 20 to 42 months [regression coefficient for conditional growth: 0.60 (95% confidence interval: 0.15; 1.06)].

Conclusion: Weight gain from 2 to 4 years is related to an atherogenic lipid profile in early adulthood, but earlier weight gain is not.

P-6A-252

Vitamin B12, folate and homocysteine in the six month old infant

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Folate and vitamin B12 (cobalamin) are important for DNA synthesis and one-carbon donation for methylation. DNA methylation is one mechanism responsible for epigenetic imprinting[1].

Objectives: To investigate some of the nutritional biomarkers of homocysteine and methyl metabolism in a cohort of infants, whose mothers were studied during pregnancy, and after birth and to assess the relationship (if any) between maternal and infant values.

Methods: Data on women and their children were collected in a prospective longitudinal study conducted at the John Hunter Hospital, Newcastle, Australia. Maternal fasting blood samples were collected at approximately 18 and 36 weeks gestation, and at 13 and 26 weeks postpartum (*n* 175). At approximately 26 weeks (six months) after birth some infants provided a non-fasting blood sample (*n* 48). All samples were assayed for plasma folate and/or red cell folate, plasma vitamin B12, and a sub-set were assayed for

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plasma homocysteine (*n* 16). Data are from healthy, singleton, term infants.

Results: A summary of the median values for each infant biomarker provided below.

Infants (6 mo)	Plasma vitamin B12 (pmol/L)	Plasma folate (nmol/L)	Red cell folate (nmol/L)	Plasma homocysteine (μmol/L)
n	48	28	23	16
Median	265.5	43.1	928	9.3
10^{th}, 90^{th} percentile	114, 441	28.7, 45.4	716, 1988	4.6, 12.9
Reference intervals	135–600	7.0–34.0	315–1420	5.0–15.0

Plasma vitamin B12 and infant folate (z-scores) were significantly lower ($P < 0.001$) for infants who were being breastfed at six months (*n* 32), compared to those receiving no breastmilk (*n* 16). This was inversely correlated ($r -0.652$) with a significantly higher (P 0.016) plasma homocysteine level (*n* 16). There were no significant differences between paired maternal and infant biomarkers at six months after birth. The infants born to mothers who took folate supplements either before and/or during pregnancy did not have higher folate z-scores than infants born to mothers who did not take them. However, the averaged value of maternal plasma folate in pregnancy inversely predicted the infant's level of plasma homocysteine at six months (R^2 0.806, P 0.023).

Conclusions: These data emphasise the potential importance of maternal nutrition in pregnancy and postpartum, as well as infant feeding mode, for programming the future health of the offspring. Biomarkers of methylation, like vitamin B12, folate and homocysteine, require further investigation in humans, especially in conjunction with studies which confirm alterations in gene expression.

1. The American Association for the Advancement of Science, www.sciencemag.org/feature/plus/sfg/resources/res_epigenetics.dtl, 25 June 2008.

P-6A-253

Synergistic role of nerve growth factor and breast milk fatty acids in mothers delivering low birth weight babies at term

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Objective: The successful progression of pregnancy depends on the complex interactions between numerous biological molecules within the uterine microenvironment. This involves an interaction of intracellular and extracellular factors including micronutrients, hormones, adhesion molecules, growth factors and immunomodulators that determine the fetal growth outcome. Nerve growth factor (NGF) is important for pre and post natal brain development. Low birth weight (LBW) is a key determinant of neonatal mortality, morbidity, subsequent growth and developmental retardation and early onset of adulthood diseases. Reports suggest that breastfeeding plays a role in preventing the neurological consequences of growth retarded babies. The present study therefore examines the association of circulating levels of NGF and breast milk fatty acids in women delivering normal birth weight babies (NBW) and LBW babies at term (≥37 weeks gestation).

Methods: Singleton pregnant women delivering NBW babies (≥2.5 kg; n = 61) and LBW babies (<2.5 kg; n = 32) at term without any pregnancy complications were recruited at Bharati hospital Pune, India. The long chain polyunsaturated fatty acids were estimated using the gas chromatograph. The omega 3 fatty acids included alpha linolenic acid, eicosapentaenoic acid and docosahexaenoic acid while omega 6 fatty acids included linoleic acid, gamma linolenic acid, di-homo-gammalinolenic acid, docosapentaenoic acid and arachidonic acid. The study was approved by institutional ethics committee and participants' informed consent was obtained. Maternal and cord plasma NGF levels were analyzed using promega kits.

Results: Maternal plasma NGF levels were significantly increased ($p < 0.01$) in LBW (359.71 ± 90.66 pg/ml) as compared to NBW (286.71 ± 110.25 pg/ml) group. Similar increase in cord NGF levels was seen in LBW (171.67 ± 114.89 pg/ml) as compared to NBW (125.85 ± 60.77 pg/ml) group. Breast milk docosahexaenoic acid concentrations were increased ($p < 0.01$) in mothers delivering LBW babies (0.29 ± 0.17 g/100 g fatty acids) as compared to NBW babies (0.20 ± 0.11 g/100 g fatty acids). Maternal plasma NGF levels showed a positive association ($r = 0.254$, $p = 0.023$, $n = 80$) with milk omega 6 fatty acids. Cord plasma NGF levels were negatively associated with baby weight ($n = 85$, $r = -0.240$, $p = 0.025$), head circumferences ($n = 84$, $r = -0.211$, $p = 0.05$) and chest circumferences ($n = 84$, $r = -0.258$, $p = 0.017$).

Conclusions: Increased levels of NGF together with the increased docosahexaenoic acid content in breast milk may have most important implication as the adaptive changes to spare/protect early brain development. Breast feeding LBW babies may be advantageous and help to prevent adverse neurological consequences in later life. Future animal studies need to be carried out to understand how NGF concentrations regulate breast milk LCPUFA composition.

P-6A-254

Late gestation undernutrition affects overall development and thyroid function in sheep

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Objective: To assess 1) whether late gestation undernutrition (LG-UN) have long-term implications for development of different organ systems and thyroid function in the sheep, and 2) whether the postnatal diet affects the phenotypical expression of any foetally derived nutritional effects.
Methods: Twenty twinpregnant ewes were fed either a NORM (~requirements for energy and protein) or LOW (50% of requirements) diet the last 6 wks of gestation (term = 147d). From 3 d to 6 mo post-partum (around puberty), twin lambs were assigned to each their feeding: CONV (hay) or HCHF (High-Fat-High-Carbohydrate: cream 38% fat + popped maize) supplemented with milk replacer 3d-8wks of life. Male lambs were slaughtered at 6mo. Female off-spring were raised on pasture from 6mo to 2 yrs (young adulthood) and then slaughtered. Total serum T3 and T4 concentrations were analysed by commercial kits. All experimental procedures were approved by The National Committee on Animal Experimentation, Denmark.
Results: Growth during the first 6 mo of life was determined exclusively by the postnatal diet. LG-UN however resulted in smaller adult body size with most tissues/organs being proportionately reduced. HCHF lambs at 6mo had vast accumulation of fat in all adipose tissue stores and in the liver ($p < 0.0001$). Liver weight (body weight corrected) was not affected, and functional hepatocyte mass was thus reduced in 6mo HCHF lambs, as was kidney weight. By 2 yrs (after $1\frac{1}{2}$ yrs on a moderate diet), postnatal diet effects had disappeared, except for less renal and more abdominal fat in HCHF compared to CONV animals. As the only organs, thyroid ($p = 0.055$) and adrenal glands ($p = 0.019$) were affected by LG-UN, both increasing in size (body weight corrected) in LOW animals. Age dependent postnatal nutrition effects ($p < 0.0001$) were observed on serum T3 and T4 levels, being highest in HCHF lambs during the differential feeding treatment, but this difference had disappeared in the young adults. Reversely, age dependent prenatal nutrition effects ($p = 0.09$ for T3, $p < 0.01$ for T4) became manifest in young adults (highest in LOW), but were absent in lambs. Accumulation of an unidentified white substance was clearly visible macroscopically in thyroids from more than half of the 2 yrs LOW animals, but from no NORM animals.
Conclusions: In our sheep model we have demonstrated long-term consequences of LG-UN on adult glucose-insulin homeostasis[1], muscle mitochondrial function[2], lipid deposition and fatty acid profiles in hepatic TAG and structural lipids. Now we also demonstrate implications for thyroid development and regulatory function, possibly involved in earlier termination of growth and smaller adult body size in LG-UN individuals. LG-UN effects were rarely detectable in adolescent lambs, where the actual postnatal rather than previous prenatal nutrition had an impact. LG-UN effects, however, became manifest in early adulthood. Effects of the extreme HCHF diet could thus in part or completely be reversed by subsequent dietary correction. Support: The Danish Council for Strategic Research, Denmark.

1. M.O. Nielsen *et al.*, *J. Diabetes* 1:A82, 2009.
2. W. Jørgensen *et al., J. Diabetes* 1:A81, 2009.

P-6A-255

Effects of restriction of prenatal and postnatal growth on skeletal muscle development

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Objective: Fetal growth restriction results in impaired organ development and programs disease in later life. We investigated the developmental timeline following intrauterine growth restriction of skeletal muscle in rats. We also used cross-fostering to determine the impact of prenatal and postnatal nutritional restraint on skeletal muscle development in 7 day old rats.
Methods: Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (*Restricted*) or sham (*Control*) surgery on day 18 of gestation in WKY rats. For the developmental timeline, offspring were killed at either day 20 of gestation or 1, 7 or 35 days after birth. For the cross-foster study, *Control* and *Restricted* pups were cross-fostered onto either a *Control* or *Restricted* mother one day after birth and killed at 7 days. Due to size restraints, hindlimb muscles were pooled within a litter according to sex for litters aged day 20 of gestation and day 1 and 7 after birth. At day 35 the whole gastrocnemius muscle was collected from individual offspring. The master regulator of mitochondrial biogenesis, PGC1α and the myogenic regulatory factors, MRF4, MyoD and myogenin mRNA expression was assessed by real-time PCR ($n = 7–10$).
Results: All offspring exposed to uteroplacental insufficiency were smaller than *Controls* at birth and all ages investigated regardless of sex ($P < 0.05$). However, *Restricted* pups cross-fostered onto a *Control* mother had improved growth such that they were not different to *Control* by day 7. At day 35 there were no differences in gastrocnemius muscle weights, either absolute or relative to body weight, across all groups. PGC1α mRNA was lower at gestational day 20 and increased across time such that expression was highest at day 35 ($P < 0.05$). At days 7 and 35, PGC1α mRNA was higher in females compared with males ($P < 0.05$) but was not different between *Control* and *Restricted* offspring at any age. MRF4 and MyoD displayed a similar developmental

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profile to PGC1α such that they were lower at gestational day 20 and increased across time and were highest at day 35 ($P < 0.05$). At day 35, MRF4 and MyoD mRNA was higher in females than males ($P < 0.05$). MRF4 mRNA was not different between *Control* and *Restricted* offspring at any age. At day 7 MyoD was higher in *Restricted* compared to *Control* offspring and showed intermediate expression in *Restricted* females but not males fostered onto a *Control* mother. Myogenin gene expression peaked at day 1 after birth and was higher than all other ages with expression at day 35 being lowest ($P < 0.05$). Similar to MyoD and MRF4, myogenin mRNA was higher in females compared to males at days 7 and 35 ($P < 0.05$). Myogenin mRNA was not different between *Control* and *Restricted* offspring at any age.

Conclusion: In conclusion, the myogenic regulatory factors have distinct developmental profiles with clear sex differences emerging at 7 and 35 days after birth. There were no differences in expression between *Control* and *Restricted* offspring except for elevated MyoD expression in *Restricted* at day 7 which was partially rescued by cross-fostering in females only. These sex differences highlight the perinatal implications for programming of adult metabolic disease and muscle function.

P-6A-256

Effect of nutrition on circulating levels of advanced glycation end products in infants

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Heating of food generates a large number of heterogeneous chemical compounds including lipoxidation and advanced glycation end products (AGEs: carboxymethyllysine = CML, methylglyoxal = MG). Recently, it has been shown that food is a major contributor to the body pool of AGEs which have been shown to have similar pro-inflammatory and pro-oxidative actions as their endogenous counterparts. It is known that circulating AGEs increase with human aging but little is known about these levels in infancy.

Objective: In the current work, we measured circulating AGE levels in newborns and during their first year of life.

Methods: Sixty healthy term singleton newborns products of normal pregnancies were recruited. A complete physical exam and a blood sample (4 ml, for AGEs and glucose) were obtained from cord blood, at 6 and 12 months plus a complete nutritional poll. AGEs were measured by ELISA using anti CML and anti MG monoclonal antibodies. Mean gestational age was 39 weeks. Main results are in the table (mean ± SD).

Time	0	6	12	Mother (at birth)
N	n = 60	n = 35	n = 32	n = 60
Weight (kg)	3.47 ± 0.38	8.26 ± 0.96	10.25 ± 0.95	74.6 ± 10
Glycemia (mg/dl)	82 ± 22	85 ± 9.5	84 ± 8	
CML (units/ml)	2.8 ± 1.3	3.4 ± 1.6*	4.5 ± 2	4.5 ± 2.6
MG (nmol/ml)	1.0 ± 0.6	1.13 ± 0.4	1.4 ± 0.6*	1.08 ± 0.6

Results: sCML levels in newborns are 60% lower ($p < 0.001$) than those in their mothers and increase during the first year of life in parallel with the initiation of food intake reaching similar levels to their mothers by 1 year. sMG levels are similar between the newborn and their mothers but increase steadily during the first year of life. There is a close correlation between AGEs levels in the mother and newborn $r = 0.74$, $p < 0.01$.

Conclusion: These results show that AGEs are part of a normal diet when infants add to breast feeding, solid food. In the future, we would like to study the relationship between specific foods intake and levels of sAGE. Moreover, long-term follow up of children born of mothers with high sAGEs may provide important metabolic clues.

P-6A-257

Maternal protein restriction during pregnancy and lactation in rats: effects on cardiac function in adulthood

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Objective: Epidemiological studies have linked intrauterine growth restriction (IUGR) with an increased risk of cardiovascular disease later in life, including hypertension and coronary heart disease. Thus, the objectives of this study were to examine the effect of IUGR in rats, due to maternal protein restriction, on cardiac function in young adulthood.

Methods: Wistar Kyoto (WKY) rats were administered a low protein diet (LPD; 8.7% casein) during pregnancy and lactation until 2 weeks postnatally; controls were administered a normal protein diet (NPD; 20% casein). At 4 weeks of age offspring were weaned and placed on standard rat chow until the experimental endpoint. At 14 weeks of age (young adulthood), cardiac function was assessed in male NPD (N = 10) and LPD (N = 11) offspring and in female NPD (N = 9) and LPD (N = 10) offspring by pressure volumetry using an anesthetized closed chest approach. From our recordings we determined mean arterial pressure (MAP), heart rate (HR) and left ventricular pressure-volume indices under baseline conditions and following dobutamine stimulation (DOB, 2–8 μg/kg/min) and volume loading (lactate solution 20 ml/min per 100 g body mass).

Results: Body weights of male and female LPD offspring were significantly smaller compared to control offspring at birth

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($P < 0.0001$) and at 14 weeks of age ($P < 0.0002$); the effects on body weight were more pronounced in females. Maternal protein restriction led to impaired regulation of cardiac output (CO) during β-adrenoceptor activation in female LPD offspring (Fig 1A), whereas regulation of CO in male LPD offspring was not different to NPD controls. Importantly, LPD females maintained a smaller end-diastolic volume ($P < 0.033$) (Fig 1B) and smaller stroke volume ($P < 0.003$) (Fig 1C) increase during DOB stimulation, resulting in a significant attenuation of the CO increase ($P < 0.029$). Arterial elastance was also significantly elevated in LPD females ($P < 0.028$), while HR, MAP and maximal and minimal rates of ventricular pressure change were not affected. Changes in indices of contractile function did not differ between groups during volume loading (stretch-dependent mechanisms).

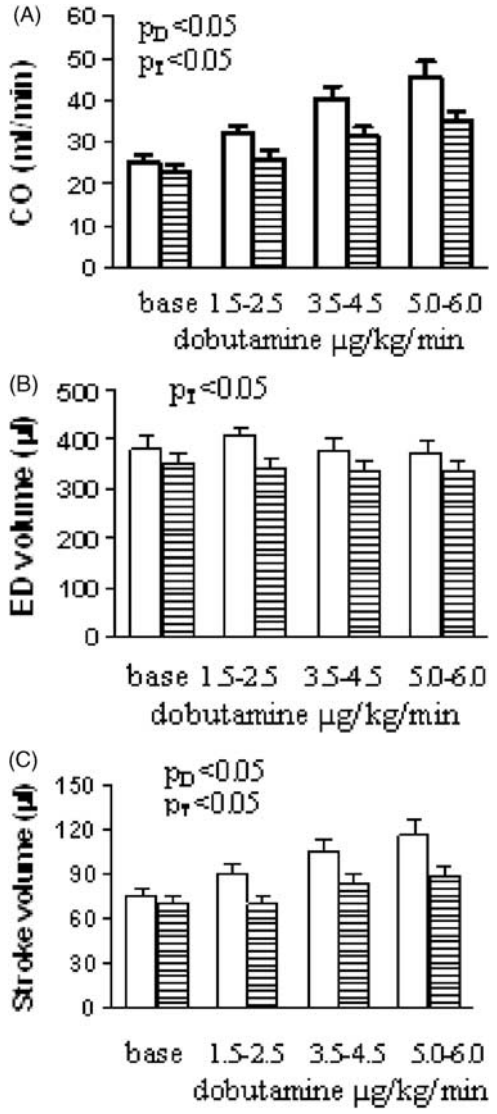

Figure 1. A) Cardiac output (CO), B) end diastolic (ED) volume and C) stroke volume (SV) during baseline (base) conditions and in response to dobutamine stimulation in female LPD and NPD offspring at 14 weeks of age. These differences in CO, ED volume and SV were not observed between male LPD and NPD offspring (data not shown). Data were analyzed usuing a two-way analysis of variance with repeated measures with treatment T (NPD and LPD) and dose D (increasing doses of dobutamine) as factors (p_D and p_T are corresponding significances). NPD offspring are represented in the open bars and LPD offspring in the striped bars.

Conclusions: The findings demonstrate that administration of a maternal low protein diet during early heart development leads to sex specific differences in heart responses to β-adrenergic stimulation in adulthood (female IUGR hearts work harder when challenged).

P-6A-258

Neonatal dietary n-3 long-chain polyunsaturated fatty acids prevent excessive fat deposition in adult male mice in an experimental model of nutritional programming

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Objective: The prevalence of childhood obesity has increased rapidly over the past decades and has a strong link to adult obesity, which is associated with several morbidities[1]. The trajectory of obesity appears to start at a preschool age[2] suggesting that early critical periods of development play an important role. The nutritional environment during fetal and neonatal life is thought to influence development of metabolic homeostasis thereby affecting susceptibility to metabolic disease. Proliferation and differentiation of pre-adipocytes, for instance, are directly affected by dietary fatty acids[3]. The objective of the present study was to investigate whether fat quality during early neonatal life has sustained effects on adult metabolic profile and body composition in a new model of nutritional programming in mice.

Methods: Male offspring of healthy, normal weight C57Bl/6j dams were subjected to an early diet containing 21 En% fat, consisting either of 100% vegetable oils (CTRL) or 80% vegetable oils and 20% tuna fish oil (n-3 LCP) from postnatal day (PN) 2 to 42. Subsequently, mice of both experimental groups were switched to a moderate Western style diet (WSD) until dissection on PN 98. Body composition was measured by dual X-ray absorptiometry at PN 42, 70 and 98. After dissection, plasma lipid profile, glucose, insulin and adipokines were measured. Weight of white adipose tissue depots and epididymal adipocyte size were also determined at PN 98.

Results: Dietary n-3 LCPs directly affected body composition as shown by a lower fat mass on PN 42 in n-3 LCP fed mice compared to CTRL fed mice (4.3 ± 0.64 g versus 3.5 ± 0.50 g, respectively; $p < 0.01$). Additionally, during WSD challenge from PN 42 to 98, beneficial effects of

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neonatal n-3 LCPs on fat accumulation persisted and the difference in body fat mass even increased between n-3 LCP and CTRL fed mice (8.4 ± 1.1 g versus 6.1 ± 1.4 g, respectively; $p < 0.001$). Moreover, mice fed n-3 LCP during neonatal development had a healthier plasma lipid profile, healthier plasma glucose homeostasis and less hypertrophic adipocytes in the epididymal fat depot at PN 98 compared to CTRL fed mice.

Conclusions: This study has shown for the first time that fatty acid composition of neonatal nutrition plays an important role in the development of body composition and metabolic homeostasis. This might be mediated by lasting effects of dietary fatty acids on development and function of white adipose tissue during neonatal life. Moreover, neonatal n-3 LCPs may protect against excessive fat deposition in a moderate obesogenic environment during adolescence and adulthood.

1. A.S. Singh *et al.*, *Obes Rev.*, 9:474–88, 2008.
2. N.J. Blair *et al.*, *Arch Dis Child.*, 92:866–871, 2007.
3. G. Ailhaud *et al., Prog Lipid Res.,* 45:203–236, 2006.

P-6A-259

Early life undernutrition in sheep induces sex- and tissue-specific effects on factors mediating insulin sensitivity and lipid handling in adulthood

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Objective: Improved insulin sensitivity may be one mechanism to accelerate growth following a period of growth retardation. We demonstrated previously that poor growth in early postnatal life, induced by nutrient restriction, enhances glucose tolerance/insulin sensitivity in female but not male adult sheep[1]. However, this may negatively affect glucose handling if it led to inappropriate adipose tissue deposition[1]. To understand the mechanisms involved in this increased glucose tolerance, this study examined components of the insulin signalling pathway (insulin receptor (IR) and glucose transporter 4 (GLUT4) in skeletal muscle and adipose tissue in our model of adult sheep exposed to undernutrition in early gestation and/or early postnatal life. Mediators of lipid handling (lipoprotein lipase, LPL) and adipocyte differentiation (PPAR-γ) were also examined in adipose tissue.

Methods: Ewes received either 100% (C, n = 36) or 50% nutritional requirements (U, n = 34) from 1–31 days gestation and 100% thereafter. Male and female offspring were then fed either *ad libitum* (CC, n = 22; UC, n = 13) or to reduce body weight to 85% of target from 12–25 weeks postnatal age (CU, n = 14; UU, n = 21) and *ad libitum* thereafter. At *post mortem* at 2.5 years, skeletal muscle and peri-renal adipose tissue were collected in liquid nitrogen. Real-time RT PCR was used to measure mRNA expression for IR and GLUT4 in both tissues, and for LPL and PPAR-γ in adipose tissue. Gene expression was normalised to mean β-actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA expression. Data (mean ± SEM) were analysed by ANOVA and linear regression.

Results: In females, postnatal undernutrition increased IR (CU and UU: 1.54 ± 0.13 *vs.* CC and UC: 1.13 ± 0.14; $P < 0.05$) and GLUT4 (CU and UU: 1.17 ± 0.06 *vs.* CC and UC: 0.94 ± 0.09; $P < 0.01$) mRNA expression in muscle, regardless of the prenatal nutrient environment. This was not observed in adipose tissue. Reduced growth rate from 12–25 weeks was directly correlated to increased IR and GLUT4 ($R^2 = -0.17$ and -0.22, respectively; $P < 0.05$) in muscle from females. In males, muscle IR and GLUT4 mRNA expression was unaffected by early life nutrition however adipose LPL mRNA expression was increased in those exposed to undernutrition in early gestation, regardless of the postnatal nutrient environment (UC and UU: 1.20 ± 0.13 *vs.* CC and CU, 0.84 ± 0.07; $P < 0.05$). PPAR-γ mRNA expression was not different between groups.

Conclusions: This study has shown sex- and tissue-specific differences in factors that regulate insulin signalling and lipid handling following early life undernutrition. The increase in IR and GLUT4 mRNA expression in skeletal muscle of adult females exposed to postnatal undernutrition suggest that the improved glucose tolerance in these animals[1] is due to increased insulin sensitivity in muscle but not adipose tissue. However, there was no evidence that this effect was also associated with factors that may predispose to inappropriate fat deposition. Rather, an increase in adipose tissue LPL mRNA expression in prenatally undernourished adult males may lead to an inappropriate balance between circulating and stored lipids, although no effects on fatness were observed in these animals[1]. Supported by British Heart Foundation and Wessex Medical Research.

1. K.R. Poore *et al.*, *Am J Physiol,* 292:E32–E39, 2007.

P-6A-260

Early life undernutrition induces opposite effects on the adrenal responses to stress in male and female adult sheep

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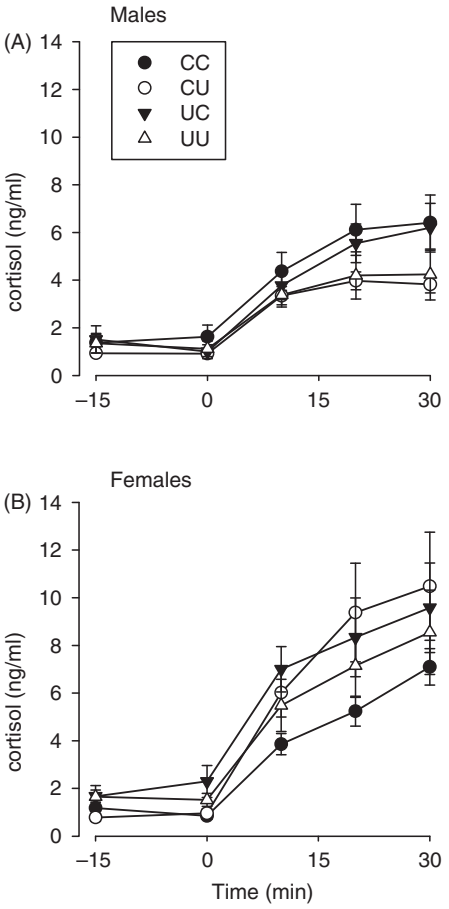
Objective: We have demonstrated previously that nutrition in early postnatal life, immediately following weaning, affects adult sheep pituitary-adrenal and adrenomedullary responsiveness during two types of stress test in a sex- and age-dependent manner[1,2]. In postnatally undernourished females, adrenocortical responses to CRF/AVP administration were enhanced in young (1.5 yr) but not mature (2.5 yr) adulthood[1], while adrenomedullary output was increased during a transport and isolation (TI) stress test at 2.5 yr[2]. In contrast, adrenomedullary output in males was unaffected by postnatal undernutrition[2], while CRF/AVP-induced cortisol output tended to be reduced, but only at 2.5 yr. This study examined the pituitary-adrenal responses to the TI stress test in our model of adult sheep exposed to undernutrition in early gestation and/or early postnatal life.

Methods: Ewes received either 100% (C, n = 23) or 50% nutritional requirements (U, n = 20) from 1–31 days gestation and 100% thereafter. Male and female offspring were then fed either *ad libitum* (CC, n = 14; UC, n = 9) or to reduce body weight to 85% of target from 12–25 weeks postnatal age (CU, n = 9; UU, n = 11) and *ad libitum* thereafter. At age 2.5 years, catheters were inserted into the carotid artery and jugular vein under general anaesthesia. The TI test involved transporting sheep in their carts from their normal holding room to a different empty room (at t_0), followed by 30 min isolation. Plasma ACTH and cortisol concentrations were measured prior to (−15, −1 min) and following transportation (10, 20, 30 min) by a chemiluminescence auto-analyser (Immulite). Data (mean ± SEM) were analysed by ANOVA and linear regression.

Results: In males, cortisol output during the TI test was reduced in groups exposed to postnatal undernutrition but this was not statistically significant when analysed over time (Fig. 1A) or as area under curve (AUC). However, in the male population as a whole (n = 20), poor growth from 12–25 weeks of age (the postnatal undernutrition challenge period) was directly associated with reduced Δ peak and AUC cortisol ($R^2 = 0.37$, $P < 0.005$ and $R^2 = 0.29$, $P < 0.05$, respectively). Accelerated growth after this time (35 weeks–1.5 yr) was also associated with lower cortisol output in males (Δ peak: $R^2 = -0.35$, $P < 0.01$; AUC: $R^2 = -0.27$, $P < 0.05$). These effects were not observed in females. ACTH output during the TI test was not different between the 4 nutritional groups in males or females.

Conclusions: In this model of sheep exposed to undernutrition in early gestation and/or early postnatal life, we suggest that adrenal responsiveness during stress is generally reduced in adult males (current study) but enhanced in adult females[1] exposed to undernutrition in the early postnatal period, regardless of the prenatal nutrient environment. These effects may mediate a link between the early life nutrient environment and later cardiovascular and metabolic abnormalities. Supported by British Heart Foundation and Wessex Medical Research.



1. K.R. Poore *et al.*, *Early Human Devel* 82:537 (F-15), 2006.
2. J.P. Boullin *et al.*, *Proc Nutr Soc* 67:E429, 2008.

P-6A-261

Maternal docosahexaenoic and arachidonic acid supplementation at low protein level affects lactational performance, survival and growth in pups

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Some studies conducted over the past decade suggest adverse effect of fish oil (n-3 PUFA) on pre/postnatal growth and survival at normal[1] and marginal protein intake[2].

Objectives: Examine the effect of docosahexaenoic (DHA) and arachidonic acid (ARA) supplementation at restricted protein intake during pregnancy and lactation on reproductive outcome, lactational performance of dams and somatic growth of pups in wistar rats.

Methods: Pregnant female rats (n = 9/group) were allocated randomly to one of the three casein diets with 18% protein (C), 9% protein (LP) or 9% protein with DHA & ARA single cell oil (LPS) throughout gestation and lactation. Litter

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size, litter weight, and mortality were recorded on d0 & d14. A sub-sample of pups was studied for presence or absence of gastric milk on d0 and for amount (ml) of milk on d14. Post-weaning growth and mortality was recorded for individual pups till adulthood. The protocol for the study was approved by Institutional Animal Ethics Committee.

Results: Litter size and weight of LPS group were marginally lower at birth. On d0, after sufficient period given for nursing the young; 2 pups/litter were randomly sacrificed for gastric milk collection. Considerably higher proportion (58%) of pups in LPS had no milk in the stomach as compared to C (11%) and LP (10%) indicating delayed or poor milk production in LPS dams. During the first week of suckling period LPS group had highest mortality (18.08%) (Figure 1). On d14, litter weights from LPS group were significantly ($p < 0.05$) lower (54.5 ± 20.6) than C (93.7 ± 32.0) as also the amount of gastric milk in pups. Even after shifting to C diet from d22, growth curves of LPS pups continued to be lower. Males showed greater growth faltering than females up to adulthood. Mortality trend observed during weaning was tracked in the post weaning period also ($p = 0.05$).

Conclusions: Higher mortality in the first week and growth deficits in subsequent weeks observed in pups of dams fed low protein DHA & ARA supplemented diet can be attributed to its adverse effects on lactational performance. The mechanisms responsible for the same warrant further investigation.

1. E. Herrera *et al.*, *Int.J. Vitam. Nutr. Res.* 76:216–224, 2006.
2. S. Joshi *et al., Nutr.* 20:465–472, 2004.

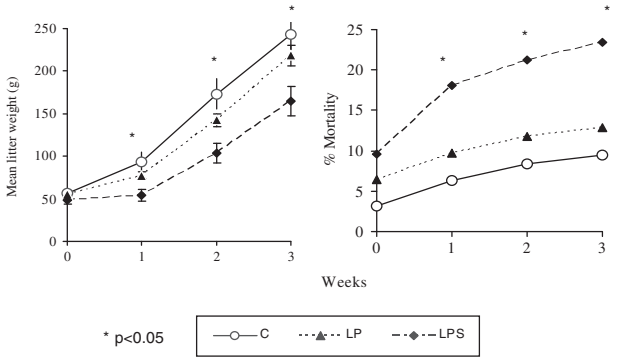

Figure 1. Growth and mortality in pups from different diet groups during lactation.

P-6A-262

Birth weight, season of birth and postnatal growth do not predict levels of low-grade systemic inflammation in Gambian adults

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Objective: Poor fetal growth and elevated low-grade systemic inflammation are two putative independent risk factors for cardiovascular disease. These findings have led to the hypothesis that the association between early life environment and cardiovascular disease may be explained by early life programming of systemic inflammation. The objective of this study was to investigate whether birth weight, weight at one year, growth velocity from birth to three months or season of birth predict adult levels of eight inflammatory markers in a Gambian sample. This study advances previous research by including a number of early life variables and measuring a wide range of adult systemic inflammatory markers.

Methods: Individuals born in three rural villages in The Gambia, and for whom early life measurements were recorded, were traced. Birth weight and weight at one year were measured by medical personnel using standard protocols and regularly calibrated equipment. Low birth weight was defined as less than 2500 g. Postnatal growth velocity was calculated as change in weight standard deviation score between birth and three months. Season of birth was defined according to whether individuals were born during the wet (July to December, inclusive) or dry (January to June, inclusive) season. Fasting blood samples were collected and levels of eight inflammatory markers (C-reactive protein, serum Amyloid A, orosomucoid, fibrinogen, α 1-antichymotrypsin, sialic acid, interleukin-6 and neopterin) measured. Adult height and weight were collected according to standard protocols. A wide range of cardiovascular disease risk factors and markers of infectious disease status were measured in all adults. The association between early life measurements and systemic inflammation was assessed using regression analysis. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: In the 320 (51.9% male) participants age ranged between 18 and 30 years (mean age 22.2 years). Seventy-eight percent of participants had a body mass index within the normal range. A higher percentage of female, compared with male, participants were classified as overweight or obese (13.6% vs. 1.8%). Less than 1% of the study population had asymptomatic malaria parasitaemia. There was no evidence that birth weight, low birth weight, season of birth or weight at one year predicted adult levels of inflammatory markers. In analyses adjusted for age and sex more rapid growth between birth and three months of age was associated with higher levels of fibrinogen, orosomucoid and sialic acid. These relationships persisted after further adjustment for adult body mass index but after full adjustment for age, sex, adult body mass index, cardiovascular disease risk factors and infectious disease status only the association with fibrinogen remained; fibrinogen increased by 0.09 (95% CI 0.02, 0.16) g/L per one unit increase in change in standard deviation score from birth to three months ($p = 0.008$).

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Conclusions: This study provides little evidence that size at birth or growth in early infancy determine levels of inflammatory markers in young Gambian adults. Support: UK Medical Research Council studentship and study grant.

P-6A-263

Breast milk sodium content in rural Gambian women: between and within women variation in the first six months after delivery

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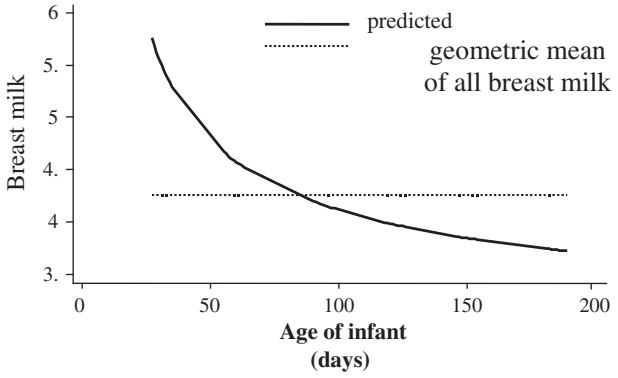
Objective: It has been suggested that infancy is a particularly sensitive period with respect to the effect of dietary sodium on future risk of hypertension. One difficulty of researching the effects of early sodium intake on later health is accurately measuring sodium intake from breast milk. In observational studies sodium content has been calculated by estimating breast milk volume consumed and assuming a fixed sodium concentration for all women at all times (a standardised measure). The objective of this study was to investigate the variation in breast milk sodium concentration in the first six months postpartum within women and to test whether the pattern of change in sodium concentration differs between women.

Methods: The study population was 197 rural Gambian mother-infant pairs. Approximately 10 mL of breast milk were collected from both breasts, by maternal manual expression, each month from delivery to six months postpartum. Sodium and potassium concentrations were measured in whole breast milk by flame photometry on a digital flame photometer with Filteau's method. Multilevel models were used to investigate whether the sodium content of breast milk changed over time within and between women. Fractional polynomials were used to identify the best-fitting functions of age to be included in the within and between variance functions. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The overall geometric mean (inter-quartile range) sodium content of all breast milk measurements (1128 measurements) was 4.26 (3.25 to 5.35) mmol/L. As indicated in the Figure, sodium levels decreased with time; the reduction was initially rapid (levels decreasing by 17.7% between 30 to 60 days post delivery). On the Figure the overall geometric mean sodium content (4.26 mmol/L) is indicated by the dashed line. A comparison of this line with the predicted sodium levels (full line) suggests that using the overall mean breast milk sodium level as an indirect measure for sodium levels at any time point would tend to underestimate the sodium levels in very early infancy (first three months) and overestimate sodium levels after three months. Assuming that breast sodium milk content was 6.5 mmol/L (the concentration calculated by McCance and Widdowson[1]) would overestimate its content at all ages in this population (6.5 mmol/L is off the upper limit of our Y axes in the Figure). Immediately after birth, there was substantial variation in breast milk sodium content between women but this reduced with time.

Conclusions: Our results suggest that it is not appropriate to use a standardised measure of breast milk sodium content when direct measurement is possible – particularly when there is a research interest in measuring sodium intake in very early infancy. Support: UK Medical Research Council, South West NHS Public Health Training Scheme, UK Department of Health.



1. The Royal Society of Chemistry and MAFF. *The Composition of Foods*. 5th ed. London: HMSO; 1991.

P-6A-264

Health policy analysis of maternal and childhood nutrition and chronic disease prevention in Chile

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Objective: To analyze maternal and childhood nutrition policies in Chile under the light of current evidence with regard to the developmental origins of health and disease.

Methods: Review of current Chilean policy aiming to improve maternal and childhood nutritional status. Afterwards, a review of the available evidence on interventions to address maternal and childhood overweight and obesity was

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carried out to establish a frame for analysis of the strategies utilized in Chile to tackle the problem. Finally, conclusions were drawn.

Results: A progressive increase in obesity and nutrition-related chronic diseases is observed in Chile[1]. Population changes in diet and physical activity are the main determinant factors; however, recent evidence suggests that specific patterns of prenatal and postnatal growth are also potential contributors[2]. Since a rising trend in obesity is observed in children under 6 years of age and pregnant women, especially among the less affluent segments of the population[1], maternal and childhood nutrition policies are particularly relevant. Current policies pay special attention to weight control during pregnancy through education and counselling, and to tackle nutrition deficiencies with dairy supplementary drinkable feeding and specific nutrients, although adherence to the latter is low[3]. Exclusive breastfeeding for 6 months increased from 16 to 43.1% between 1993 and 2002, showing an inverse association with maternal work[4]. Policies to address childhood obesity include reformulation of the National Complementary Food Program[5] and joint actions with the educational sector to promote healthy nutrition and physical activity among preschool children[6].

Conclusions: Current policies might not reverse the rising trend of maternal and childhood overweight and obesity. The majority of the strategies implemented in Chile are centred on individual responsibility, even those targeting children. These approaches tend to blame the victim for poor health outcomes, without taking into account environmental determinants of the problem. Research findings regarding socio-cultural aspects involved in feeding practices during pregnancy and early childhood should be taken into account in order to improve educational interventions. A more ecological approach, as well as upstream population-based interventions, should be encouraged. Research on policies related to regulation of food processing (salt, sugar and fat content) of products manufactured and marketed for children is necessary.

1. Vio, F. *et al. Public Health Nutr*. 11:405–412, 2007.
2. Uauy, R. *et al. Ann Med*. 40:11–20, 2008.
3. Ministerio de Salud Chile. *Manual de Atención Personalizada en el Proceso Reproductivo*. 173–177, 2008.
4. Atalah, E. *et al. ALAN*. 59:374–379, 2004.
5. Uauy, R. *et al. JN*. 131:893S–899S, 2001.
6. Salinas, J., Vio, F. *Rev Chi Nutr*. 2:164–117, 2002.

P-6A-265

Adolescent build and diabetes: The Guangzhou Biobank Cohort Study

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Objective: An epidemic of diabetes is emerging in recently developed and developing Asia despite relatively low levels of obesity and ischemic heart disease (IHD). Muscle mass reduces vulnerability to type 2 diabetes, for which adolescence is a key developmental window. We examined the association of adolescent build with diabetes in a cohort who grew up in a developing country.

Methods: We used linear multivariable regression in 19,505 older (⩾50 years) Chinese from the Guangzhou Biobank Cohort Study (phases 2 and 3) to examine the adjusted associations of recalled adolescent relative weight at 15 and 20 years (light (n = 6,100), average (n = 10,954), heavy (n = 2,451), i.e. build, with diabetes and waist-hip ratio. We also examined whether the associations varied by sex.

Results: Adolescent build had no sex-specific associations with later life diabetes but did with waist hip ratio. In later life relatively heavy adolescents had a lower risk of diabetes (odd ratio 0.85, 95% confidence interval (CI) 0.73 to 0.99) compared to light adolescents adjusted for age, sex, life course socio-economic position, lifestyle and linear growth (leg length and seated height). Similarly adjusted, heavy adolescents also had higher waist-hip ratio, particularly in men (mean difference 0.01, (95% CI 0.004 to 0.02) rather than women (0.006, 95% CI 0.002 to 0.009).

Conclusions: Relatively heavy build in adolescence may be associated with a lower risk of later life diabetes, perhaps via greater muscle mass, although the same exposure was also positively associated with central obesity particularly in men. Childhood physical activity to build muscles may be relevant to diabetes prevention. Although, the underlying physiology processes are unknown, we speculate that a dual process may exist whereby nutritionally and inter-generationally driven increases in pubertal sex steroids increase men's risk of IHD (via central obesity and lipids), but decrease men and women's risk of diabetes (via muscle mass). Support: The University of Hong Kong (HKSAR), Guangzhou Public Health Bureau (China), Guangzhou Science and Technology Bureau (China), The University of Birmingham (UK).

P-6A-266

Perinatal programming of appetite control – determination of gastric ghrelin expression and effects on intracellular energy sensing within the gut

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Objectives: Periods of restricted prenatal growth followed by accelerated rapid postnatal growth can determine whole body energy homeostasis, thereby determining an individual's risk of later obesity. The gut is a system which may be important in this process but has not been fully investigated to date. Therefore, we examined the effect of changes in pre and postnatal growth on the gene expression of the appetite regulation hormone ghrelin, together with intracellular markers of energy sensing and mitochondrial bioactivity.
Methods: Pregnant twin-bearing sheep were either fed to requirements (R; n = 20) or nutrient restricted to 60% of this amount (N; n = 20) from 110 days up to term (~147 days). Ten offspring in each group were then reared by their mother as singletons in order to promote postnatal growth (accelerated weight gain – A). Ten twin offspring from each group were reared by their mother together in order to restrict postnatal growth (standard weight gain – S). After weaning, all offspring were kept in a control indoor environment up to 17 months of age and then humanely euthanased to enable tissue sampling of the abomasum (true stomach in the sheep). This was washed, snap frozen in liquid nitrogen and kept at -80C until analysis of mRNA abundance for the genes encoding for ghrelin, the acetyl CoA carboxylase, AMP related kinase (AMPK-α2), PPAR gamma coactivator-1, uncoupling protein (UCP) 2 and the leptin receptor (Ob-R) by real-time PCR. Appropriate institutional animal ethics committee approval was obtained.
Results: Offspring born to N mothers were lighter at birth ($p < 0.01$) and, irrespective of postnatal growth rate, gene expression for ghrelin was raised in these offspring as adults (C: 1.0 ± 0.3; N $2.3 + 0.6$ a.u. ($p < 0.05$)). Interestingly, although the in utero environment had no effect on any of the genes related to energy sensing examined, their expression was all markedly upregulated in those offspring showing lower postnatal growth e.g. AMPK-α2 (A: 0.7 ± 0.3; S: 10.4 ± 3.8 a.u. ($p < 0.01$)) and mitochondrial bioactivity e.g. UCP2 (A: 0.6 ± 0.5; S: 15.6 ± 4.0 a.u. ($p < 0.01$)).
Conclusions: We have shown, for the first time, that exposure to caloric restriction during the period of maximal fetal growth is related to long term alterations in gastric ghrelin gene expression and that this is not abolished by reducing postnatal growth. In addition, we have established that modulation of early postnatal growth induced long term modifications in energy sensing within the stomach which were accompanied by mitochondrial adaptations. These striking effects suggest that the energetic environment during the neonatal period may be critical in determining long term gastric function.

P-6A-267

Neonatal hyperleptinaemia programmes adrenal medullary leptin signaling pathway, adrenal morphology and catecholamines *in vitro* release in rats

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Objective: We have shown that leptin serum concentration in early life is an important factor for adequate future development of the offspring in different experimental models of programming. Leptin treatment to normal fed pups during the first half of lactation programmes for hyperleptinaemia with central leptin resistance, higher catecholamines synthesis and release and higher blood pressure and heart rate in adult rats[(1,2)]. The relationship between leptin and catecholamines is well described in cultured chromaffin cells and our previous results are according to them. The aim of the present study was to evaluate if the leptin treatment on lactation alters the adrenal morphology and if there is a direct effect of leptin on adrenal medullary function in the programmed animals.
Methods: Wistar male rats were injected with 50 μL of saline or leptin (8 μg/100 g of body weight, daily, for the first 10 days of life). Rats were weighted during the experimental period. They were sacrificed when reached 150 days-old. Leptinaemia was determined by a rat leptin specific radioimmunoassay. Adrenal glands were collected for morphological analysis. Adrenal medullae were carefully isolated for the *in vitro* assay using leptin as a secretagogue and for western blotting analysis of the leptin receptor (OBR) and other leptin pathway signaling proteins (JAK2, STAT3, SOCS3 and p-STAT3).
Results: As expected, the leptin group had lower body weight during the treatment (−10%, $p < 0.05$) but higher body weight (+10%, $p < 0.05$) and hyperleptinaemia (+78%, $p < 0.05$) at adulthood. Adrenal glands from leptin group presented higher weight (+15%, $p < 0.05$) and hypertrophy of cortex and medulla. The leptin group had lower content of OBRb (−61%, $p < 0.05$) and JAK2 (−29%, $p < 0.05$) with higher expression of p-STAT3 (+2x, $p < 0.05$). Leptin stimulated catecholamines release in both groups, but programmed rats showed a lower response to leptin stimulation *in vitro* (−27%, $p < 0.05$).
Conclusions: The higher catecholamines synthesis and secretion in the leptin programmed rats observed in our previous study (2) does not seem to be a consequence of the direct effect of leptin upon the medullae. Supporting this data we found lower expression of OBRb and JAK2 in the medullae from leptin group as we had already found in the hypothalamus (1). We suggest that leptin increases adrenal medullary function through sympathetic nervous system activation. The higher content of p-STAT3 may be related

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to another signaling pathway (leptin-independent). Hypertrophy of adrenal glands may be a consequence of the trophic role of leptin already demonstrated for the central nervous system in early life. Besides, p-STAT3 content has been related to proliferative and anti-apoptotic events in other cell types which could also contribute to the growth of the chromaffin cells in this model. Support: FAPERJ and CNPQ.

1. F.P. Toste *et al.*, *Brit J Nutr*, 95:830–837, 2006.
2. I.H. Trevenzoli *et al.*, *J Physiol*, 580:629–637, 2007.

P-6A-268

Cognitive performance in 9–10 year old children in South India: no relationship with breast-feeding duration or age of introduction of complementary foods

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Objective: Several studies have suggested a beneficial effect of infant breast-feeding on childhood cognitive function[1,2]. Our main objective was to examine whether duration of breast-feeding and age at introduction of complementary foods are related to cognitive performance in 9–10 year old school going children in South-India.

Methods: We examined 514 (249 boys and 265 girls) healthy children from the Mysore Parthenon birth cohort for whom breast-feeding duration (6 categories from <3 to $\geqslant 18$ months) and age at introduction of complementary foods (4 categories from <4 to ≥ 6 months) were collected at the 1st, 2nd and 3rd year annual follow-up visits. Their cognitive function was assessed at a mean age of 9.7 years using 3 core tests from the Kaufman Assessment Battery for children and additional tests measuring long-term retrieval/storage, attention and concentration, visuo-spatial and verbal abilities. We also collected data on a variety of potential confounders like maternal age, parity, BMI and height in pregnancy, gestational age at birth, birthweight, gender, children's current age height and BMI, parents' area of residence, educational attainment and current socio-economic status. Associations of breast-feeding duration and age at introduction of complimentary foods with cognitive ability were examined by multiple linear regression analysis using stata version 10. The study was approved by the Holdsworth Memorial Hospital, Mysore, research ethics committee and informed verbal consent was obtained from parents and children.

Results: All the children were initially breast-fed and very few (2.3%) stopped breast-feeding before the age of 3 months. The mode for duration of breast-feeding was 12–17 months (45.7%) and for age at introduction of complementary foods 4 months (37.1%), similar in boys and girls. Girls scored better than boys in tests of word order (short-term memory) ($p = 0.03$), pattern reasoning (planning and fluid reasoning) ($p = 0.003$), verbal fluency-names (broad retrieval ability, speed and flexibility of verbal thought process) ($p < 0.001$), and coding-Wechsler Intelligence Scale for Children-III (visual-motor processing speed and coordination, short-term memory, attention and concentration) ($p < 0.001$). There were no associations between duration of breast-feeding, or age of introduction of complementary foods, and cognitive function, either unadjusted or after adjustment to a variety of confounding variables listed above.

Conclusions: Our study does not suggest a beneficial effect of longer duration of breast-feeding on later cognitive ability in this population, most of whom were breast-fed for over a year. There was no evidence of an association between the age at introduction of complementary foods and cognitive development. Support: The study was supported by the Parthenon Trust, Switzerland, the Wellcome Trust and Medical Research Council, UK.

1. Michaelsen KF *et al.*, *Adv Exp Med Biol*, 639:199–215, 2009.
2. Anderson JW *et al.*, *Am J Clin Nutr,* 70:525–535, 1999.

P-6A-269

Poor postnatal nutrition alters the effects of leptin, neuropeptide Y and a melanocortin receptor agonist on food consumption in later life

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Objective: Susceptibility to obesity and metabolic disease can be programmed early in postnatal life by the level of nutrition during suckling. Studies in rats have shown increased susceptibility with maternal overnutrition and a resistance to obesity with undernutrition. The protective effect of a low-protein maternal diet is associated with reduced feeding and lean mass. It occurs despite the offspring having low circulating leptin, low pro-opiomelanocortin (POMC) in the arcuate nucleus and high neuropeptide Y (NPY) – changes which may be expected to stimulate food consumption. This study aimed to investigate the effects of centrally administered leptin, NPY and the melanocortin 3/4-receptor agonist, melanotan II (MT-II), on food intake and energy expenditure in adult rats that had been undernourished during suckling.

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Methods: Male Wistar rats from dams fed either a normal chow (control) or a low-protein diet during suckling (postnatal low protein, PLP) were implanted with an intracerebroventricular cannula into the third ventricle and leptin, NPY or MT-II at maximal and two or three sub-maximal doses were administered from 12 weeks of age. Food was weighed after 2, 4, 6 and 24 hours.
Results: Leptin at the mid-range dose of 2.5 μg reduced food consumption and body weight over 24 hours more in the PLP group than in controls (75 ± 5 *vs.* $93 \pm 5\%$ of the food consumption following saline administration, $p < 0.05$; 13.3 ± 2.2 *vs* 4.4 ± 2.0 g body weight loss, $p < 0.01$). The dose of NPY required to double food intake during the first 6 hours in the PLP group was twice that in the controls (0.82 ± 0.22 *vs.* 0.40 ± 0.06 nmol, $p < 0.001$). Furthermore, NPY, except at the maximal dose of 2.5 nmol, did not increase food intake significantly over 24 hours in the PLP offspring, whereas it increased food intake in control offspring. The dose of MT-II required to halve food intake over the first 4 hours in the PLP offspring was half that in the controls (0.093 ± 0.002 *vs.* 0.184 ± 0.002 nmol, $p < 0.01$).
Conclusions: Rats suckled by dams fed on a low protein diet have reduced food consumption and bodyweight, accompanied by an increased sensitivity to both leptin and an MC3/4-R agonist and a decreased sensitivity to NPY. This suggests that these animals have activated anorexigenic neural pathways and less active orexigenic pathways, which may contribute to the resistance to diet-induced obesity. Support: Biotechnology and Biological Sciences Research Council UK.

P-6A-270

A design for investigating the association of birth weight, weight change during life course with adult hypertension in Hong Kong women

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"Fetal origins hypothesis" by Barker[1] in 1990 is a well known hypothesis. One of the key finding was that low birth weight was associated with higher adult systolic blood pressure. However, in 2002, Huxley *et al*[2] argued that birth weight had little relevance in determining blood pressure levels in later life. But after that, several studies[3,4] argued against it until now. In addition, some experts think that change in size between birth and current rather than fetal biology itself affect the blood pressure, which means the fetal origins hypothesis must be weighed against a "postnatal origins hypothesis"[5]. Therefore, it is important to explore what is the interaction between later body weight change and fetal programming on the effect of BP.
Objectives: Of this study is to determine whether: Low birth weight predicts higher blood pressure/risk of hypertension in later life; The impact of birth weight on later blood pressure is modified by adult BMI (BMI at age 18 and current BMI); Adult BMI (BMI at age 18 and current BMI) has independently association with blood pressure/risk of hypertension.
Method: Female Registered Nurses and Enrolled Nurses who are memberships of Association of Hong Kong Nursing Staff (AHKNS) are considered as the target population. AHKNS has all the members' home address, they will be asked to measure their waist circumference using a mailed tape measure and record in a card, then send back the cards and inform us if they willing to participate the study. The participants will be invited to the Women's Health Centre of CUHK to fill out a self-administrated questionnaire, after that, anthropometric measurements are conducted. The main exposures are birth weight, BMI at age18 and current BMI; the main outcomes are blood pressure and hypertension. Current height, weight, blood pressure will be obtained by field measurement; other important self-report variables will be checked by medical records for validity. This study is approved by the Faculty Ethics Sub-committee.
Significance: Using a mailed tape measure to invite the target population attending study, it may be more cost-effective than a general population-based survey, and improve the response rate; it is easily be performed. From our knowledge, this is the first study in Hong Kong to use a life course epidemiology method to verify the "Fetal origins hypothesis" in Chinese population, and determine how import that the effect of weight change during a person's life course on later life outcome. It allows us to find some evidences for developmental origins disease, and then give the better suggestions for primary prevention of some later life outcomes.

1. Barker, D.J., BMJ, 301:1111, 1990.
2. Huxley, R. *et al*, *Lancet*, 360:659–665, 2002.
3. Gamborg, M., *et al*., *Am J Epidemiol*, 166:634–645, 2007.
4. Davies, A.A., *et al*., *Hypertension,* 48:431–436, 2006.
5. Lucas, A. *et al*, *BMJ,* 319:245–249, 1999.

P-6A-271

Evaluation of feeding practices education for mothers whom children (aged between 6 and 36 months) suffered form improper growth in district 19 of city of Tehran

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Objective: This study was undertaken to assess and report the Successfulness of the implemented feeding practice education for Mothers by measuring the Ante optometric index among their children during education.
Methods: All mothers whom children had including Criteria referred to Ayat health center during 2001–2003. They

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Educated by trained health workers using Face to Face education and Holding Role playing workshops and All data needed for the study gathered using a researcher – administrated check list during the Implementation of the educational project.
Results: In this study, 1096 mother was educated and 300 finished the educational course successfully and their children's growth indexes measured in this study were normal after complete education. 152 (50.7%) and 148 (49.3%) of these children were male and female respectively. All these children' growth charts were in normal range after their mother finishing the implemented Educational course.
Conclusions: The result of the current study showed that Implementation of proper feeding practices educations can be considered as an effective method for improving growth indices among children with malnutrition.

P-6A-272

Association between catch-up in the first years of life and overweight/obesity at 10 years of age, in a low-income community in São Paulo, Brazil

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In the last years it has been shown that weight gain velocity is a determinant of future obesity. These findings are mainly from developed nations, and few studies from developing countries showed this association[1,2].
Objective: To test if the weight gain velocity during different periods of age (0–6 m, 6–12 m, 12–24 m, 2–5y and 5–10y) is associated with overweight/obesity at 10 years of age.
Method: A historic cohort was made, based on clinical records from the Social Project of Einstein Hospital at the Paraisopolis Slum. All children, who were enrolled in the health service between October 1998 and August 1999 (845) were selected to participate in the study. Exclusion criteria were having some base health condition (neurological or endocrinological diseases), being malnourished and not having complete records. A final sample of 378 eutrophic and overweight/obese children was analysed. Weight gain velocity was categorized according to the quartile of its distribution. A multivariate logistic analysis looked for the independent effect of weight gain in each age period. The outcome was having a BMI at age 10 greater than 1.5 z-score. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The mean birth weight was 3.15 kg (1.33–4.89); 95% of the children were term at birth and 66% had a vaginal delivery. The median length of breastfeeding was 4 months and of exclusive breastfeeding was 2 months. The mean z-score of BMI at age 10y was 0.3 (−1.25–+3.74). After adjusting for birth weight and for being small-for-gestational-age, the effect on the BMI at age 10y of passing to a higher quartile of weight gain in every age period was OR 4.51 (1.97–10.32) at 0–6 m; 2.20 (1.19–4.06) at 6–12 m; 1.30 (0.78–2.19) at 1–2y; 4.23 (2.24–7.98) at age 2–5y and 1.96 (1.14–3.37) at age 5–10y (reciprocally adjusted).
Conclusions: Weight gain during the first 6 months of life was the main determinant of overweight/obesity at age 10y in children from a low income community in São Paulo, Brazil.

1. J.C.K. Wells *et al. Int J Obesity*, 29:1192–1198, 2005.
2. E.M. Taveras *et al. Pediatrics*, 123:1177–1183, 2009.

P-6A-273

Solid introduction and growth in the first two years of life in formula-fed children

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Objectives: The global population recommendation of WHO to start complementary foods after age 6 months is a balance between positive effects of breastfeeding and nutritional requirements of the child. A potentially negative effect of early solid introduction could be more rapid weight gain in infancy which is associated with later obesity. We tested the hypothesis that the timing of solid introduction has no influence on growth during the first two years of life.
Methods: The study is based on a double blinded, randomized controlled trial comparing two groups of children fed cow's milk formula with either higher or lower protein content for the first year of life. Eligible for study participation were apparently healthy, singleton, term infants. Children were recruited in five countries (Belgium, Germany, Italy, Poland, Spain). Standardized anthropometric measurements were taken at recruitment, and at 3, 6, 12, and 24 months. Type of feeding given to the child and the week of introducing solids were asked at 3, 6 and 9 months. Furthermore, infant food intakes were recorded by prospective three day weighed food records at monthly intervals from the infant ages one to nine months. The week of solid introduction was categorized in 4 groups: $<=12$ weeks, 13 to 16 weeks, 17 to 23 and >23 weeks. Anthropometric results were expressed as z-scores relative to the growth standards of the World Health Organization for exclusively breastfed children. Linear regression analysis was applied to test the effect of type of feeding on z-scores at 24 months. Multilevel growth models were used to construct longitudinal models of anthropometric z-score trajectories of each child over the first 2 years of life. The study was approved by the ethics committees of all study centres. Written informed parental consent was obtained for each infant.

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Results: Of originally 1090 formula-fed children included in the study 687 (63%) completed the study until 24 months of age. In 682 children all anthropometric measurements and in 671 also the week of solid introduction were available. The median age at solid introduction was 19 (25.–75. perc. 17–21) weeks. Almost a quarter of the children received solids before 17 weeks and 60% between 17 and 23 weeks of age. The timing of solid introduction was significantly associated with country of study centre, gender, parental nationality, marital status, and maternal smoking behaviour. Those children becoming slimmer between baseline and 3 months of age were fed solids earlier. Solid introduction did not predict anthropometric measures at 24 months of age, growth trajectories, however, differed significantly between the children: those children introduce to solids in the first 12 weeks catched up growth between 3 and 6 months whereas those introduced to solids >23 weeks of age grew slower and stayed on lower trajectories.

Conclusion: Solid introduction did not influence the weight or length at 24 months. However, the growth pattern differed significantly by the timing of solid introduction with a catch-up growth in those introduced to solids in the first 12 weeks.

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P-6A-274

Perinatal supplementation and birth outcomes: does it produce "better" kids? A review of the literature

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Objectives: Long lasting health impact of the intrauterine environment on the developing foetus has been shown for cardiovascular disease and type II diabetes. Deficiencies in macro- and micronutrients during pregnancy have been shown to be associated with adverse outcomes including maternal complications, low birth weight, increased morbidity and mortality. Under-nutrition can affect the foetus in a number of ways, depending on the time of gestation and organ/system development. Much of the literature has examined retrospective studies of cohorts with dietary deficiencies due to war or famine. On the other hand, little is known about an "enhanced" or nutritionally optimal intrauterine environment on the development of the foetus. That is, does a nutrient-rich intrauterine environment (e.g. by supplementation) lead to "better" outcome (e.g. birth outcomes, physical and cognitive development) for the newborn? The *purpose* of this review was to examine the literature on supplementation and nutrient status during pregnancy in relation to pregnancy/birth outcome, foetal development and child development.

Methods: The literature was searched in the databases of PubMed, OVID Medline, AMED, Embase, HealthStar, PsycInfo, GlobalHealth, Cochrane Library, BioMed Central, CINAHL Plus, and Google Scholar, and included human studies, English only text, and no date limit for publication. Search terms used were "pregnancy + supplement [exp. dietary/vitamin] + pregnancy outcome [exp. child outcome]" or "cognition" or "psychomotor" for randomized trials and cohort studies. Titles and abstracts were cursorily examined for search terms. Citations that contained the search terms and/or reflected the terms of interest were included in the review.

Results: A total of 554 references were found after searching the databases with the key terms. After titles were examined for search terms, 75 references were identified. Of the 75 citations, abstracts were reviewed and 58 studies were selected to be appropriate for the review. Of the 58 studies reviewed, 40 looked at nutrient supplementation during pregnancy and child outcome (19) or pregnancy/birth outcome (21); 18 examined nutrient status during pregnancy in relation to child development (4), foetal development (4) or pregnancy/birth outcome (10). Overall, studies found that supplements of fish oils (DHA and EPA) or a multivitamin were associated with mental/cognitive/motor development in children, while supplements of single nutrients such as vitamin A, zinc, or iron were not associated with birth or child outcomes. In studies of nutrient status, the common finding was higher levels of nutrients such as vitamin C, E, B12, folate, and omega 3 fatty acids prenatally were associated with positive birth/pregnancy outcomes and child development.

Conclusion: An enhanced intrauterine environment through supplementation and/or improved dietary intake may be associated with better birth and child outcomes. The strongest evidence seemed to support the omega 3 fatty acids and multivitamins. However, a number of studies had small sample size, short follow up durations and unclear measurement outcomes that made interpretation of the findings problematic. Further research to examine the use of multivitamin and omega 3 supplementation on pregnancy outcome and child development is warranted.

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P-6A-275

The role of serum and breast milk adiponectin in determining maternal and infant adiposity – study methodology

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Objective: Recently, adiponectin is found at high concentrations in human breast milk and proposed to lower the risk of childhood obesity in exclusively breastfed infants[1]. However, the functional role of breast milk adiponectin on maternal and infants obesity is not well explored. Hence, this cohort study aims to investigate the association between maternal blood and breast milk adiponectin concentrations and its relationship with maternal and infant adiposity.
Methods: A total of 320 pregnant women who will be practicing exclusive breastfeeding for at least 2 months and their newborns will be recruited from the Obstetric and Gynecology Clinic, USM Hospital. Mothers with gestational age more than 32 weeks, infant birth weight less than 2500g and complications during pregnancy, delivery or perinatal period will be excluded. Maternal and infant anthropometry will be measured, maternal intakes (food frequency questionnaire) and physical activities (International Physical Activity Questionnaire) will be assessed, maternal blood will be analysed for routine biochemistry tests, adiponectin protein (using ELISA kit) and adiponectin gene polymorphism will be investigated. Breast milk sample will be analysed for adiponectin hormone concentrations (using ELISA kit). Active follow-up for mother and infants will be performed at age 2 months, 4 months, 6 months, 8 months, 10 months and 12 months. All data will be stored and analysed using SPSS program. Appropriate institutional ethics committee clearance and participants' informed consent will be obtained.

Subjects selection (n = 320)

↓

Baseline

Maternal sociodemographic data, clinical characteristics, dietary intake (FFQ), physical activity (IPAQ), anthropometric measurements (weight, height, neck and mid-upper-arm circumferences, skinfold thicknesses, body composition), blood pressure, biochemical investigations (fasting blood glucose, lipid profiles, serum adiponectin) and gene analysis (AdipoQ gene polymorphism).

↓

Follow up after delivery and at age 2, 4, 6, 8, 10 and 12 months

Maternal anthropometric measurements (weight, height, waist, hip, neck and mid-upper-arm circumference, skinfold thicknesses, body composition), blood pressure and biochemical investigations (blood glucose, lipid profile, serum adiponectin, milk adiponectin).
Neonatal anthropometric measurements (weight, length, head, neck, mid-upper-arm, chest, abdominal and hip circumferences, skinfold thicknesses). Placental weight (after delivery only).

↓

Sample and data analysis

Figure 1. Flow chart of research activities.

Results: Complete results shall be made available for publication by July 2011.
Conclusions: Findings from this study will enlighten researchers and the public on the beneficial effect of adiponectin from breast milk on subsequent child weight. Possible collaborative research works are welcomed.

1. J. Bronsky *et al.*, *Clin Chem,* 52:1763–70, 2006.

P-6A-276

Relation of some factors with body mass index in 2–6 years old Iranian children

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Objective: Obesity is one of the most widespread difficulties of children around the world. Prevalence of obesity at age 2–6 years is estimated about 15% in Iran and knowledge of some relation factors is very important.
Methods: This is a cross-sectional study carried out 2009.The data were collected by a checklist containing 28 questions and measurement of body mass index (BMI) as well as 430 children. Independent variable was BMI and dependent variable were nutrition during first year after birth, parents education, occupation of mother, duration of watching TV and playing computers, birth weight, parents BMI, nutritional score, smoking of parents, marriage condition of parents and physical activity. The process of data collection started from 21st of Feb until 25th Apr 2009 and sampling method was random allocation. Content validity was used in order to determine the validity of this questionnaire and for data analysis, descriptive and analytic statistics were used. Data analysis was completed by the aid of the SPSS and statistics soft wares.
Results: The finding of research showed that the prevalence of obesity among the samples was 33%. The finding also showed that nutrition during first year after birth, parents education, occupation of mother, duration of watching TV and playing computers and birth weight were considered as related factors with BMI in 2–6 years old.
Conclusion: Regarding the results of the study and the deep effect of children obesity, investigating the relation factors with BMI of children can be modified with educational programs.

1. S. Fadai. *NMRJ*, 19:53, 2009.
2. N. Carpenter. *WICJ*, 5:10–12, 2006.
3. A. Mellin. *Obstet Gyecol J*, 11:17, 2006.

P-6A-277

The mechanisms of insulin resistance in adipocytes from intrauterine growth retardation rats with catch-up growth

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Objective: In the past decade, several epidemiological studies have shown a relationship between intrauterine growth retardation (IUGR) and insulin resistance(IR). Many studies emphasize that IR is an early manifestation of the mechanisms by which catch-up growth may predispose to other diseases in later life. We developed a catch-up growth IUGR (IUGR-CG) model of Sprague-Dawley rats. To investigate insulin resistant index (IRI), glucose tolerance test, the expression of GLUT4 in the adipocytes and the proliferation and differentiation of the preadipocytes at 4-week-old IUGR rats with catch-up growth.
Methods: IUGR-CG animal model was established by maternal nutrition restriction during pregnancy. 5 newborn IUGR pups were breast-fed by a maternal and 8 normal newborn control pups were breast-fed by a maternal. Recorded the measurement of body weight and body length at every week. 20 IUGR-CG pups and 24 AGA pups randomly selected were to fast overnight and plasma samples were collected for testing of fasting plasma glucose, insulin and triglyceride. HOMA-IR indexes of IUGR-CG group and AGA group were calculated. Glucose tolerance was measured by intraperitoneal injection of glucose (2 g/kg) after an overnight fast. Adipocytes and preadipocytes isolated from epididymal and perirenal adipose tissues of 4-week-old offspring by collagenase digestion. Investigated the expression of GLUT4 in the fluorescence staining adipocytes by confocal microscopic and the proliferation of preadipocytes with crystal violet.
Results: Body weight, body length of IUGR-CG rats were significantly lower than AGA rats at birth and 1-week-old, but body weight of IUGR-CG rats (33.60 ± 3.80 g, 59.26 ± 3.02 g, 74.69 ± 5.81 g) were significantly higher than AGA rats 29.83 ± 3.01 g, 50.83 ± 2.14 g, 71.23 ± 2.75 g) at 2 to 4-week-old. Body length of 2-week-old IUGR-CG rats had no significant difference with AGA rats, even higher than AGA rats at 3-week-old, but lower than AGA rats at 4-week-old. BMI of IUGR-CG rats were significantly lower than AGA rats at birth, but significantly higher than AGA rats at 4-week-old. Fasting plasma glucose had no significant difference between the two groups. Fasting plasma triglyceride (1.29 ± 0.42 mmol/L), insulin (7.01 ± 1.43 mU/L) and IRI (1.32 ± 0.41) of IUGR-CG rats were significantly higher than AGA rats (0.75 ± 0.40 mmol/L, 5.08 ± 1.35 mU/L, 0.83 ± 0.20). Plasma glucose of IUGR-CG rats was higher than AGA rats after 30 min in glucose tolerance test. The expression of GLUT4 in the adipocytes had no significant difference between the two groups. Contrast to AGA, the proliferation of preadipocytes of IUGR-CG rats had an increasing trend.
Conclusions: The body weight, body length and BMI of IUGR-CG rats had a significantly change. There were overweight, hypertriglyceridemia, insulin resistance, impaired glucose tolerance and the proliferation of preadipocytes had a increasing trend in IUGR-CG rats, catch-up growth early in life born intrauterine growth-restricted may be a major risk factor or early manifestation for later obesity, type-2 diabetes and cardiovascular diseases.

P-6A-278

Early weight gain and outcomes at 9 years in very low birth weight children

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Objective: Postnatal growth during infancy and early childhood has implications for adult health. In preterm infants, growth rate after discharge from neonatal intensive care is positively correlated with developmental outcome. It is not known whether rapid growth of preterm infants early in life predisposes them to obesity, cardiovascular disease, and insulin resistance in later life, as is the case in infants born at term. The objective of this study was to determine the influence of weight gain during infancy and early childhood on systolic BP, BMI, and IQ at age 9 years.
Methods: Sixty-five children participated in a follow up study of a randomized placebo-controlled trial of postnatal dexamethasone to decrease ventilator dependence. Dexamethasone exposure had no effect on BP, BMI, or IQ at follow up. Time periods were defined as infancy (nursery discharge to 1 year of age adjusted for prematurity) and early childhood (1 to 4 years). Rates of weight gain (grams/week) were calculated for each period. Z-scores were calculated using US population norms. Multivariate analysis was used to determine the association between rate of weight change during each period and SBP Z-score, BMI Z-score, and verbal and performance IQ at age 9 years. Appropriate institutional ethics committee clearance and participants' assent and guardians' informed consent were obtained.
Results: Characteristics of the participants are shown in the Table.

Characteristic	Mean	Standard Deviation
Birth weight, g	796	195
Discharge weight, g	2,261	437
1 year adjusted weight, g	8,974	1,348
4 year weight, g	17,030	4,006
Δ weight discharge – 1 year, g/week	117	21
Δ weight 1–4 years, g/week	43	16
9 year BMI Z-score	0.19	1.34
9 year SBP Z-score	1.18	1.02
9 year verbal IQ	92	19
9 year performance IQ	86	16

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The rates of weight gain during infancy and early childhood were positively associated with BMI Z-score at age 9, with an increase in BMI Z-score of 0.23 and 0.58 per 10 gram/week increase in rate of weight gain, respectively ($p = <0.01$). These associations persisted in multivariate analyses that adjusted for gender, antenatal steroid exposure, bronchopulmonary dysplasia, major cranial ultrasound abnormality, and maternal education. The rates of weight gain were not associated with either systolic BP Z-score or IQ at 9 years of age.

Conclusion: Rates of weight gain during infancy and early childhood in children born with very low birth weight are important determinates of later BMI. Since childhood BMI tracks to adulthood and is a risk factor for cardiovascular and metabolic diseases, we speculate that rapid weight gain between ages 1 and 4 years may contribute to the development of higher blood pressure and insulin resistance that has been observed in adults born with very low birth weight. Support: The General Clinical Research Center of Wake Forest University Baptist Medical Center MO1-RR07122, the Intramural Research Support Core of Wake Forest University, the Brenner Center for Child and Adolescent Health, and NIH grant number PO1HD047584.

P-6A-279

Reduced risk for body size and shape -related symptoms in young adults born premature – Helsinki study of very low birth weight adults

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Objective: Patients with anorexia and bulimia nervosa are more likely to have a history of obstetric complications[(1,2)] and premature birth[(1,3)]. We tested whether being born prematurely with very low birth weight is associated with symptoms of body dissatisfaction, drive for thinness and bulimia – endophenotypes of eating disorders – in young adulthood.

Methods: 163 premature VLBW subjects (birth weight <1500 g) and 189 controls born at term (not small for gestational age) completed a 22-item Eating Disorder Inventory (EDI) questionnaire. Multiple linear regression and adjustments for a set of covariates were performed. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: EDI total scores were lower (less symptoms) in VLBW subjects than in controls born at term in both sexes. The fully adjusted difference was −12.4% (95% CI −21.1%, −2.7%), $P = 0.01$ among women, and −10.5% (95% CI −19.8%, −0.2%), $P = 0.05$ among men. Of the covariates, higher body mass index (BMI) and higher score in Beck's Depression Inventory contributed significantly to a higher EDI total score in both sexes. In women, also father's BMI, and among men, earlier timing of puberty and shorter height were associated with a higher EDI total score. 134 VLBW adults and 138 controls underwent dual-energy X-ray absorptiometry. Results were similar when we adjusted for fat percent in these subjects. Analyses of subscales revealed that the difference between VLBW adults and controls was observed in each EDI subscale, although the difference was statistically significant for body dissatisfaction in women only. The numbers self-reporting anorexia or bulimia nervosa were similar between VLBW and term subjects.

Conclusions: Young adult women born prematurely with very low birth weight have less body size and shape -related symptoms than their peers born at term. The same phenomenon seems to exist among men, although the evidence remains less clear. We found no evidence for a difference in manifested eating disorders, for which the study had, however, limited power. The study has been supported by the Foundation for Paediatric Research, Finland.

1. S. Cnattingius *et al. Arch Gen Psych.,* 56:634–638, 1999.
2. A. Favaro *et al. Arch Gen Psych.,* 63:82–88, 2006.
3. D.L. Foley *et al. Am J Med Gen.,* 105:426–431, 2001.

P-6A-280

Plasma levels of ghrelin and leptin in children: relationship with age and body mass index

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Objective: Leptin and ghrelin are hormones related to regulation of food intake and consequently body weight control. Normal values of these hormones are subject to a great variability as has been described so far. Due to the lack of studies regarding serum levels of total and acylated ghrelin and leptin in young children, this study aimed to assess these three hormones and compare with body mass index (BMI) in eutrophic children aged 4 months -10 years old.

Methods: Cross-sectional study evaluated 118 children aged 4 -129 months old with BMI-for-age values were within −2.0 and +2.0 SD z-scores for children under 5 years and within −2.0 and +1.0 SD for children over five years, according to WHO 2009.[1,2] Subjects were enrolled from a population

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referred to minimal surgeries and healthy otherwise. All subjects were categorized into 4 groups: 0–24 months (n = 22); 25–60 months (n = 37); 61–96 months (n = 36) and 96–129 months (n = 23). Blood samples were collected following a minimum of 3-hour and a maximum of 14 hours fasting period. Total and acylated ghrelin and leptin concentrations were assessed by ELISA commercial kit (Linco Research, St Charles-MI, US). Appropriate institutional ethics committee clearance and participants informed consent were obtained.

Results: Overall mean age was 61.5 ± 35 months, 64 boys (54.2%). Mean value of BMI was 15.7 ± 1.3 Kg/m^2 (range 12.9–18.9 Kg/m^2). Median (25–75% quartiles) values of total ghrelin, acyl ghrelin were 1427.42) pg/mL (1024.65–1893.23) 290.71 pg/mL (207.41–465.42. Median (25–75% quartiles) values of leptin were 1.55 ng/mL (1.11–2.88), respectively. There was a weak negative correlation between ghrelin (total and acylated) levels and age ($r = -0.454, -0.338$; $p < 0.001$). There was a weak positive correlation between leptin levels and BMI ($r = +0.458$; $p = 0.001$).

Conclusions: Ghrelin and leptin levels do not seem to show great variability in eutrofic subjects ranging from 4 months to 10 years old. This study might be helpful to provide data for the standardization of ghrelin and leptin serum levels in healthy children.

1. WHO Anthro for personal computers, version 3, 2009: Software for assessing growth and development of the world's children. Geneva: WHO, 2009 (http://www.who.int/childgrowth/software/en/).
2. WHO AnthroPlus for personal computers Manual: Software for assessing growth of the world's children and adolescents. Geneva: WHO, 2009 (http://www.who.int/growthref/tools/en/).

P-6A-281

Assessment of the factors effecting malnutrition in children between 0–3 of age, and effect of education on decreased malnutrition in district 19 of Tehran municipality in 2002–2006

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Objective: In general, malnutrition is observed in all ages, but the most amount of outbreak is observed among breast feeding and pre-school children. According to the 1998 statistics, 27.2%, 23.8%, 8.3% of children under 5, suffered from stunting; underweighting and wasting.

Methods: At first in The present interventional-comparative study, the prevalence of different kinds of malnutrition in 400 was determined .The subjects were 0–36 months of age, under health care special in health-care treatment centers of district 19 over the period 78–80 .Then the same study was conducted on 400 subjects of the same age as the first group, from the health care and treatment centers of the same district, requiring special care (having either once descending growth curve, or two consecutive times horizontal growth curve; or slow disproportionate growth compared to reference curve over three consecutive periods of care); referred to a nutrition consultation unit where their mothers were consulted and trained in nutritional facts. The children of the later group had already been under continuous growth surveillance. Then the two groups were compared. In both groups regular systematic methods of sampling was applied.

Results: Upon the intervention, the findings showed a noticeable decrease in various kinds of malnutrition among the age groups of 18, 24 and 36 months compared to the observed group, showing a higher weight mean among the subjects being consulted.

Conclusions: The results show that the health-related problem could be solved utilizing the potentials health care and treatment centers in preparing various methods of education such as holding workshops, training sessions, face to face training, role playing and using a pamphlets and presenting training based on the compiled training package in accordance with the family's economic and social conditions and the need of target group (child growth, breast feeding, food habits, complementary nutrition).

P-6A-281B

Being born small reduces the number of cardiomyocyte nuclei which can be increased by improving postnatal nutrition and growth

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Objective: Cardiomyocytes cease proliferating soon after birth when they become terminally differentiated. A reduced complement of cardiomyocytes at birth can adversely affect the functional and remodelling capacity of the heart. We aimed to determine whether uteroplacental insufficiency and fetal growth restriction reduces the number of cardiomyocyte nuclei in hearts of male rat offspring, and if so, whether this can be overcome by restoring early postnatal nutrition during lactation.

Methods: We studied male offspring from mothers that underwent bilateral uterine vessel ligation (Restricted) to induce intrauterine growth restriction or sham surgery (Control) on day 18 of gestation. Control and Restricted pups were cross-fostered onto Control (normal lactation) or Restricted (impaired lactation) mothers 1 day after birth. At 7 days of age cardiomyocyte nuclei number was determined

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stereologically. Cardiac mRNA expression was quantified by real-time PCR.

Results: There was a significant reduction ($p = 0.02$) in body weight and absolute, but not relative, heart weight of Restricted-on-Restricted offspring on postnatal day 7. Growth restriction was accompanied by a 28% reduction ($p < 0.05$) in total cardiomyocyte nuclei number. Providing a normal lactational environment to restricted offspring (Restricted-on-Control) improved postnatal growth such that body and heart weights were not significantly different compared to Control-on-Control offspring. This improved nutrition and growth was associated with a restoration of cardiomyocyte nuclei number to that in Control-on-Control offspring. There were no differences in cardiac mRNA expression for growth factors (*Igf1*, *Igf1r* and *Igf2*) or differentiation/maturation markers (*Gata4*, *Anp*, *Myl2* and *Myh7*) across the 4 cross-foster groups. All cross-fostering groups had increased cardiac mRNA expression of At1aR and At1bR compared to Control-on-Control offspring ($p < 0.05$) which may be associated with altered cardiac growth. An upregulation of cardiac *Bcl2* and *Cmyc* is suggestive of a compensatory increase in proliferation and reduction in apoptosis in the cross-foster groups.

Conclusions: Growth restriction due to uteroplacental insufficiency adversely impacts on heart cardiomyocyte nuclei number postnatally. Importantly, improvement of lactational nutrition, at a time when the cardiomyocytes are still undergoing proliferation, prevents the deficit in cardiomyocyte number associated with growth restriction.

P-6B-282

Segmental sodium reabsorption in low protein programmed hypertension in the juvenile rat

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Objective: Programmed hypertension induced by maternal protein restriction during pregnancy in the rat is associated with increased renal expression of the $Na^+K^+2Cl^-$ type 2 (NKCC2) transporter in the offspring. The increase in sodium transporter expression has led to the suggestion that sodium retention underlies this form of hypertension[1]. However, we have recently reported[2] that, contrary to expectations, absolute and fractional excretion of sodium (FE_{Na}) are increased in rats with programmed hypertension. This increase in sodium excretion was associated with a 20% reduction in whole kidney Na^+:K^+ATPase activity and the loss of sodium pump expression in the inner medulla of the kidney. The aim of the current study was to quantify segmental sodium reabsorption *in vivo* in order to determine whether increased expression of NKCC2 leads to altered sodium handling by the thick ascending limb of the loop of Henle.

Methods: Programmed hypertension was induced by exposure to a low (9%, LP) maternal protein diet during gestation (N = 5 litters); controls (C) were exposed to an isocalorific 18% protein diet (N = 5 litters). After birth, dams were returned to standard chow and nursed their litters until weaning. Four weeks old male offspring received LiCl-supplemented rat chow (12 mmol/kg chow) for 48 h prior to making standard clearance measurements under Inactin anaesthesia (100 mg/Kg i.p.). Servo-controlled fluid replacement (0.154 M NaCl) was employed: after 90 mins equilibration and a 30 min control period, rats received either amiloride (AM: 2 mg/kg/h) for 1 h followed by AM and bendroflumethiazide (BF: 1.25 mg/kg/h) for 1 h or AM and BF for 1 h followed by AM and BF and furosemide (FUR: 2.5 mg/kg/h) for 1 h. Appropriate institutional ethics committee clearance was obtained.

Results: Mean arterial pressure was increased in LP rats (102 ± 1) compared with controls (87 ± 2 mmHg $P < 0.001$). Glomerular filtration rate did not differ between control (0.60 ± 0.17) and LP rats (0.52 ± 0.08 ml/min/100 g bwt). During vehicle infusion FE_{Na} was increased in LP rats (Table); however, there was no significant difference between LP and control rats during infusion of any combination of diuretic drugs.

Conclusions: These data show that despite increased expression of the loop transporter NKCC2, FE_{Na} is greater in LP rats. Assessment of segmental sodium reabsorption suggests that the greater sodium loss was incurred in the inner medullary collecting ducts, as FE_{Na} only differed in the absence of diuretic drugs. These data are consistent with our earlier report of the loss of Na^+:K^+ATPase expression in the inner medullary collecting ducts.

FE_{Na} (%)	Vehicle	AM	AM + BF	AM + BF + FUR
C (n = 6–14)	1.7 ± 0.5	6.9 ± 0.7	11.2 ± 1.9	34.3 ± 4.5
LP (n = 8–17)	3.0 ± 0.3*	7.6 ± 1.6	11.4 ± 1.7	30.9 ± 3.3

FE_{Na} by the proximal tubule (FE_{Li}: C 37.4 ± 7.1 vs LP 49.2 ± 5.8%) and end proximal fluid delivery (C_{Li}: C 201.6 ± 27.0 vs LP 246.1 ± 28.0 µl/min/100 g bwt) tended to be greater in LP rats, but this did not achieve statistical significance.

1. J. Manning. *Am. J. Physiol.*, 283:F202–F206, 2002.
2. S.H. Alwasel, N. Ashton. *Clin. Sci.*, 117:75–84, 2009.

P-6B-283

Dysregulation of fetal kidney notch signaling pathway contributes to reduced nephrogenesis in growth restricted newborns

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Objective: In human and animal studies, intrauterine growth restriction resulting from maternal under-nutrition (MUN)

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leads to fetal and adult nephropenia (reduced glomerular number), elevation in adult arterial blood pressure, and potential progression to renal disease. Thus, reduced nephrogenesis represents an underlying mechanism for hypertension. We have shown that MUN causes dysregulation of several key fetal (E20) nephrogenic signaling pathways, including ureteric branching and mesenchymal-to-epithelial transformation related signaling pathways. To investigate putative developmental signaling pathways in nephrogenesis, we performed DNA microarray analysis. From these data we have identified the intriguing possibility of dysregulation of the Notch signaling pathway. Notch signaling is regulated by both co-activators (MAML1, SKIP) and co-repressor (CTBP1) which ultimately controls cell fate in embryogenesis, particularly in pretubular aggregate formation. In this study we investigated the dysregulation of Notch signaling and potential downstream effectors.

Methods: Pregnant rat dams were fed either ad libitum diet (control) or were 50% food restricted (MUN) from embryonic day (E10). At E20, male offspring fetal kidneys ($n = 4$ each MUN and control) were RNA hybridized using rat Agilent DNA microarrays to determine mRNA expression. The second kidney from each animal was used for protein expression. Data was considered statistically significant by student t-test at $p < 0.01$ for microarray analysis and $p < 0.05$ for Western blot analysis.

Results: Microarray results showed of the 41,000 oligonucleotides representing unique rat mRNA transcripts, 3547 transcripts were both detectable and differed statistically between control and MUN ($p < 0.01$). At the chosen significance threshold of 1.5 fold, 430 transcripts were increased and 49 transcripts were decreased by MUN. Ratios ranged from 10.3 fold increased to 2.2 fold decreased. Of the differently expressed transcripts, 198 were known genes and these were analyzed for placement into ontological groups and specific signaling pathways. Four significantly dysregulated genes were detected in the Notch signaling pathway, with down-regulation of Notch2 (0.55 fold, $p = 0.01$), and co-activators MAML1 (0.55 fold, $p = 0.01$) and SKIP (0.56 fold, $p = 0.01$), and upregulation of co-repressor CTBP1 (6.8 fold, 0.01). Western blots were performed for Notch2, SKIP, CTBP1 and the downstream effector gene HEY1. Consistent with microarray results, Notch2 protein expression was down-regulated (0.71 fold, $p = 0.01$) as was its downstream target (HEY1, 0.86 fold, $p = 0.01$). Additionally, CTBP1 protein expression was up-regulated (2.0 fold, $p = 0.04$), although SKIP protein was unchanged.

Conclusions: MUN leads to altered expression of nearly 200 known genes in the E20 fetal kidney, suggesting that gestational programming has a large impact on nephrogenesis. In particular, the reduced Notch signaling pathway, which is critical for the determination of cell fate in the developing nephron, suggests that kidney nephron reduction in MUN may occur through alteration of Notch pathway activity during fetal nephrogenesis.

P-6B-284

Effect of maternal low protein diet on kidney ultrastructure, function and arterial blood pressure in adult male rat offspring

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Objective: The aim of present study was investigate the effects of maternal low protein diet on glomerular filtration rate (GFR), urinary sodium handling and blood pressure response, related to morphologic and ultrastructural analysis in adult male offspring.

Methods: Virgin female Wistar rats were fed during pregnancy a normal-protein diet (NP 17% casein) or protein-restricted diet (LP 6% casein). Male renal function tests were performed on the last day at 16 weeks of age and Creatinine and Lithium clearance were carried out. The systemic arterial pressure (AP) was measured in conscious 6, 8, 10, 12, 14 and 16-week-old rats by tail cuff method. Data are presented as mean ± SD and analyzed using appropriate ANOVA. *Post hoc* comparisons between selected means were made by Bonferroni's contrast test. The current study used scanning (SEM) and transmission (TEM) electron microscopy to assess the glomerular ultrastructural morphologyfor correlation with functional data.

Results: In 16 weeks-old LP rats AP increased from 116.2 ± 6.5 mmHg to 137.9 ± 6.9 mmHg ($P < 0.01$), whereas AP in NP rats showed a smaller and non-significant rise from 114 ± 7.4 mmHg to 128.8 ± 8.7 mmHg. In LP, AP rise was significant after 12 weeks of age. Fractional urinary sodium excretion (FE_{Na}) was significantly lower in LP rats, when compared with the NP intake age-matched group (LP: $0.32 \pm 0.012\%$ vs. NP: $0.48 \pm 0.087\%$, $P < 0.05$). The decreased FE_{Na} in LP rats was accompanied by decreased proximal sodium excretion (FEP_{Na}) and fractional potassium excretion (FE_K) ($P < 0.05$). The GFR did not significantly differ among the groups. The figure shows SEM micrographs of glomeruli. **A:** Three dimensional organization of the outer surface of podocytes (p) surrounding capillaries in a control rat. **a:** Detail of a podocyte showing primary (1) and secondary (2) processes and pedicels (arrow) among which filtration slits can be seen. **B:** LP rat glomerulus with an intensive cohesive arrangement and bulbous and crushed podocytes. **b:** Note the irregular surface of cell body with enlarged processes, pseudocyst formation (*), wide and club-shaped pedicels, and reduced number of filtration slits. TEM analyze confirm these results and show electron-dense clumps of amorphous bodies in the cytosol of podocyte processes.

Conclusions: The current data suggest that changes in renal functions are conducive to excess hydroelectrolyte kidney

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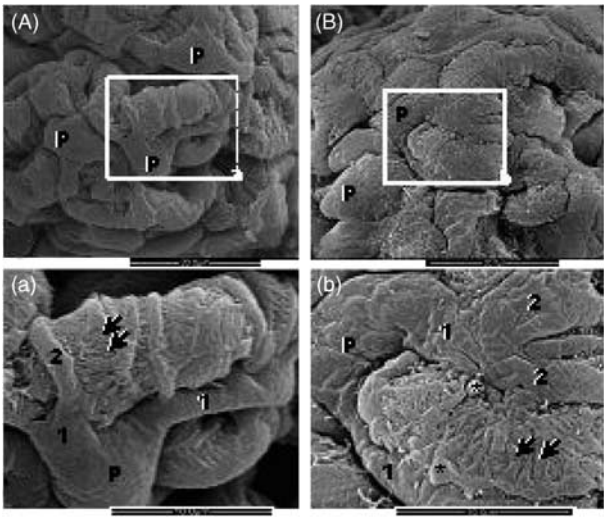
reabsorption, and this way might contribute to programming of adult hypertension. Despitefinding podocyte hypertrophy and foot process effacement or simplification representing a reduction in the complexity of the usual interdigitating pattern of cell–cell connections and apparent diminution of filtration capacity, there was no GFR alteration. These morphological changes could be attributed to an adaptation for reduced nephron number and, consequently glomerular hyperfiltration and overflow in LP offspring, and could account for the breakdown of optimal glomerular filtration barrier functioning. Thus, the current study demonstrates for the first time glomerular morphologic and functional stress that may contribute to premature podocyte senescence as well as glomerular pathology. Support: FAPESP.



P-6B-285

Transgenerational effects of maternal protein restriction on glucose homeostasis, blood pressure and kidney morphology

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Substantial body of evidence suggests that poor intrauterine milieu elicited by maternal nutritional disturbance may programmed susceptibility in the foetus to later development chronic disease, and one of the most interesting features of fetal programming is the evidence from several studies that the consequences may not be limited to the first generation offspring and it can be passed transgenerationally.
Objective: The objective of this work is to study the effects of maternal protein restriction in kidney development, blood pressure and glucose homeostasis in F1 and F2 generations.
Methods: Female (F0) Wistar rats fed either a normal protein diet (control diet, C, 19 g protein/100 g diet), or a low protein diet (restricted diet, R, 5 g protein/100 g diet). The offspring were termed according to the period and the types of diet dams were fed, gestation (first letter) and/or lactation (second letter): CC, RC, CR, RR. Three-mo-old F1 females were bred to proven males, outside the experiment, to produce F2 offspring. At weaning, F2 offspring were divided by gender. Blood pressure, body mass were measured weekly. Blood sample were collected and glucose, insulin and leptin were determined. Homeostasis model assessment for insulin resistance (HOMA) was calculated with the baseline values. Left kidney was analyzed by stereology at birth and at six months, the following parameters were measured: glomeruli number, cortex/medulla (C/M) ratio and glomerular diameter.
Results: RC1 were born with low birth weight, but afterwards they had catch up reaching CC1 weight. The increased glycaemia in RC1 was associated with insulin resistance. CR1 and RR1 showed impairment of the growth with no changes in glucose metabolism. RC2 showed high body weight at birth, sustained all over the experiment in male group. F2 generation showed more alteration in glucose metabolism than F1 generation. CR2 and RC2 had hyperglycaemia accompanied by hyperinsulinaemia and insulin resistance in both genders. CR2 showed increase in body adiposity with hyperleptinaemia. RC1, CR1 and RR1, both genders, showed an increased blood pressure and a decreased glomeruli number associated with glomerular hypertrophy, males showed a greater loss compared to females. The high blood pressure values persisted in F2 with renal consequences only in males.
Conclusions: Low-protein during gestation improves body mass, fat mass and growth rate in F1 rats, and has adverse effects on glucose and leptin metabolism resulting in insulin resistance in adult F1 and F2 offspring. Low-protein during lactation has adverse effects on glucose, insulin and leptin metabolism resulting in insulin resistance in adult F2 offspring. These findings suggest that low-protein during gestation and/or lactation can be passed transgenerationally to the second generation. Support: CAPES, CNPq, FAPERJ (Brazil).

P-6B-286

Early indication of essential hypertension in adolescent offspring of hypertensive parent/s

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Essential Hypertension is the most common form of hypertension in adults[1] and a major cardiovascular risk factor making it a public health concern in the Indian subcontinent[2]. Notwithstanding low prevalence of hypertension in children and adolescents, evidence shows that hypertension

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begins to develop during the first two decades of life[3,4]. School based blood pressure screening and measurement of height and weight revealed a prevalence of 4.5% which was higher than in the past[5]. Tracking of blood pressure over time, therefore, can become a useful tool for early detection of vulnerable kids from amongst their peers. Family history and hereditary disposition are strong determinants of hypertension.
Objective: To prospectively study 8 to 18 year old offspring of parent(s) with essential hypertension from a closed community hospital catering to employees of Mumbai Port Trust.
Methods: 177 children of 113 parent(s) (88Fs and 33 Ms) from 100 families attending l OPD for essential hypertension have been randomly selected (every fifth consenting family attending Hypertension clinic of the hospital) and studied during the period of one year. Standard parent and child history sheets were filled out by the author. Family tree and family history was obtained at length. Blood Pressure measurements were done at least every 3 months on each child. At each visit 3 recordings at an interval of at least 10 minutes were done after ensuring that the child was relaxed. Anthropometry was done to calculate BMI. Data was analyzed using SSPC/PC+ statistical package. Chi square test was applied for correlation.
Results: While no child had frank hypertension, 32 children had high-normal blood pressure (>90 and <95 percentile)as compared to the rest. Of these, 7 children had either parent or grandparent hypertensive, 3 had both parents and both grandparents hypertensive, and 4 had both parents hypertensive. There was a significant relationship between BMI and blood pressure in all children. There was significant relationship between Mother's (but not Father's) blood pressure values and children's blood pressure values.
Conclusions: This simple clinical study shows the need for a vigorous approach to primary prevention of hypertension amongst offspring of hypertensive parents with routine or frequent monitoring of blood pressure and BMI.

1. Bernstein D. Systemic Hypertension. In: *Nelson's Textbook of Pediatrics*, Ed. Beharman, Kliegman and Jenson. Saunders, 17th Ed, pp 1592, 2004.
2. R. Gupta, *J. Human Hypertension*, 18:73–78, 2004.
3. S.L. Chadha *et al. Ind.Heart Journal*, 51:178–182.
4. S.H Zinner *et al. Hypertension*, 7:411–416, 1985.
5. Sorof J.M.*et al. Pediatrics* 113:475–482, 2004.

P-6B-287

High salt diet during pregnancy alters renal and circulating renin-angiotensin system of male and female Wistar rats offspring

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Objective: it is known that morbidity-mortality of adult subjects is influenced by the intra-uterine environment. Exposure to an excess of salt during the perinatal period leads to high blood pressure in adult offspring (Nutr Metab Cardiovasc Dis. 2003; 13:133–139).The objective was to evaluate the renal and circulating renin angiotensin aldosterone system in adult male and female offspring of dams fed high salt diet during pregnancy.
Methods: female Wistar rats received normal (NSD 1.3%), high 1 (HSD1-4.0%) or high 2 (HSD2- 8.0% NaCl) salt diet during pregnancy. All offspring were fed NSD from birth until adulthood, independent of maternal diet. Tail cuff blood pressure (tcBP), serum aldosterone (Aldo), plasma renin activity (PRA), renal renin activity (RRA), renal renin granules (RG), renal cortical (C-mRNA) and medulla (M-mRNA) renin gene expression were determined in 12-weeks-old male and female offspring. * $p < 0.05$ vs HSD1-M; & $p < 0.05$ vs HSD2-M; § $p < 0.05$ vs NSD-M; # $p < 0.05$ vs NSD-M; @ $p < 0.05$ vs NSD-F.
Results (mean±SE):

Parameters	Male offspring			Female offspring		
Maternal diet	NSD	HSD1	HSD2	NSD	HSD1	HSD2
tcBP(mmHg) n = 30/group	119.4 ± 2.8	121.2 ± 2.8	120.8 ± 2.4	117.4 ± 2.6	110.7* ± 3.3	112.7[&] ± 2.7
Aldo (pg/mL) n = 8/group	410.0 ± 35.8	267.9[§] ± 55.4	302.0[§] ± 24.9	1005.0[#] ± 153.3	680.9* ± 161.7	988.9[&] ± 210.2
PRA(ng/mL/h)n = 8/group	4.2 ± 0.4	2.8 ± 0.4	2.2[§] ± 0.6	4.2 ± 0.7	4.7 ± 1.0	4.0 ± 0.7
RRA(ng/mg/tecido/h)n = 8/group	9.0 ± 0.8	15.9[§] ± 1.5	16.4[§] ± 1.8	6.0 ± 1.2	3.6* ± 0.7	6.7[&] ± 1.2
RG (%)n = 8/group	60.7 ± 5.0	40.9[§] ± 3.9	38.4[§] ± 4.2	65.8 ± 4.2	52.9 ± 4.9	46.4[@] ± 4.4
C-mRNA(renin/β-actin)n = 8/group	1.5 ± 0.2	1.1 ± 0.1	1.0[§] ± 0.1	1.3 ± 0.2	1.3 ± 0.2	1.2 ± 0.2
M-mRNA(renin/β-actin)n = 8/group	0.9 ± 0.1	0.5[§] ± 0.03	0.6[§] ± 0.06	0.7 ± 0.1	0.5 ± 0.03	0.8 ± 0.1

Conclusion: High salt diet during pregnancy leads to sexual dimorphic alterations of the renal and circulating renin-angiotensin-aldosterone system and blood pressure in adult Wistar rats offspring. Support: FAPESP.

P-6B-288

Renal vasoactive hormones in adult offspring from diabetic rats: response to high sodium diet and nitric oxide inhibition

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Objective: Foetal exposure to an abnormal intrauterine environment, such as that caused by maternal diabetes,

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results in long-term postnatal abnormalities, including changes in renal function and vascular reactivity in the mature offspring. The mechanisms of these alterations are not elucidated, but it has been observed a permanent change in nitric oxide (NO)-related vascular response. Previous studies have demonstrated that nitric oxide synthase (NOS) inhibition during high-salt (HS) intake leads to the development of salt-sensitive hypertension and to abnormal renal function. We hypothesized that diabetic offspring (DO) would exhibit an abnormal renal response to NOS inhibition, particularly under HS intake. Considering that the kidney synthesizes several vasoactive systems, the aim of the present study was to explore changes in renal hormones in adult DO exposed to HS diet, with or without NOS inhibition.

Methods: At day 7 of pregnancy, Sprague-Dawley rats were randomized to a control group or to a streptozotocin (STZ Upjohn, single injection 45 mg/kg) group, obtaining maternal glucose levels between 300–400 mg/dl. After delivery, pups were weighted and reduced to 8 per dam; control mothers fostered STZ pups. At week 12, male and female offspring from both groups were randomized to normal sodium (NS) or HS diet (1% NaCl in the drinking water); at week 14, groups were again randomized to receive a NOS inhibitor (L-NAME, 50 mg/kg/d dissolved in tap water) or vehicle for two additional weeks. All animals were studied at week 16. Blood pressure was recorded by tail-cuff pletysmography; animals were placed in individual metabolic cages for urine collection and, under general anaesthesia, blood was withdrawn from abdominal aorta.

Results: Weight at birth was reduced in DO (Female: Control = 6.4 ± 0.1 *vs* DO = 5.9 ± 0.2 g; Male: Control = 6.8 ± 0.07 vs STZ = 6.3 ± 0.2 g, $P < 0.05$); net body weight was still lower in DO at week 16. Blood pressure increased with L-NAME, but exhibit no difference between control and DO. As expected, urine volume increased with HS diet in control females (NS = 5.1 ± 1.5 vs HS = 40 ± 11 ml/16h), but this change was attenuated with L-NAME. DO had an attenuated diuretic response to HS. Female and male DO had higher basal renin activity than their corresponding controls. Under all experimental conditions, aldosterone levels were higher in male than in female rats. Urinary kallikrein activity was higher in male than in female rats, but no significant difference was observed between DO and control rats. L-NAME treatment reduced kallikrein in all groups except in female control rats. Kallikrein/renin ratio increased with HS intake in both female and male control rats, but remained unchanged in DO. Response to L-NAME was reduced in DO, particularly in male rats (Control NS: 901 ± 204 to 1602 ± 366; DO NS: 846 ± 257 to 1127 ± 192).

Conclusions: Present data suggest that prenatal hyperglycaemia alters renal vasoactive hormones in response to HS diet and also modifies the response to L-NAME treatment. Thus, intrauterine exposure to high glucose could increase the vulnerability to environmental stressors. Support: FONDECYT 106.0720.

P-6B-289

Evaluation of renal function in the preterm neonate: evidence of microalbuminuria during the first month of life

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Objective: Preterm infants are born at a time when nephrogenesis, the formation of nephrons in the kidney, is still on-going. Therefore, being born prematurely is likely to impact on renal function. To date there is little known as to the effect of preterm birth on renal function, and how renal functional development may be influenced by various pre- and post-natal factors. Hence, the aim of the current study is to evaluate renal function in preterm neonates during the first month of life.

Methods: Preterm babies were recruited from Monash Newborn neonatal intensive care unit at the Monash Medical Centre (Clayton, Victoria, Australia) and were stratified into three gestational age groups: ⩽28 weeks (extremely preterm; n = 16), 29–31 weeks (very preterm; n = 21) and 32–36 weeks (moderately preterm; n = 17). Twenty-four hour urine collection began 72 hours following the time of birth, through collection of the babies' nappies, and continued for the following five days. Nappies were fitted with a cotton pad and nappy liner prior to use in order to facilitate clean urine extraction via a hydraulic press. Urine was pooled per 24 hours, and from these extracted samples creatinine clearance (CrCl) and the fractional excretion of sodium (FeNa) was calculated. On day 7, clean spot urine samples taken using a urine collection bag were also analysed for urinary albumin excretion. Further 24 hour urine collections and spot urine samples were taken on postnatal days 14, 21, and 28. Appropriate institutional ethics committee clearance and informed parental consent were obtained.

Results: During the first week of life, CrCl increased with increasing gestational age at birth while FeNa decreased with increasing gestational age. By one month of age, CrCl had increased significantly ($p = 0.03$) and FeNa had decreased significantly ($p = 0.002$) in all groups. Importantly, albumin to creatinine ratios were higher than normal (>3.5 mg/mmol) in almost all preterm babies, with the extremely preterm group having significantly higher ($p = 0.02$) levels compared to the older gestational age groups. Albumin to creatinine ratios were highly variable between individuals, and there were some cases of pathological proteinuria on day 7 of life with total urine protein > 500 mg/L.

Conclusions: The findings of this ongoing study demonstrate that renal function in the preterm neonate is significantly

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affected by gestational age at birth. The high urinary albumin levels observed in the preterm neonate may be indicative of glomerular damage and/or structural immaturity of the glomerular filtration barrier. This project is supported by the National Health and Medical Research Council of Australia (NHMRC).

P-6B-290

Effects of antenatal endotoxin exposure on kidney development in rats

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Objectives: Chorioamnionitis is one of the major causes of preterm delivery. It may also be associated with impairment of various fetal organs such as brain (white matter injury) and lungs (chronic lung disease). Some recent studies revealed that fetal growth restriction due to intrauterine undernutrition might cause impairment of kidney development. However, we do not know how chorioamnionitis influences developing kidneys. Therefore, we studied effects of antenatal intra-amniotic injection of lipopolysaccharide (LPS) on growth and development of the kidneys.

Methods: At 20 d gestation, pregnant SD rats were anesthetized, the uterus exposed, punctured and 0.1 μg LPS dissolved in 0.1 mL saline injected into each amniotic sac. In the control group, 0.1 mL saline was injected. At 22 d (term), the fetuses were delivered spontaneously and vaginally. At 0 and 28 days, the pups were euthanized and the kidneys harvested, weighed and fixed with formaldehyde. Some of the kidneys collected at birth were dried in an oven for dry/wet weight ratio. The 3 μm thick paraffin embedded samples were stained with hematoxylin-eosin, microscopic images taken and saved for morphometric examination. We analyzed morphometric parameters such as volume ratio of cortex to medulla (C/M), numerical density of glomeruli in the cortex (Ng/C) and mean glomerular volume (Vg).

Results: LPS pups had higher perinatal mortality rate than controls (42/73, 58% vs 12/82, 15%; $p < 0.01$). At birth, kidney weights were lighter ($0.028 +/- 0.002$ vs $0.021 +/- 0.002$; g, $p < 0.01$) and the dry/wet weight ratio of the kidney tended to be lower in the LPS group than in controls (0.13 ± 0.04 vs 0.17 ± 0.06, $p = 0.24$). C/M also tended to be decreased in the LPS group compared to controls (3.8 ± 1.8 vs 4.4 ± 1.9, $p = 0.31$). At 28 days, LPS treated pups tended to have lower Ng/C (202 ± 48 vs 154 ± 52; / mm^3, $p = 0.35$) and higher Vg (12.2 ± 2.0 vs 17.3 ± 2.7; $\times 10^{-5}\, mm^3$, $p = 0.23$).

Conclusions: A relatively low dose (~ 20 μg/kg) intra-amniotic LPS had significant deleterious effects on fetal rats. Antenatal exposure to LPS resulted in lighter kidneys at birth, which was likely to be associated with higher water content and reduced cortex volume. LPS exposure may have impaired nephron endowment (fewer and larger glomeruli) and may potentially influence renal function in later life. *Acknowledgments:* We thank Drs Y. Shimizu and Y. Goto for assistance in preparation of histology samples and N. Koyama, S. Okada, M. Ryumae and Y. Saikatsu for daily care of experiment animals.

P-6B-291

Maternal smoking during pregnancy affects fetal kidney size. The Generation R Study

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Objective: An adverse fetal environment may lead to smaller kidneys, which predispose the individual to subsequent development of kidney disease and hypertension in adult life[1–4]. We examined whether maternal smoking during pregnancy, as important determinant of an adverse fetal environment, directly influences kidney development in third trimester of pregnancy.

Methods: In a population-based prospective cohort study among 1,031 children followed from early fetal life onwards, we assessed maternal smoking during pregnancy (not, first trimester only, continued) using questionnaires. Fetal kidney characteristics (left and right length, width, depth and combined volume) were measured by ultrasound at a gestational age of 30 weeks. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Of all mothers, 9.4% smoked in first trimester only, and 15.9% continued smoking during pregnancy. Compared to non-smoking, first trimester only was not associated with any fetal kidney characteristics. We observed a curved-shaped association between the number of cigarettes smoked in third trimester of pregnancy with fetal kidney size (see figure). Compared to non-smoking, smoking less than 5 cigarettes per day was associated with larger combined kidney volume (difference 2.04 (95% Confidence Interval 0.73, 3.36) cm^3), while smoking more than 10 cigarettes per day was associated with smaller combined kidney volume (difference −1.99 (95% Confidence Interval −3.86, −0.11) cm^3). We did not find any associations between maternal smoking during pregnancy and deepest pocket of amniotic fluid, as reflection of kidney function. Adjusting these associations for fetal sex, gestational age, estimated fetal weight, alcohol use during

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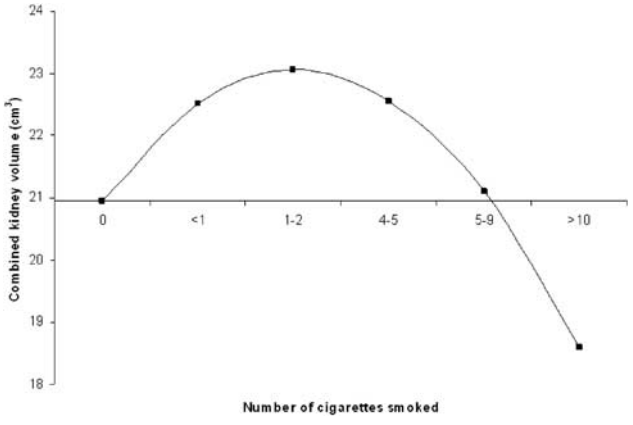
pregnancy, maternal educational level, maternal height and maternal pre pregnancy weight did not materially influence the effect estimates.

Conclusion: This study showed for the first time that maternal smoking during pregnancy affects kidney development in fetal life with a curved shaped relationship. Further studies are needed to assess the underlying mechanisms and whether these developmental adaptations have postnatal consequences for kidney function and blood pressure. The first phase of the Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam; Erasmus University Rotterdam; and The Netherlands Organization for Health Research and Development (ZonMw). Additional support was provided by a grant from the Dutch Kidney Foundation (C08.2251).



1. P. Bateson *et al.*, *Nature,* 430:419–421, 2004.
2. S.A. Hinchcliffe er al., *Br J Obstet Gynaec,* 99:296–301, 1992.
3. B.M. Brenner, GM Chertow. *Am J Kidney Dis,* 23:171–175, 1994.
4. B.M. Brenner *et al.*, *Kidney Int,* 49:1774–1777, 1996.

P-6B-292

Renal and hormonal changes in offspring born to ewes fed high salt during pregnancy

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Objective: To determine the effect of high maternal salt intake during pregnancy on renal physiology of the offspring.

Methods: Animals used in this experiment were born to ewes that were fed either a high-salt diet for the final 3 months of pregnancy ('high-salt offspring') or from a control group ('control offspring'). Blood samples were taken from the lambs at birth, at 2 weeks of age and at 4 months of age when weaned. Animals were weighed at birth, weaning and at the end of the experimental period. Following weaning, the physiological consequences of ingesting high amounts of salt were tested by feeding the lambs (n = 60) *ad libitum* amounts of either a control (1.5% NaCl) or a high-salt (10.2% NaCl) diet over a 16-day treatment period. Over the last four days of the 16-day period, blood samples were collected twice daily. Voluntary feed intake and water intake were measured throughout the treatment period, and urinary output was measured over the last four days of the experiment. The lambs were euthanased at the end of the experiment and their kidneys collected for analysis.

Results: The high-salt offspring had a lower plasma renin concentration (6.47 *v* 8.43 ng/mL/h, SE 0.42; $p < 0.001$) than control offspring, regardless of the diet they were fed. Similarly, renin mRNA was more lowly expressed (21%) in the high-salt offspring ($p = 0.033$). However, plasma aldosterone was higher in the high-salt offspring than in the control offspring (49.4 *v* 43.6 pg/mL, SE 1.02; $p < 0.001$). The high-salt offspring gained less weight during the experimental period compared to the control offspring ($p = 0.064$), and there was an interaction between their foetal origin and treatment diet ($p = 0.088$). They also excreted less urine ($p = 0.094$), however, there was no difference in the total urinary sodium excretion between both groups. Foetal origin significantly altered glomerular density in kidney tissue, with the high-salt offspring having 17% fewer glomeruli than the control offspring ($p < 0.001$) without significant differences in the kidney weights between the salt and control offspring. No significant differences were observed in glomerular filtration rate (GFR) or organ (kidney, liver and lung) weights between the two groups of offspring. Feeding the high-salt diet during the 16-day treatment period, in both the high-salt and control offspring, lowered plasma renin and aldosterone concentration, and increased kidney weights, urinary sodium excretion and total urinary output, as was expected.

Conclusions: This study showed that high salt intake during pregnancy affected the offspring's renal physiology, changing their ratio of aldosterone to renin concentration. It is possible that this is the result of an altered responsiveness or sensitivity to renin. The implications of the decline in glomerular density are yet to be determined. Future studies are needed to see if the observed epigenetic effects are passed down through subsequent generations.

P-6B-293

The reduced glomeruli number is associated with increased glomerular volume and attenuated RAS activity in maternal food restriction rats

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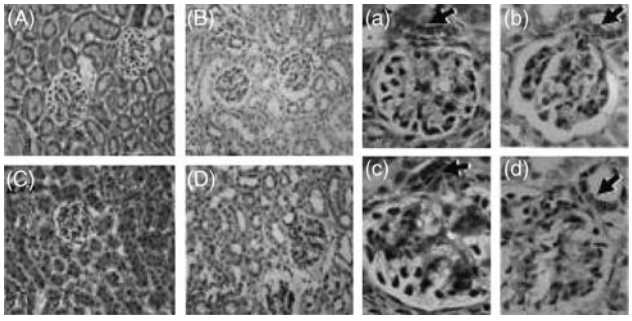
The renin-angiotensin system (RAS) is a coordinated hormonal cascade in the control of cardiovascular, renal, and adrenal function that governs body fluid and electrolyte balance, as well as arterial pressure.

Objective: The present study aims to investigate the effects, in juvenile male rats, of food restriction *in uteru* investigating nephron number, glomerular volume and its possible association with angiotensin II (angio II) receptors (ATR) expression.

Methods: The daily food supply to pregnant rats was measured and one group received normal quantity of food (NF) while the other group received 50% of that (FR50). The birth weight (BW) of male offspring was measured. At 23rd day, the rat was perfuse for tissue fixation and the kidneys removed, weighted and the volume estimated by Cavalieri's principle. Fractionators'method was used to estimate glomeruli number in histological slices. The slices were also processed to ATR type 1 and 2 (AT1R and AT2R) immunolocalization.

Results: The FR50 offspring presented significant reduction in birth BW (5.67 ± 0.16 vs. 6.84 ± 0.13 g, $P < 0{,}001$). Our results show that at 23rd day of life, FR50 offspring has fewer (18%) glomeruli per kidney when compared with NF group. Despite the smaller nephron number and enhanced cortical area, the FR50 group animals have similar total glomerular volume. In the figure **A** and **a,** we have AT2R/NF localization in basolateral tubular pattern, in glomeruli and macula dense (arrow). In **B** and **b** is the same but in RF50; in the figure **C** and **c** we have represented AT1R/NF localization in apical tubular surface, in glomeruli and macula densa (arrow). **D** and **d** is the same but in RF50. These figures shows that in FR50 cortical enhanced area is in consequence of rise in internal and external tubules diameter. This morphological aspect is indicative of an elevation in the intratubular sodium concentration. The patent diminution in afferent arterioles ATR expression in FR50 is indicative of rise in both glomeruli blood flow and ultrafiltration. Additionally, this reduced expression was observed in mesangium that may result enhance in glomerular capillary area and filtration coefficient. Consequently we may have a rise in sodium proximal concentration that may explain the prominent enlargement of your internal diameter. In FR50 the reduced ATR expression was also observer in macula dense suggesting possible impairment in tubule-glomerular feedback and enhance of: rennin and angiotensin circulating; vasoconstriction; blood pressure. The angio II acts in an autocrine or paracrine fashion to modulate proximal tubular transport and, in physiological circumstance, promote proximal ATR-dependent sodium reabsorption but, in FR50 the important tubular ATR decrease observed denote the impairment in this response with consequent rise in distal sodium and water delivery provoked by the rejection of this ion by the proximal nephron.

Conclusions: We may affirm that gestational food restriction is related to juvenile RAS activity impairment, which may be involved in genesis or in adaptation to diminution in nephron number but, in any of these two hypotheses, is clearly involved in adult hypertension establishment. A renal function study is necessary to confirm these morphological and immunohystochemical results. Support: FAPESP support.



P-6B-294

Reduced nephron number is a common phenotype of maternal protein and iron restriction in Wistar and Hooded Lister rats

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Objective: A wealth of epidemiological evidence indicates that the intrauterine environment is a determinant of lifelong physiological and metabolic function and therefore related to risk of disease in adult life[1]. Global nutrient restriction[2], in particular protein restriction[3], can reduce the total number of nephrons formed in developing rat kidneys. This has been linked to hypertension in adulthood[4]. Iron deficiency anaemia is the most prevalent micronutrient deficiency on a global scale, affecting approximately two thirds of the world population, with pregnant women particularly at risk. 65% of the world population are at risk of protein intakes below the UK RNI for pregnancy[5]. Whilst hypertension has been observed in offspring of rats that are iron deficient in pregnancy, an effect on renal morphology has not previously been reported. Therefore the objective of this study was to identify if a reduction in nephron number was common across two established rat models of hypertensive programming: a maternal low protein (MLP) model developed in Wistar (W) rats and a maternal iron deficiency (FeD) model in the Rowett Hooded Lister (RHL) strain[6].

Methods: 30 W and 35 RHL rats were assigned to a diet group as shown in the table below. Groups 1–2 and 5–6 were weaned onto standard laboratory chow and fed either a CP

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(18% casein) or MLP (9% casein) diet after successful mating with a stud of the same strain. From day 13 of pregnancy (GA13) all animals were fed CP until birth, when all experimental diets were replaced with chow. Iron diets commenced at weaning for 4 weeks pre-mating. At GA13 FeD (7.5 mg Fe/kg) diet was replaced with FeC (50 mg Fe/kg) for the remainder of pregnancy. One of the male offspring from each litter was culled at 16 weeks of age and the left kidney fixed in buffered formalin. Total nephron number was determined using a maceration method.

Results: As shown in the table, nephron number was significantly reduced in Wistars subjected to protein restriction and iron deficiency *in utero*. An even greater reduction was seen in restricted RHL offspring.

Group	1	2	3	4	5	6	7	8
Strain	W	W	W	W	RHL	RHL	RHL	RHL
Diet	CP	MLP	FeC	FeD	CP	MLP	FeC	FeD
n	9	8	5	8	9	6	10	10
Nephrons/ kidney	24806 ± 2045	15862 ± 1468*	27621 ± 1135	17581 ± 2116*	29351 ± 1373	18198 ± 1984*	29378 ± 1172	20584 ± 3261*

CP = control protein; FeC = control iron; MLP = low protein; FeD = iron deficient. Data are shown as mean ± SEM. * indicates significantly different to appropriate control group of same strain ($P < 0.01$).

Conclusions: For both strains, protein and iron restriction in early to mid gestation resulted in a common phenotype of a reduced nephron complement in adult male offspring. This experimental design now provides a powerful tool to examine common pathways which may drive the programming of renal development and associated disease risk, allowing assessment of the genes, proteins and pathways for which expression is disturbed across both strains and dietary protocols.

1. D.J. Barker. *Philos Trans R Soc*. 359:1359–1356, 2004.
2. M.C.P. Franco *et. al. J Cardio Pharmacol*. 39:369–377, 2002.
3. S.C. Langley-Evans *et al. Life Sci*. 64:965–974, 1990.
4. H. Mackenzie, B. Brenner. *Am J Kidney Disease*. 26:91–98, 1995.
5. S.C. Langley-Evans *et al. Clin Sci*. 91:607–615,1996.
6. L. Gambling *et al. J Physiol*. 552:603–610,2003.

P-6B-295

High-salt intake during pregnancy is associated with blood pressure and renal structural and functional alterations in adult female offspring

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Objective: to evaluate the effects of high salt intake during pregnancy on blood pressure (BP) and renal function and structure of adult female offspring.

Methods: normal- (NS, 1.3%) or high-salt (HS, 8% NaCl) diet was given to Wistar rats during pregnancy. During lactation all dams received NS diet as well as the offspring after weaning until the 21st week of age. Starting on the 21st week of age, 50% of each group was fed high-salt (hs, 4% NaCl) diet until 36 weeks of age (NShs, HShs). The other 50% was maintained on a NS diet (NSns and HSns) for the same period. Results (mean ± SEM, $p < 0.05$, n = 5–8/groups): tail-cuff BP (TcBP-mmHg) was higher in HShs (117 ± 4) compared to NShs (106 ± 2). No differences were observed in TcBP among HSns and NSns offspring groups. Kidney weight (g/kg) was higher in HShs (3.7 ± 0.1) compared to HS (3.3 ± 0.1). Twenty-four hour urinary protein excretion (mg/100 g/day) was higher in HShs (0.9 ± 0.1) compared to HS (0.5 ± 0.05). Serum aldosterone levels (pg/ml) were lower in NShs (824 ± 62) compared to NSns (1128 ± 114). This difference was not observed between HShs (919 ± 60) and HSns (988 ± 172) offspring groups.

Conclusions: according to these data, high salt diet during pregnancy is associated with BP and renal abnormalities in adult female offspring. Offspring from HS dams are hyperresponsive to salt overload in adulthood and this mis-adaptation is associated with a non-modulation of serum aldosterone levels. Support: FAPESP and CNPq.

P-6B-296

Maternal protein restriction affects gene expression profiles in mouse kidney at weaning with implications to the regulation of lifespan

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Objectives: Nutritional conditions and growth rate during early life can influence later health and lifespan. We previously demonstrated that low birth weight resulting from maternal protein restriction during pregnancy followed by catch-up growth in rodents was associated with shortened lifespan whereas protein restriction and slow growth during lactation increased lifespan[1,2]. The current study aims to understand molecular mechanisms by which maternal protein restriction influences longevity.

Methods: Three groups of animals were established by subjecting mice to the same maternal protein restriction regime that is known to influence lifespan: control animals were the offspring of dams fed a diet containing 20% protein throughout pregnancy and lactation, postnatal low protein (PLP) animals were offspring of control dams but nursed by dams fed an isocaloric low protein diet (containing 8% protein) and recuperated animals were offspring born to dams who were fed the low protein diet during pregnancy but were cross-fostered to control dams during lactation. Kidneys were collected at weaning (21 days of age) from these three groups (n = 8 for

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each group). Total RNA was extracted using TRI reagent and purified using an RNeasy Mini Kit. RNA samples were used to generate cRNA which were subsequently fragmented and hybridized to the Affymetrix GeneChip Mouse Genome 430 V2.0 Array (>45,000 probe sets). Microarray data were analyzed using Affymetrix GeneChip Operating Software and GeneSpringGX 7.3. Quantitative RT-PCR was employed to verify microarray results for specific genes of interest. Protein expression was measured by Western blotting.

Results: 36 genes in PLP and 52 genes in recuperated kidneys were up-regulated, 54 genes in PLP and 302 genes in recuperated kidneys were down-regulated compared to control tissues. Pathway analysis using Ingenuity Pathway Analysis revealed that these genes are involved in diverse biological functions such as cellular growth/proliferation, cell signaling, and metabolism. Quantitative RT-PCR verification of 14 selected genes showed that changes in gene expression detected by microarray were robust and reproducible. Among the genes up-regulated in PLP kidneys was heme oxygenase-1 (HO-1). HO-1 has cytoprotective functions which are mediated by antioxidant and anti-inflammatory effects of the enzyme reaction products. Western blot analysis showed that HO-1 protein level was also significantly ($P < 0.001$) up-regulated in PLP kidneys. Among the genes down-regulated in recuperated kidneys were Lon peptidase 2 and FOXO3a which have been shown to be involved in the regulation of the ageing process through their respective functions on protein homeostasis and expression of antioxidant enzymes.

Conclusions: Maternal protein restriction can influence gene expression profiles which in turn can affect many biological pathways. Up-regulation of HO-1 in PLP kidneys may suggest that tissues of these long-lived mice are equipped with a better cytoprotective function. In contrast down-regulation of Lon peptidase 2 and FOXO3a in the kidneys of recuperated offspring may suggest that protein homeostasis and resistance to oxidative stress are compromised in these shorter lived mice.

1. B.J. Jennings *et al.*, *FEBS Lett.* 448: 4–8, 1999.
2. S.E. Ozanne, C.N. Hales. *Nature* 427: 411–412, 2004.

P-6C-297

Single nucleotide polymorphisms in the leptin and leptin receptor genes are associated with fetal growth trajectories and adolescent cardiovascular disease precursors

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Objective: Leptin is a key adipokine reflecting the general state of long-term energy stores. Its effects are related to eating behaviour and satiety leading to alterations of body composition. Abnormalities in leptin quantity and function can result in metabolic syndrome (obesity, atherogenic dyslipidemia, hypertension, and insulin resistance). There is increasing evidence of the role of leptin in fetal growth with abnormal leptin levels measured at birth in neonates who have experienced intrauterine growth restriction[1]. The aim of this study was to assess the associations between single nucleotide polymorphisms (SNPs) in leptin and the leptin receptor genes and antenatal growth and adolescent cardiovascular disease precursors.

Methods: 1079 population-based adolescents from the Western Australian Pregnancy Cohort (Raine Cohort) underwent serial ultrasound biometry of fetal head circumference (HC), abdominal circumference (AC) and femur length (FL) at 18, 24, 28, 34 and 38 weeks' gestation. Anthropometry was measured at birth, including head, chest, and abdominal circumferences, birth length, birth weight, and ponderal index. Cardiovascular disease precursors were assessed at age 14, including body mass index, fasting total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, homeostasis model assessment of insulin resistance (HOMA-IR) and systolic blood pressure. DNA samples were obtained and the leptin (*LEP*) and leptin receptor (*LEPR*) genes were tagged with five and 26 SNPs respectively. Linear mixed effects models were used to analyse ultrasound anthropometrics (HC, AC, FL and HC/AC) and random effects were fitted for each individual for slope and intercept. Association between *LEP* and *LEPR* polymorphisms and anthropometric parameters at birth and 14-years and cardiovascular disease precursors at 14-years were investigated using multivariate linear modelling, including terms for diet, exercise, smoking and socioeconomic status. P-value of <0.05 was considered significant. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Three SNPs in *LEP* were associated with significant alterations to fetal growth. The minor alleles in *LEP* SNPs rs11763517 and rs11760956 were associated with reduced fetal AC growth trajectories, birth-weight and AC on day-2 of life. Conversely, *LEP* SNP rs3828942 was associated with an increase in fetal AC and FL growth trajectories. None of the five SNPs in *LEP* were associated with cardiovascular disease precursor in adolescence. Of the 26 SNPs in *LEPR*, 12 *LEPR* SNPs were associated with changes in fetal growth trajectories, both increased and decreased. Two SNPs (rs9436737 and rs1892535) were associated with an increase in body mass index at 14-years in male adolescents; neither was associated with BMI in female adolescents. Nine SNPs in *LEPR* were associated with changes in lipid profile, including adverse and beneficial changes. *LEPR* SNP rs3762274 was associated with increase in AC and birth-weight as well as HOMA-IR at 14 years.

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Conclusions: Multiple SNPs in *LEP* and *LEPR* are associated with fetal growth trajectories and adolescent precursors of cardiovascular disease. These associations were different in males and females and require detailed evaluation and replication in other pregnancy cohorts. These data support the concept that complex gene-environment interactions underlie the developmental origins insulin resistance.

1. Chiesa C, *et al. Clin Chem*. 54:550–558, 2008.

P-6C-298

Associations of 48 genetic adult height loci with fetal femur length

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Objective: Recent genome-wide association studies have identified 57 single nucleotide polymorphisms (SNPs) in 48 different loci which affect adult final height. However, each individual SNP has only limited effect on total body size, which may be due to the fact that body stature is a highly complex trait comprised of several separate phenotypes, such as torso and leg length. Final adult height is also known to be partly determined by early growth patterns. We therefore hypothesized that a number of these SNPs are associated with fetal femur length.

Methods: This study was embedded in the Generation R Study, a population-based prospective cohort study. Polymorphisms were selected from the recently published genome-wide association studies on adult height. Genotypes of the 57 SNPs were extracted from the Illumina HumanHap 610 chip. Femur length was determined in second and third trimesters of pregnancy by fetal ultrasound. Data was available in 1194 and 1218 children of Dutch/Northern European ancestry for second and third trimesters, respectively. All analyses were performed under an additive model adjusted for sex and gestational age and were Bonferroni corrected for multiple testing. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Mean femur length in second and third trimesters was 33.4 mm (SD = 3.38) and 57.5 mm (SD = 2.99), respectively. Only 2 of the 57 SNPs analyzed were associated with femur length in utero. Each A-allele of rs10906982 (ADAMTSL3) was associated with an increase of 0.26 mm in femur length in both the second ($p = 3.6*10^{-4}$) trimester and 0.29 mm in third ($p = 1.8*10^{-3}$) trimester. This association remained after Bonferroni correction. A weaker association was also found for each G-allele of rs4713858 (ANKS1) in both the second (0.33 mm, $p = 1.6*10^{-3}$) and third (0.26 mm, $p = 0.049$) trimesters, though this association did not endure Bonferroni correction.

Conclusions: This study indicates that at least one, and possibly two, of the identified SNPs previously associated with adult height influence femur length as early as from second trimester of pregnancy onwards. The relative effects in standard deviations scores are up to twice as large as the effect sizes found previously on final adult height. Data from a larger sample and replication of these findings in the Raine Study will be presented. Larger studies with longer follow-up and studies with other body stature measures are necessary to identify in what exact way these identified polymorphisms influence final height.

P-6C-299

Effect of maternal protein restriction *in utero* on offspring lung micro-RNA expression

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Objectives: micro-RNAs (miRNA) are short nucleotides that can regulate the expression of genes at the post-transcriptional level. miRNAs have an important role in fetal lung development, modulating developmental timing, cell fate determination and apoptosis. The lung undergoes several stages of development, extending into early life, that are sensitive to alterations in the developmental environment. We hypothesised that restricting the amount of protein available to the developing fetus will result in alterations to lung miRNA expression that will persist into later life.

Methods: Pregnant Wistar rats were allocated to either control (C, 18% casein) or protein restricted (PR, 9% casein) diet for the duration of the pregnancy. Lung tissue was harvested from 120 day old male offspring (C group, n = 7; PR group, n = 7). miRNA expression was assessed by miRXplore^{TM} microarrays, the cut-off was determined as ⩽1.7 fold change in expression. Changes in expression were correlated to miRNA gene clusters to determine whether miRNA expression was controlled by cis-elements acting at shared promoter regions. A miRNA gene cluster was defined as two or more miRNA genes less than 10 kb apart in non-genic regions.

Results: 15 miRNA were up-regulated (1.8–16.1 fold) and 13 miRNAs down-regulated (1.8–13.7 fold) in response to

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maternal PR. A further 9 miRNA genes were only expressed in PR lung and another 6 miRNAs were only detected in controls. miRNA gene cluster expression profile analysis revealed that the miR-181a-1 miR-181b-1 cluster, is down-regulated in response to PR. All other expression is independent of miRNA gene clustering e.g. alteration of a single miRNA (bold in table) or two miRNAs with in one cluster demonstrating reciprocal expression (underlined in table).

Expression grouping	miRNA name (rno-miR-xxx)
↑ in PR	22*, 24-1, 33A, 92B, **100**, 124, †126*, 130B, 142-3P, †186, 218, **374**, **450A-2**, 484, **494**
↓ in PR	17-3P, **23A**, **98, 99A**, 101B, 122, 140*, 151*, [181A*, 181B], 301-A, 362-3P, 674-3P
PR only	**LET-7D***, 10B, 20A, 142-5P, 331-5P, 340*, †365, **450A-1**, 664
Control only	9*, 129*, 153, 190, 378*, 384-3P

†miRNA expression confirmed by qPCR; [ ] miR-181a-1 miR-181b-1 cluster.

Conclusions: We have shown changes to key miRNAs that are known to be involved in critical stages of lung development and that these changes persist into later life. miRNAs exhibiting altered expression in response to PR include members of the miR-17-92 gene family, such as miR-20a that is expressed in the mesenchyme during the pseudoglandular stage of lung development and a member of the LET family (LET-7D); that are involved in developmental timing. Therefore, miRNAs are a potential mechanism of plasticity in fetal lung developmental. Future work will complete qPCR validation, determine miRNA profiles in younger offspring and seek to establish which post-transcriptional and/or epigenetic mechanisms (e.g. gene promoter-DNA methylation) could account for these observations.

P-6C-300

The vitamin D receptor is related to growth in subjects born very preterm

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Objective: Epidemiological studies have indicated that polymorphisms of the vitamin D receptor (VDR) are associated with body growth. The effects of polymorphisms of the VDR gene can be examined best in infancy, when growth is fastest. However, this has not been investigated in children born very preterm. No data are available about a possible association with early catch-up growth, which these infants show to a variable extent after discharge from the hospital.

Methods: A cohort of 344 children born very preterm, recruited from the POPS-cohort (consisting of 94% of all subjects born very preterm (GA <32wks) or with a low birth weight (<1500g) in the Netherlands in 1983) was studied from birth to 19 years. We chose four common SNPs of the VDR: *c1521g* (promoter region 1a), associated with more promoter activity; *Fok1* (exon 2), associated with a more active receptor, *Bsm1* (intron between exon 8 and exon 9), and *Taq1* (exon 9). The genotypes were analyzed in a recessive model. Length (0-2 yrs) or height (2-19 yrs), weight and head circumference (HC) were measured at birth, 3, 6, 12 and 24 months, and at 5 and 19 years. All measurements were converted to standard deviation scores (SDS) using national references. Early catch-up growth was defined as length-SDS (3–24 months) minus birth length-SDS.

Results: Each of the SNPs was in Hardy-Weinberg equilibrium. The minor allele frequencies for *c1521g*, *Fok1*, *Bsm1* and *Taq1* were 0.40, 0.36, 0.41 and 0.40 respectively. The GG-genotype (minor allele) of *c1521g* was associated with faster growth, when compared to carriers of the C-allele. At 3 months and 19 years, the difference in height-SDS was 0.45 (95% CI 0.02–0.87) and 0.41 (95% CI 0.06–0.75) respectively. For *Fok1*, height-SDS at 19 years was 0.38 higher for the AA-genotype, when compared to carriers of the G-allele (95% CI 0.01–0.75). HC-SDS was larger for the GG-genotype of *c1521g* at 6 months (0.43, 95% CI 0.10–0.77) and at 5 years (0.26, 95% CI −0.03–0.55). For *Fok1,* the difference in HC-SDS at 5 years was 0.34 (95% CI 0.03-0.65). For *Bsm1* and *Taq1*, no differences in height-SDS or HC-SDS were observed between homozygotes for the minor allele and carriers of the major allele. Weight-SDS did not differ significantly for the four SNPs. Catch-up growth during the first 24 months showed no differences for *c1521g* and *Fok1*. For *Bsm1,* the differences between the TT-genotype versus carriers of the C-allele were 0.58 (95% CI 0.02–1.15), 0.82 (95% CI 0.29–1.35), and 0.68 (95% CI 0.13–1.24) at 6, 12 and 24 months respectively. For *Taq1*, the differences between the GG-genotype versus carriers of the A-allele were 0.56 (95% CI 0.01–1.12), 0.84 (95% CI 0.33–1.035), and 0.65 (95% CI 0.11–1.20) at 6, 12 and 24 months respectively.

Conclusions: The GG-genotype (minor allele) of *c1521g*, and the AA-genotype (minor allele) of *Fok1* are associated with a larger height in young adulthood and a greater head circumference in childhood. The TT-genotype of *Bsm1* and the GG-genotype of *Taq1* are associated with early catch-up growth in infancy.

P-6C-301

Impact of the parity on very low birth weight rate in the Southern Brazil

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Objective: There is a significant association of Very Low Birth Weight – VLBW (<1,500 g) with primiparity. There are no data related to VLBW trends in Brazil although findings from some large surveys carried out in small and medium-sized cities have demonstrated an increase in Low Birth Weight – LBW (<2,500 g) rates over the last 30 years. The aim of this study was to evaluate the impact of parity on the secular trend of VLBW in the city of Porto Alegre, a large city in a developed area in Southern Brazil, and their potential determinants of this trend during the 1990s and early 2000s.
Methods: This is a registry-based study. Data were obtained from birth certificates of all live births in the city from 1994 to 2005. The variables analyzed were: VLBW as the dependent variable and parity as independent variable. Maternal age and schooling, type of delivery, type of hospital, number of live births, gestational age, newborn gender, and unemployment rate were included in the model as covariables. Poisson regressions were performed in order to assess the influence of some independent variables on VLBW. The incidence ratio rate (IRR) using Poisson regression was calculated to identify possible trends in parity and VLBW rates. Appropriate institutional ethics committee clearance were obtained.
Results: A total of 257,740 singleton newborns were delivered in the city during the period, with a steady reduction in the total number of live-births per year. The results showed a small but significant increase in VLBW (P for trend = 0.049). There was a significant trend towards adequacy for gestational age per birth weight, suggesting a reduction in rates of intrauterine growth restriction (IUGR). The crude relative risk of VLBW per year reinforces a significant increase in the probability of VLBW and in the main risk factors related to VLBW mothers with low levels of schooling, public hospitals, primiparity and multiparity. Primiparity remained as a significant risk factor and the interaction between type of hospital and type of delivery indicated that vaginal delivery in private hospitals represents the lowest risk, with risk increasing steadily from mix to public hospitals. In the latter, caesarean section was always associated with high rates of VLBW.
Conclusion: These results show that Southern Brazil is facing a demographic transition demonstrated by a significant decrease in number of live-births. This was associated with an increase in primiparity rates, effectively contributing to an increase in VLBW rates. *Acknowledgements:* to Denise Aerts, Juarez Cunha, Gehysa G.Alves and Rui Flores of Secretaria Municipal de Saúde de Porto Alegre.

P-6C-302

Glucocorticoid receptor-9beta polymorphism is associated with blood pressure and heart growth during early childhood. The Generation R Study

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Objective: Glucocorticoid receptor-9β polymorphism (rs6198) is associated with the susceptibility for cardiovascular disease. The aim of this study was to examine whether the GR-9β variant is also associated with blood pressure and heart growth in early childhood.
Methods: This study was embedded in a population-based prospective cohort study from fetal life onwards. Left cardiac structures (aortic root diameter, left atrial diameter and left ventricular mass), shortening fraction and heart beat were measured postnatally at the ages of 1.5, 6 and 24 months. Blood pressure was measured at 24 months of age. Analyses were based on 857 children. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The distribution of the GR-9β genotype showed 75.1% homozygote reference, 23.5% heterozygote and 1.4% homozygote variant subjects. No differences in cardiovascular outcomes were observed at the ages of 1.5 and 6 months. At the age of 24 months, homozygote variants showed an increased systolic blood pressure of 2.65 mmHg (95% CI: 0.16, 5.14), an increased heart rate of 9.14 beats per minute (95% CI: 0.22, 18.1) and an increased left ventricular mass of 5.01 grams (95% CI: 1.32, 8.71) compared to homozygote references. This means an increase of 2.6%, 8.6% and 16%, respectively. GR-9β polymorphism was significantly associated with left ventricular mass growth during the first 2 years.
Conclusions: Our findings suggest that genetically determined differences in cortisol sensitivity exposure affect cardiovascular development in early life. Future studies are needed to replicate these findings and should assess whether these relations persist during later life and are associated with development of cardiovascular disease.

P-6C-303

Molecular markers of predictive value for phenotypic outcomes associated with low birth weight

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Objective: Epidemiologic studies in human cohorts and animal studies have shown that low birth weight (LBW) is a valuable predictor of later health issues, such as hypertension, ischemic heart disease, glucose intolerance, insulin resistance, type II diabetes, obesity and reproductive disorders. However, the parameters which determine LBW are poorly defined and the clinical evaluation of LBW associated phenotypes remains imprecise in the absence of objective measures. We hypothesize that LBW associated phenotypes should result from variable patterns of gene expression that discriminate LBW populations from counterparts considered to be of Optimal Birth Weight (OBW) and reflect the impact of environmental cues during prenatal development. LBW is often a consequence of intrauterine growth retardation (IUGR) during foetal development.

Methods: To investigate potential prognostic markers of LBW associated phenotype present at birth, we have compared patterns of gene expression in LBW and OBW subjects by global gene expression arrays. A total of 38 subjects were recruited based on birthweight criteria (LBW $<$ 2500 g, 3200 $<$ OBW $<$ 3600 g) from a case/control study diagnosed with IUGR *in utero*; from which umbilical cord samples were collected at the time of birth, flushed free of blood and snapped frozen in liquid nitrogen. RNA extracted from whole cord tissue was hybridized to an Agilent 4×44 K gene expression array. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Unbiased cluster analysis revealed distinct populations of subjects and a total of 429 differentially expressed genes ($P < 0.05$, fold change $\geqslant 2$).

Conclusions: We anticipate that differences in cord gene expression will correlate to phenotypic distinctions between LBW and OBW babies and provide markers of prognostic utility allowing prediction of health status later on in life. Given that early development *in utero* is considered a critical period in which epigenetic marks such as DNA methylation are susceptible to environmental modulation, variable gene expression in LBW is likely to be reflected in differential promoter methylation status in critical genes. For future analysis, genes of potential biological significance will be validated by quantitative RT-PCR analysis and subject to subsequent DNA methylation analysis by Sequenom EpiTyper MassArray. Support: A*STAR and NHG (NHG/SIG-07072).

P-6C-304

Cytosine-Adenosine $(CA)_n$ repeats polymorphism in IGF-I gene and early growth in infants born appropriate and small for gestational age

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IGF-I gene polymorphisms might alter IGF-I level[1] resulting in decreased foetal and postnatal growth, accelerated weight gain in infancy[2] and increased risk for diabetes mellitus type 2 and cardiovascular diseases in adulthood[3].

Objective: We analyzed the association between Cytosine-Adenosine $(CA)_{10-24}$ repeats polymorphism in promoter region of the IGF-I gene and early growth in infants with birth weight appropriate for gestational age (AGA) and small for gestational age (SGA).

Methods: All neonates were born at term, 196 of them were AGA and 26 SGA. Blood for DNA analyses was obtained from placental part of umbilical vein. Genotyping was performed using fragment analyses of IGF-I gene promoter region. The data about postnatal growth in the group of AGA children were obtained at the age of 18 months, in SGA children at 12 months. The Medical Ethics Committee of 1st Medical Faculty of Charles University, Prague, approved the study. Written informed consent was obtained from parents of all participating infants.

Results: No differences in the frequency of wild type allele with $(CA)_{19}$ repeats and polymorphisms with $(CA)_{<19}$ or $(CA)_{>19}$ repeats were observed between AGA and SGA children. The average birth weight and length in AGA wild type $(CA)_{19}$ homozygotes were lower in comparison with AGA carriers of various $(CA)_n$ polymorphisms but all observed anthropometric differences disappeared at the age of 18 months. In SGA children, no differences were found between number of $(CA)_n$ repeats and anthropometric parameters both at birth and at the age of 12 months.

Conclusions: Although $(CA)_n$ repeats polymorphism in IGF-I gene might affect prenatal growth in AGA children, our results have not shown any impact of variable number of $(CA)_n$ repeats in IGF-I gene on postnatal growth, absence of significant differences in SGA children in the present study indicate that probably other genetic and environmental factors may infuence postnatal growth. With financial support of the Internal Grant Agency of the Ministry of Health of the Czech Republic NR 9374-3/2007.

1. I. Rietveld *et al*, European Journal of Endocrinology, 148:171–175, 2003.
2. E. Landman *et al*, Pediatrics, 118:2374–2379. 2006.
3. N. Vaessen *et al*., Diabetes, 50:637–642, 2001.

P-6C-305

Mitochondrial DNA 16189T $>$ C variant is associated with lower body mass index of children at the age of 18 months

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A mitochondrial DNA (mtDNA) variant 16189T > C is associated with insulin resistance in European adults and type 2 diabetes mellitus in Asians[1,2,3] and with lower ponderal index (kg/m^3) at birth[4], which is a risk factor for insulin resistance.

Objective: In our prospective study we analyzed the association between mtDNA variant 16189T > C and early growth in infants with birth weight appropriate for gestational age (AGA) and small for gestational age (SGA). In addition, insulin resistance was analysed in SGA children at the age two years.

Methods: 191 AGA and 53 SGA children were enrolled. Blood for DNA analyses was obtained from placental part of umbilical vein. Genotyping was performed using the PCR-RFLP. The data about postnatal growth in AGA children were obtained at the age of 18 months, in SGA children at the age of 24 months. The blood for glycaemia and insulin levels were obtained and HOMA indexes were counted. The study was approved by Ethical Committee and an informed consent was obtained from parents.

Results: No differences in the prevalence of mtDNA variant 16189T > C were found between group of AGA neonates (common T variant 85.3%, C variant 14.7%) and SGA newborns (T 86.8%, C 13.2%). Although the ponderal index at birth was not different in neonates with and without mtDNA variant 16189T > C, the AGA children with mtDNA variant 16189T > C had significantly lower body mass index at the age of 18 months ($P < 0.05$). In SGA children, no association was found in glucose and insulin levels and HOMA index and mtDNA variant 16189T > C.

Conclusions: The results of our study suggest, that mtDNA variant 16189T > C is associated with lower BMI in toddler's period, but it plays only a minor role in the pathophysiology of diabetes type 2. Support: IGA-NR 9374-3/2007 and VZ 64165.

1. P. Chinnery *et al*, *Lancet*. 366:1650–51, 2005.
2. K. Park *et al*, *Diabetologia*, 51:602–608, 2008.
3. J. Poulton *et al*, *Hum Mol Genet.* 11:1581–83, 2002.
4. K. Casteels *et al*, *Lancet,* 353:1499–1500, 1999.

P-6C-306

Two new independent loci are associated with size at birth

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Objective: Identifying genes involved in birth outcomes presents some particular challenges. Notably, the maternal intrauterine environment is often considered to be of primary importance, while two genomes (maternal and fetal) may interact with it, and with one another, to influence any given trait. Despite this, it is important to understand the etiology of fetal growth. High and low birth weights are associated clinically with considerable perinatal risk and epidemiologically with later life chronic disease. The identification of such genes is likely to provide important insights into fetal growth processes.

Methods: We analyzed genome-wide association data from 10,623 participants, born of singleton pregnancies at ⩾37 weeks' gestation, from 5 European studies: Avon Longitudinal Study of Parents and Children (N = 1,418); Northern Finland 1966 Birth Cohort (N = 4,333); 1958 British Birth Cohort (N = 3,264); Netherlands Twin Registry (N = 414); Generation R (N = 1,194). After quality control, a total of 2,427,548 directly-genotyped and imputed SNPs were available for meta-analysis. We tested the association of each SNP with birth weight, assuming an additive model and adjusting for sex and gestational age.

Results: Two new statistically independent loci were found to be strongly associated with birth weight ($P = 1 \times 10^{-10}$ and $P = 8 \times 10^{-8}$). Both signals had a similar estimated per-allele effect size of about 0.09SD (approx. 90 g difference between the two homozygous groups). Further work is needed to establish the causal genes.

Conclusions: Our study provides evidence that genetic factors contribute to normal variation in birth weight. A difference in birth weight of 90g between homozygous groups is equivalent to the effect of a mother smoking 3 cigarettes per day in the third trimester of pregnancy. Replication efforts are underway, and further studies will be necessary to assess the role of maternal genotype at these loci and to investigate potential maternal-fetal genotype interactions.

P-7A-307

Maternal prepregnancy weight change in relation with birthweight and risk of adverse pregnancy outcomes

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Objective: Maternal prepregnancy BMI is associated with pregnancy outcomes and fetal growth. Maternal nutritional status early in pregnancy affects maternal adaptation to pregnant state and fetal growth potential. Weight change in the years before pregnancy may be another significant modulator of maternal nutritional status at the early stage of pregnancy. Thus the aim of this study was to investigate the association of maternal prepregnancy weight change with birthweight and adverse pregnancy outcomes, while taking into account maternal BMI before pregnancy.

Methods: Data come from the ongoing French EDEN mother-child cohort. Women's weight at age 20 years, just before pregnancy, after delivery, birthweight and pregnancy outcomes were collected for 1756 mother-child pairs. An average yearly weight change was computed from the weight difference between 20 years and age at pregnancy and divided in 3 categories with balanced number of observations: <0.1 kg/year ($n = 589$), 0.1–0.75 kg/year (reference category) ($n = 580$), $\geqslant 0.75$ kg/year ($n = 587$). The association between prepregnancy weight change (PPWC) with birthweight or adverse pregnancy outcome were analysed with multivariate linear or logistic regressions adjusted for maternal age at pregnancy, height, weight gain during pregnancy, smoking, parity, centre, newborn gender and prepregnancy BMI. Interaction between PPWC and maternal prepregnancy BMI was tested and were not significant. Appropriate institutional ethics committee clearance and patients consent were obtained.

Results: Birthweight was lower in women with PPWC < 0.1 kg/year (3247 g 95% CI 3215–3279), than in women who gained 0.1–0.75 kg/year (3305 g CI 3273–3337, $p = 0.01$). However, a prepregnancy weight gain higher than 0.75 kg/year did not show an additional increase in birthweight (3295 CI 3260–3330). OR for small for gestational age infant (SGA) was associated with PPW: compared to the reference category, OR for SGA was 1.72 (95% CI 1.11–2.68,) for PPWC < 0.1 kg and 0.97 (0.55–1.7) for PPWC $\geqslant 0.75$ kg/year. Risk of large for gestational aged (LGA) was not significantly associated with higher PPWC categories. Adverse pregnancy outcomes were also related PPWC for the lower and higher PPWC categories ORs for gestational diabetes were respectively 0.62 CI 0.33–0.17, and 1.74 CI 1.00–3.03 (p trend 0.002), OR for gestational hypertension 0.90 CI 0.44–1.82 and 1.76 CI 0.91–3.40 (p trend 0.04). For hospitalization during pregnancy there was an increased risk only in the higher category compared to reference: 1.52 CI 1.10–2.12.

Conclusions: The findings of this study suggest that, independently of maternal BMI, maternal prepregnancy weight change is positively associated with maternal risks during pregnancy while a prepregnancy weight loss increases the risk of SGA babies. Although we did not find an interaction according to maternal prepregnancy BMI, these results may not apply to underweight or obese women who were too few in our data set (8.3%(146) and 8.2% (144) respectively) for reliable estimation. *Acknowledgements:* ID was supported by the French A.N.R and the EDEN study by several funding sources.

P-7A-308

Fructose consumption during gestation alters maternal metabolism and fetal expression of CD36 and FABPpm

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Objective: Over the past several decades there has been a global increase in the intake of fructose due in part to increased consumption of fructose-sweetened beverages such as soda pop and fruit juices[1]. High fructose intake has been shown to promote body weight gain along with derangements in glucose homeostasis and lipid metabolism in both rodent and human models[2,3]. In addition, obesity and malnutrition during pregnancy have been associated with increased susceptibility to cardiovascular and metabolic disease in the offspring. Despite this knowledge, the effects of fructose consumption on pregnant mothers and their offspring have not been extensively studied. Therefore, the objectives of this study were to examine the effects of fructose intake during gestation on maternal health, fetal growth and fetal cardiac energy metabolism.

Methods: Female rats were randomized into one of two groups: 10% fructose solution consumed during gestation (FR; $n = 11$) or tap water (CNTL; $n = 12$). Maternal blood was collected for determination of glucose, insulin, and triglyceride concentrations before gestation, and on gestational days (GD) 12 and 19. Dams were weighed and ended on GD 20. Fetuses and placentas were harvested and their weights were recorded. Fetal blood was collected for determination of glucose and insulin concentrations and fetal hearts were harvested to determine protein expression of the fatty acid transport proteins CD36 and FABPpm using immunoblot analysis.

Results: Maternal weight, placental weight, fetal weight and litter size were not different between FR fed rats and CNTL rats. However, plasma glucose was significantly elevated on GD 19 in FR fed rats compared to CNTL rats (FR: 7.12 ± 1.0 mmol/L; CNTL: 5.43 ± 0.8 mmol/L; $p < 0.05$) and plasma insulin was increased on GD 12 (FR: 2.40ng/ml $\pm$ 1.6 ng/ml; CNTL: 1.33 ± 0.5ng/ml; $p < 0.05$). Moreover, plasma triglyceride was elevated on GD 12 (FR: 3.21 ± 1.0 mmol/L; CNTL: 1.59 ± 0.8 mmol/L; $p < 0.01$) and GD 19 (FR: 9.89 ± 1.7 mmol/L; CNTL: 4.76 ± 1.1 mmol/L; $p < 0.01$) in FR fed rats compared

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to CNTL rats. These results support that FR feeding during pregnancy results in detrimental alterations in maternal glucose and lipid metabolism. Fetal plasma insulin and glucose concentrations did not differ between the groups. However, protein expression of the fatty acid transport proteins CD36 and FABPpm was significantly reduced in offspring of FR fed rats compared to CNTL rats. This suggests that FR feeding alters cardiac substrate supply in offspring, which might impede fetal cardiac energy metabolism.

Conclusions: Taken together, our data indicate that fructose intake during gestation leads to detrimental alterations in maternal glucose and lipid metabolism and might delay maturation of energy metabolism in the newborn heart.

1. K.J. Duffey, B.M. Popkin. *Am J Clin Nutr*, 88: 1722S–1732S, 2008.
2. S.S. Elliott *et al.*, *Am J Clin Nutr*, 76:911–922, 2002.
3. K.L. Stanhope *et al., J Clin Invest,* 119:1322–1334, 2009.

P-7A-309

A maternal diet rich in fat results in preferential elevation in amniotic fluid lipid concentrations

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In addition to lifestyle and genetic factors the maternal environment plays a role in programming disease in later life. Models of maternal diets high in fat demonstrate that offspring are programmed with a metabolic-like syndrome, but understanding of how maternal fat intake alters fetal development and lipid metabolism is limited. Amniotic fluid lipid content reflects fetal lipid status, allowing us to probe fetal lipid metabolism under conditions of altered maternal dietary intake. We hypothesise that exposure to high levels of saturated fats during development will alter fetal metabolism of lipids, resulting in increased accretion of fatty acids and altered fetal growth.

Objective: To determine the effects of maternal fat consumption in pregnancy upon lipid profiles in maternal (maternal plasma) and fetal (amniotic fluid) compartments.

Methods: Female Sprague-Dawley rats (n = 4–7 per group) were fed either a control (7% canola oil) or lard rich (HF) (3% canola oil and 20% lard) diet for 3 weeks prior to mating and throughout pregnancy until embryonic day 20 (E20). Dams were euthanised, a blood sample taken and the uterus removed. Amniotic fluid was collected and frozen. Embryos and placentas were dissected and weighed. Lipid profiles were determined in amniotic fluid and maternal plasma by performing lipid extraction followed by liquid chromatography mass spectrometry. Internal standards were used to quantify diacylglyceride (DG), triacylglyceride (TG) and phosphatidylcholine (PC) species and expressed as pmol/ml. Data were compared by 1-way ANOVA with maternal diet as the factor. Data were first analysed as total (measured) DG, TG and PC, then individual species were compared. Data are presented as mean ± SEM.

Results: At E20, offspring of HF dams were significantly heavier than controls (HF 3.1 ± 0.04 g vs. control 2.9 ± 0.04 g, $P < 0.005$). Placental weights did not differ ($P < 0.87$) between groups. Total maternal DG ($P < 0.94$), TG ($P < 0.11$) or PC ($P < 0.51$) did not differ in dams consuming HF or control diets. Conversely, there were striking differences in the lipid concentrations measured in amniotic fluid: DG ($P < 0.02$), TG ($P < 0.0001$), and PC ($P < 0.014$) were significantly raised following *in utero* exposure to maternal HF diets. Further analysis of lipid species in TGs demonstrated an increase in saturated fatty acids in offspring of HF dams (HF 576 ± 76 pmol/ml n = 6 vs. control 311 ± 54pmol/ml n = 4, $P < 0.01$) and mono-unsaturated fats (HF 712 ± 71pmol/ml n = 6 vs. control 323 ± 56 pmol/ml n = 4, $P < 0.01$). Of the 33 TG species measured, 23 were significantly ($P < 0.05$) elevated in offspring of HF rats.

Conclusions: Offspring of HF dams are significantly heavier in late gestation than age-matched controls suggesting an altered growth trajectory. Maternal consumption of a HF diet in pregnancy has only a modest affect on maternal plasma fat concentrations, however there are profound increases in amniotic fluid fatty acid profiles, suggesting that placental transfer or fetal utilisation of lipid species is altered under conditions of dietary excess. Further analysis of placentae and fetal organs will better our understanding of the underlying mechanisms. Support: National Heart Foundation of Australia Fellowship (PF 06M-2766) and Monash Fellowship to JAA.

P-7A-310

Leptin receptor and insulin receptor mRNA levels are reduced in pancreas of male rat offspring following maternal high fat nutrition

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Epidemiological and experimental studies have highlighted a relationship between an adverse prenatal environment and long-term metabolic consequences, in particular obesity and leptin and insulin resistance. We have recently shown that maternal high fat feeding results in obesity and hyperinsulinemia and hyperleptinemia in offspring independent of postnatal diet[1].

Objective: The present study therefore examined possible alterations in the adipoinsular feedback loop as a result of altered mRNA levels of insulin receptor (IR) and leptin

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receptor (ObRb) in pancreas of male offspring following maternal high fat nutrition.

Methods: Wistar rats (day 120) were assigned to 1 of 3 experimental groups: 1) Controls (CONT): dams fed a standard chow diet pre-conceptionally and throughout pregnancy and lactation; 2) MHF group: fed a HF diet from weaning until conception and throughout pregnancy and lactation, and 3) PLHF: dams fed a chow diet until conception and a HF diet throughout pregnancy and lactation. Birthweights were reduced in MHF and PLHF offspring and pups were hypoleptinemic and hypoinsulinemic compared to CONT pups. At weaning, offspring were placed onto either the standard chow or HF diet for the remainder of the study (160 days).

Results: MHF and PLHF offspring had significantly increased total body fat compared to CONT animals even when fed a standard chow diet postnatally and these effects were exacerbated in offspring fed the HF diet. The increased adiposity in MHF and PLHF offspring was paralleled by elevated fasting plasma insulin and leptin. We now report that dams fed HF either pre-conceptionally and/or during pregnancy and lactation produced offspring with significantly reduced pancreatic mRNA levels of ObRb compared to CONT offspring ($p < 0.05$). Post-weaning high fat nutrition did not further alter pancreas ObRb expression in any of the groups suggestive of a direct maternal nutritional effect. Interestingly, there was no difference in ObRb levels between the MHF and PLHF phenotypes, suggesting that offspring effects due to HF diet exposure during pregnancy and lactation are independent of pre-conception HF nutrition. IR expression was significantly increased in pancreas of HF-fed CONT offspring compared to chow-fed CONT animals but there was no effect of postnatal diet in MHF or PLHF offspring. IR expression was significantly reduced in MHF and PLHF offspring fed the HF diet compared to CONT HF animals ($p < 0.05$).

Conclusions: There were no statistical differences in IR mRNA levels within or between MHF and PLHF offspring groups, suggesting that maternal pre-conception diet was not a determinant in offspring IR gene expression. These data further reinforce the importance of maternal nutrition during critical windows of development and show that maternal HF feeding alone can induce a markedly obese phenotype with altered ObRb and IR mRNA levels in mature offspring, completely independent of relatively healthy postnatal nutrition.

1. Howie GJ, *et al.*, J Physiol. 587:905–15. 2009.

P-7A-311

Intra-uterine exposure to maternal diabetes is associated with higher adiposity and insulin resistance and clustering of cardiovascular disease risk markers in Indian children

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Objective: To test the association of maternal gestational diabetes mellitus (GDM) and gestational glucose and insulin concentrations in the absence of GDM, with adiposity, insulin resistance and cardiovascular risk factors in the young offspring.

Methods: Children (N = 630, 41 born to mothers with GDM) whose mothers completed an oral glucose tolerance test during pregnancy had detailed anthropometry at birth and at least annually until 9.5 years at the Holdsworth Memorial Hospital (HMH), Mysore. Systolic (SBP) and diastolic blood pressure (DBP) and plasma glucose and insulin concentrations were measured at 9.5 years of age in 455 children whose fathers' diabetes status was determined at 5-year follow-up. The HMH research ethics committee approved the study, and informed consent was obtained from parents and children.

Results: At birth, offspring of diabetic mothers (ODM) were larger in all body measurements than controls (babies of non-GDM mother and non-diabetic father). At 9.5 years, female ODM were larger in all anthropometric measurements including triceps and subscapular skinfold thickness ($P < 0.001$). The difference in skinfold measurements between ODM and controls increased with age, in girls (Figure 1). Female ODM also had higher glucose (30-minute, $P = 0.002$) and insulin concentrations ($P < 0.01$ for all) and higher SBP ($P = 0.02$). Insulin resistance (HOMA; $P < 0.01$) was higher in both male and female ODM compared to control children. These associations were independent of maternal BMI. Among offspring of diabetic fathers, girls had larger subscapular skinfold thickness ($P = 0.006$), and boys had higher HOMA ($P = 0.03$) compared to respective controls; associations were not independent of father's BMI. In control children, subscapular skinfold thickness was positively related to both maternal and paternal BMI ($P < 0.001$). There were similar positive associations between both parents' glucose/ insulin concentrations, and offspring adiposity and insulin resistance independently of parent's BMI.

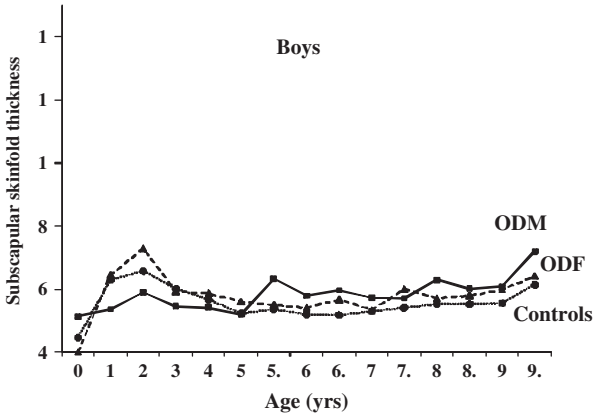

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Figure 1. Median subscapular skinfold thickness for ODM, offspring of diabetic fathers (ODF) and controls 0–9.5 years.

Conclusions: Maternal diabetes during pregnancy is associated with higher adiposity and cardiovascular disease risk markers in the offspring. The stronger associations than in the offspring of diabetic fathers suggest an additional effect of the intra-uterine environment. The associations in control offspring are not conclusive of an intra-uterine adverse effect of lesser degrees of maternal glycaemia. Support : The study was supported by the Parthenon Trust, Switzerland, the Wellcome Trust and the Medical Research Council, UK.

P-7A-312

Maternal obesity in the rat permanently influences sympathetic control of blood pressure in offspring: evidence for selective leptin resistance

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Objective: Leptin plays an important role in the central control of appetite, and through activation of hypothalamic efferent sympathetic pathways to the kidney, is also implicated in obesity-related hypertension. We have recently reported hyperphagia and increased adiposity in the adult offspring of obese rats, associated with region specific hypothalamic leptin resistance in the arcuate nucleus[1]. Here we have investigated the blood pressure longitudinally from weaning, and the potential role of leptin and sympathetic control pathways.

Methods: Female Sprague Dawley rats were maintained prior to mating, and throughout pregnancy and suckling on either a standard chow diet or a semi-synthetic energy-rich and highly palatable obesogenic diet, (16% fat, 33% simple sugars). All offspring were maintained on standard chow and at 30 days, before the onset of obesity, hyperphagia and hyperleptinaemia, blood pressure and heart rate were recorded using mouse radio-telemetry probes (DSI PhysioTel® PA-C10) and cardiovascular responses to restraint stress and a leptin challenge (10 mg/kg i.p.) determined. Heart rate variability (HRV) was derived from the telemetry record by autoregressive spectral analysis at 30 and 90 days, and at 90 days, baroreflex sensitivity was derived from blood pressure responses to phenylephrine and sodium nitroprusside (SNP). The role of sympathetic activity was investigated by administration of propanolol and terazosin (10 mg/kg i.p.), and renal noradrenaline and renin.

Results: Weanling (postnatal day 30) offspring of obese dams (OffOb) demonstrated increased systolic blood pressure compared to offspring of control (OffCon) (SBP [mmHg, mean ± SEM] male OffOb, 129.1 ± 0.9 *Versus* OffCon, 118.6 ± 0.9, $n = 6$, $P < 0.05$; female OffOb, 131.5 ± 0.3 *Versus* OffCon, 124.6 ± 0.5, $n = 6$, $P < 0.05$) with exaggerated cardiovascular responses to restraint stress ($P < 0.01$) and a >4 fold increase in renal tissue noradrenaline content ($P < 0.001$). Low frequency HRV oscillations, indicative of sympathetic tone, were significantly increased in male and female OffOb *Versus* OffCon ($P < 0.05$). Leptin administration caused a greater rise in blood pressure in OffOb than OffCon ($P < 0.05$). Renal renin was increased at both timepoints. At 90 days reflex tachycardia to SNP was reduced in OffOb rats, (slope of linear regression, OffOb -1.16 ± 0.06 Versus OffCon -2.24 ± 0.07 bpm/mmHg, $P < 0.05$) with increased responsiveness to PE ($P < 0.01$). Mixed α and β blockade normalized blood pressure in OffOb.

Conclusions: Early onset hypertension in offspring of obese rats is associated with evidence for increased sympathetic tone and enhanced sensitivity to leptin. These animals demonstrate an apparent 'selective leptin resistance' as described in obese rodents[2] which show a reduction in the inhibitory action of leptin on food intake, with preservation of the pressor response. However, in the offspring of obese rats this occurs prior to the onset of obesity. This study implicates a central role of the hypothalamus in the origin of hypertension in the offspring of obese dams. Support: The British Heart Foundation (PG/06/067/21009) and Tommy's Charity.

1. Kirk, S.L., *et al. PLoS ONE*, 4:e5870, 2009.
2. Rahmouni K *et al. Diabetes*, 54:2012–2018, 2005.

P-7A-313

Combined associations of preconceptional body mass index and gestational weight gain on foetal growth

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Objective: Preconceptional nutritional status of women, calculated using body mass index (P-BMI), and gestational weight gain (GWG), are important determinants of birth weight[1]. Their joint effects have not been assessed in Chilean data using recently developed criteria for classifying P-BMI and GWG.

Methods: Prospectively collected anonymous data from a Santiago maternity ward surveillance system was used. Single, term, non-smoking and uncomplicated pregnancies from 11,266 women delivering their newborns between 2000 and 2004 were included in the analysis. The new criteria to classify women's nutritional status based on P-BMI[1], was applied: underweight (<18.5), normal (18.5–24.9), overweight (25–29.9) and obese (≥30). A recently used criteria for GWG classification was also applied, as: low (< 10 kg), medium (10–15 kg), high (16–19 kg), very high (≥20 kg)[2]. Relative risks (RR CI 95%) for birth weight <3000 g, as a proxy for IUGR, and ≥4000 g, as a proxy for FM, were calculated for each category of the combined P-BMI and GWG classifications; non-risky subjects were defined as those born from normal P-BMI women having medium GWG. Appropriate institutional ethics committee clearance was obtained.

Results: A significant reduction in the RR of IUGR was observed with high and very high GWG in underweight women: RR (CI 95%) = 0.42 (0.2–0.89) and 0.34 (0.15–0.79), respectively. In normal P-BMI women the RR of IUGR was inversely related to GWG: RR (CI 95%) = 1.41 (1.19–1.66), 0.80 (0.67–0.96) and 0.48 (0.36–0.62) for low, high and very high GWG, respectively. Also in normal women the RR of FM was directly related to GWG: RR (CI 95%) = 0.38 (0.26–0.55), 1.40 (1.16–1.70) and 2.35 (1.95–2.82) for low, high and very high GWG, respectively. On the other hand, high and very high GWG was related to an increased risk of FM in overweight women: RR (CI 95%) = 1.44 (1.16–1.80) and 2.11 (1.70–2.61), respectively. These effects were similar in obese women. Interestingly, a low GWG in both overweight and obese women was associated to a reduction of FM risk: RR (CI 95%) = 0.76 (0.61–0.96) and 0.56 (0.44–0.73), respectively. However, restriction of GWG in these over nourished women was associated to an increased RR of IUGR: RR (CI 95%) 1.29 (1.00–1.68) and 1.66 (1.01–2.74), for overweight and obese women, respectively.

Conclusions: Heavier women may benefit from avoiding high and very high GWG, which is associated with a relatively low increase in the risk of IUGR; the later results may be improved using proportionate to maternal height GWG[3]. High GWG in underweight women does not appear to have deleterious consequences for their infants and low GWG was clearly associated with IUGR, as recently reported[2].

1. Institute of Medicine. National Research Council. Weight gain during pregnancy: re-examining the guidelines. Washington, DC: National Academy Press, 2009.
2. E.A. Nohr *et al. Am J Clin Nutr*, 87:1750–1759, 2008.
3. F. Mardones, P. Rosso. *Matern Child Nutr,* 1:77–90, 2005.

P-7A-314

Intervention that decreases pre-pregnancy obesity recuperates effects of maternal obesity and high fat diet on adipose tissue and glucose tolerance of rat male offspring

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Objective: Maternal pre-pregnancy BMI is a major determinant of adverse rat male offspring (OFF) metabolic outcomes resulting from maternal obesity (MO)[1]. Although rodent models of developmental programming of OFF, adipose tissue (AT) and glucose tolerance (GT) have been extensively studied no studies exist on effectiveness of interventions to reduce food intake prior to pregnancy on OFF outcomes. We induced MO prior to rat pregnancy with high fat diet (F) and determined if MO reversal before mating recuperates effects on OFF AT and GT.

Methods: After weaning female Wistar rats randomly received either control (C) rodent diet 5001 (Teklad – 5%F) or high fat (F −25%F added to C). One month before breeding 50% of F females were recuperated (R) on C diet for the rest of the study including pregnancy (P) and lactation (L) while remaining F were fed F during P and L. At postnatal day (PND) 120 all three groups were bred and remained on their pre-pregnancy diet in P and L. Litters were adjusted to10 pups/dam. Body and subcutaneous fat weight, serum leptin (RIA) and triglycerides (TG) were averaged in two random male OFF per litter at weaning (21 PND). OFF visceral fat cell size and gonadal F mass were measured at 150 PND. An iv GTT was performed at 120 PND. Data M ± SEM, analysis ANOVA, n = 5 mothers/group.

Results: Maternal breeding weight was higher in F vs C with R intermediate (C: 213 ± 9^{a}, F: 262 ± 13^{b} and R: 232 ± 7^{ab} g, $p < 0.01$). F maternal serum leptin at weaning was higher than C and R (C: 0.8 ± 0.1^{a}, F: 3.8 ± 0.1^{b}, R: 1.2 ± 0.1^{a} $ng.ml^{-1}$, $p < 0.001$). Pup weights were similar at birth and weaning (Fig. 1A) when F pups had more fat (Fig. 1B), serum leptin (Fig. 1C) and TG (Fig. 1D). 150 PND visceral fat cell size was greatest in F, least in C with R intermediate (Fig. 1E) as was gonadal fat mass (data not shown). 120 PND serum GTT glucose was recuperated in R (Fig. 1F) (insulin in progress).

Conclusions: Dietary intervention that decreases maternal weight from 123% of C to 109% of C before P continuing

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through P and L, recuperates adverse OFF AT, leptin, TG, fat cell size and glucose intolerance.

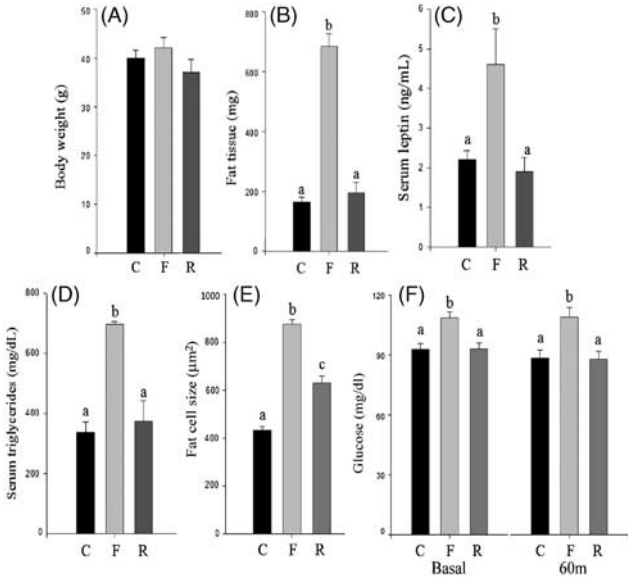

Figure 1. Male OFF. PND 21 (A) weight (B), fat weight, (C), serum leptin (D) triglycerides. 150 PND (E) AT visceral fat cell size. 120 PND (F) blood glucose in iv GTT. C, F and R defined in text. M ± SEM; n = 5. Different letters, $p < 0.05$.

1. Catalano PM, *et al. Diab Care,* 32:1076–1080, 2009.

P-7A-315

Fetal growth in the obese patient

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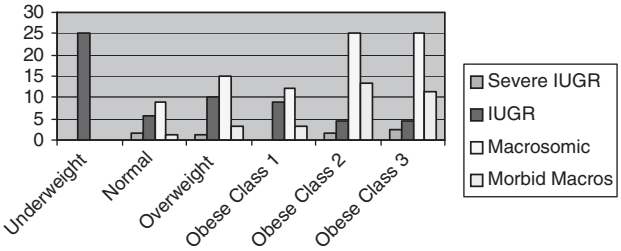
Objective: Middlemore Hospital has a diverse ethnic population with a high incidence of obesity. Many of these obese patients have large babies. We wanted to know whether there was increased Growth Velocity of the Abdominal Circumference (GVAC) in these patients and when in pregnancy this started. Growth velocity between trimesters has been described in the prediction of fetal growth abnormalities[1].

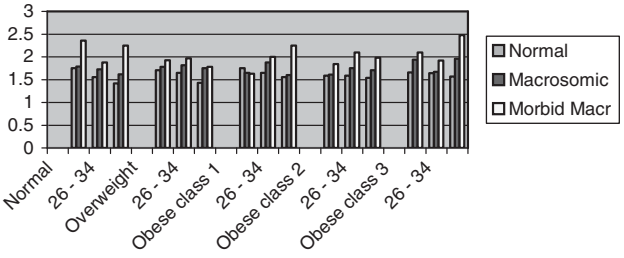
Method: This was a retrospective analysis of 611 patients who had more than one fetal biometry ultrasound, maternal height and weight recorded and who delivered over a 3-month period. Birthweight was customised from maternal ethnicity, height and weight. BMI was calculated using ethnic specific BMI and classified into BMI groups according to WHO criteria. GVAC was determined in mm/day by dividing abdominal circumference measurement by the number of days between the examinations.

Results: Our range of BMI in this group was underweight - 1.96%, normal – 29.62%, overweight – 26.19%, Obese class 1–23.89%, Obese class 2–11.13%, Obese class 3–7.21%. This gives an above normal weight percentage of 68.42%. The ethnic groups differed across the BMI ranges with Obese class 3 represented by Pacific Island, Maori and European mothers. There were no Indian or Asian mothers in this group. In the underweight group there were no Pacific Island patients. The BMI showed increasing Pacific Island groups and decreasing Indian and Asian groups with increasing obesity.

The incidence of macrosomia and IUGR in different BMI groups is as shown.



Morbid macrosomia is a fetal weight >100% on customised chart. Obese class 2 and 3 patients have a much higher rate of macrosomic and morbidly macrosomic babies. GVAC shows increasing velocity across all trimesters with increasing obesity in babies who become macrosomic and morbidly macrosomic.



Conclusion: Morbid macrosomia starts early in the pregnancy with increasing Abdominal Circumference Growth Velocity. This is more pronounced with increasing maternal obesity and is consistent with an early origin for fetal and childhood disease.

1. Salomon L.J. *et al. J Mat-Fet Neonat Med*, 17:193–197, 2005.

P-7A-316

Maternal obesity in the mouse leads to a phenotype with similarities to that observed in non-alcoholic fatty pancreas disease

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Objective: Increasing prevalence of pancreatic adenocarcinoma (PAC) may relate to rising rates of obesity and dysmetabolism. Non-Alcoholic Fatty Pancreas Disease (NAFPD), a phenotype similar to obesity induced Non-Alcoholic Fatty Liver Disease (NAFLD), may link obesity and PAC, a mechanism implicated in NAFLD and hepatocellular carcinoma. However, it is not known whether there is a developmental influence of maternal obesity on incidence of NAFPD. Our objective was to determine effect of maternal obesity on pancreatic histology and expression of fibrosis in the offspring and ascertain contributions of the intra/extra-uterine periods.
Methods: Female C57BL/6J mice were fed either a standard chow (3% fat, 7% sugar) or a highly palatable, hypercalorific diet (16% fat, 33% sugar) for six weeks and throughout pregnancy and lactation. Offspring were cross-fostered for suckling to dams on the same or opposite diet. All offspring were weaned to a control diet. At three months of age, pancreas weights and pancreatic triglyceride content were determined. Pancreatic TGF-β1 and collagen 1-α2 mRNA expression (fibrotic markers) were assessed by RT-PCR. Histological analysis of pancreatic fat, inflammation and fibrosis was performed by a gastrointestinal pathologist, blinded to the identity of the groups. Systolic blood pressure (SBP) and restraint stress responses were assessed by radiotelemetry in female offspring at 3 months as determinants of sympathetic nervous system (SNS) activation, a putative pro-fibrogenic pathway.
Results: Significant increases in body weight, tissue triglyceride content and markers of fibrosis (TGF-β and collagen gene expression) were observed in offspring of both control and obese dams that critically were exposed to maternal obesity in the suckling period. SBP at rest and in response to restraint stress were also elevated, confirming a dysmetabolic phenotype (tables).
Conclusion: Exposure to maternal obesity during the suckling period conveys a dysmetabolic phenotype with a phenotype similar to that observed in NAFPD in man. Should this occur in man maternal obesity could be as predisposing factor to development of offspring pancreatic adenocarcinoma.
Acknowledgements: Funded by EARNEST, EU, The Wellcome Trust and Tommy's Charity.

	Offspring of lean suckled by lean dam (Mean ± SEM)	Offspring of obese suckled by obese dam (Mean ± SEM)	P value
Mean body weight (g)	25.98 ± 0.91	29.53 ± 0.75	<0.05
Tissue triglyceride content (mmol/L)	0.65 ± 0.05	2.74 ± 0.32	<0.0001
Relative TGF-β1 gene expression	0.017 ± 0.008	0.065 ± 0.006	0.0025
Relative collagen gene expression	0.923 ± 0.004	1.013 ± 0.032	NS
Night time SBP (mmHg)	123.2 ± 1.1	134.3 ± 2.1	<0.0001
Restraint stress SBP (% Δ baseline)	115.2 ± 1.4	133.6 ± 0.6	<0.0001

	Offspring of lean suckled by lean dam (Mean ± SEM)	Offspring of obese suckled by obese dam (Mean ± SEM)	P value
Mean body weight (g)	25.98 ± 0.91	34.12 ± 0.98	<0.0001
Tissue triglyceride content (mmol/L)	0.65 ± 0.05	2.63 ± 0.75	<0.001
Relative TGF-β1 gene expression	0.017 ± 0.008	0.067 ± 0.007	0.0025
Relative collagen gene expression	0.923 ± 0.004	1.094 ± 0.0193	0.005
Night time SBP (mmHg)	123.2 ± 1.1	133.4 ± 0.8	<0.0001
Restraint stress SBP (% Δ baseline)	115.2 ± 1.4	128.5 ± 2.0	<0.0001

P-7A-317

Evidence for offspring myocardial dysfunction arising from maternal obesity in a murine model

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Objectives: We have recently reported a novel murine model of developmental programming by maternal obesity-induced by diet, leading to offspring hyperphagia, insulin resistance, increased adiposity and hypertension[1]. Neonates from obese dams showed evidence of cardiac remodelling with increased heart weight and hypertrophy and hyperplasia. In this study we investigated the functional impact of early cardiac remodelling in adult offspring of obese mice (OffOb) using small animal high frequency ultrasound.
Methods: Female C57BL/6J mice were fed either a standard chow diet (3% fat, 7% sugars) or a highly palatable, obesogenic diet (16% fat, 33% sugars) for 6 weeks prior to mating and throughout pregnancy and lactation as previously described[1]. Offspring were weaned onto standard chow. Myocyte mRNA expression was assessed by RTPCR and microarray. At 6 months, Small Animal Micro-Echocardiography Imaging was performed employing the Vevo 770® v1.2, with a RMV 707B scanhead (Visualsonics, Canada).
Results: At gestational day 18, up-regulation of both GATA-4 and BMP-10 genes was observed in fetal hearts from offspring of obese dams (OffOb) Versus controls (OffCon), consistent with cardiac myocyte proliferation. At 28 days microarray showed marked upregulation in OffOb of all 16 genes in the array related to oxidative phosphorylation (KEGG pathway analysis, Z score 7.0. Significance assumed if Z score <-2 and $>+2$). At 28 days and 3months of age, OffOb hearts were heavier than OffCon, but this difference had resolved at 6 months, consistent with the cardiac dilatory phase of heart failure. Micro-Echocardiography Imaging at 6 months, revealed an altered cardiac phenotype in OffOb *Versus* OffCon characterised by a significantly reduced ejection fraction (EF [mean ± SEM] OffCon, 72.3 ± 2.3

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Versus OffOb, 62.6 ± 3.4, n = 6, $P = 0.04$) and fractional shortening (FS [mean ± SEM] OffCon, 41.6 ± 2.4 *Versus* OffOb, 33.9 ± 2.5, n = 6, $P = 0.04$, and evidence of increased ventricular internal dimension at systole (LVID;s [mean ± SEM] OffCon, 2.29 ± 0.2 *Versus* OffOb, 2.94 ± 0.2, n = 6, $P = 0.057$). This morphology, indicative of cardiac dilation, may reflect the second, ''decompensatory'' phase of myocardial failure.

Conclusions: Fetal and neonatal cardiac remodelling resulting from maternal obesity preceded functional abnormalities in later life, consistent with the early stages of heart failure. Micro-echocardiography at 6 months of age showed evidence of cardiac dilatation in OffOb, a recognised transitional phase in development of cardiac failure. The role of adulthood hypertension in elucidation of the adult phenotype requires investigation. Up-regulation of mitochondrial genes involved in oxidative phosphorylation pathways implicate oxidative stress in fetal cardiac myocyte proliferation and in the origin of the cardiac dysfunction observed. Support: The British Heart Foundation (PG/06/081/21195) and Tommy's Charity.

1. Samuelsson, A.M., *et al. Hypertension*, 51:383–392, 2008.

P-7A-318

Maternal and offspring outcomes of pregnancies associated with severe obesity: study design and preliminary data from the hormones and inflammation in obese pregnancy study

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Objective: Recent studies estimate that more than 1:5 pregnant women are obese[1]. This has significant implications for maternal and fetal health. Mothers are at increased risk of developing complications such as gestational diabetes mellitus, pregnancy-induced hypertension, pre-eclampsia and delivery complications including caesarean section. For the offspring, short- and long-term risks of maternal obesity include congenital anomalies, stillbirth, and early onset childhood obesity and metabolic syndrome[1]. Little is known about the physiological and hormonal changes that occur in pregnancies associated with severe obesity and how these impact on offspring development and obesity and metabolic risk. The best weight-management advice for severely obese women for optimal maternal and offspring health is not known[2]. We aim to carry out a detailed study of severely obese pregnant women and their offspring.

Methods: We are recruiting severely obese pregnant women (body mass index (BMI) > 40 kg/m^2) and normal-weight controls (BMI < 25 kg/m^2) to a research clinic. Women are given advice about weight maintenance and characterised in detail in each trimester and post-partum in terms of body composition (serial measurements of BMI, fat mass by four-site skin-fold thickness measurements and bio-electrical impedance, and booking waist, hip, mid-arm and mid-thigh circumferences), metabolic profile (including oral glucose tolerance tests, glucocorticoid hormone levels and inflammatory markers), blood pressure, stress and anxiety levels, diet (by food frequency questionnaire and food diary), exercise (by questionnaire and accelerometry). Placenta and cord blood are collected at delivery, and mode of delivery and intrapartum complications recorded. Babies are assessed at birth, 3 and 6 months to study growth, weight, body composition, and development. Preliminary analyses of data from the first 85 obese women attending the clinic are presented. Data are mean (sd). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: At the first visit (16–18 weeks gestation) women were of mean weight 121.3(16.89) kg and BMI 45.4(5.29) kg/m^2 with blood pressure 115/68(9.9/6.7) mmHg. Pregnancies were terminated in 2 women due to fetal anomaly, 6 women developed pre-eclampsia, 8 women developed gestational diabetes. By 36 weeks gestation, there were increases in weight (+3.4(4.96) kg, p = 0.002), BMI (+1.24(1.85) kg/m^2, p = 0.003), blood pressure (systolic +12.5(10.17) mmHg, p = 0.002; diastolic +11.09(9.6) mmHg, p = 0.003) and glucose (fasting +0.17(0.32) mmol/l, p = 0.0003, 2 hour glucose +0.41(0.22) mmol/l). Mean calorie intake was 3386(1484) kcal/day with higher proportions of fat and refined carbohydrate, and lower proportions of essential micronutrients, than recommended for healthy pregnancy[2]. Exercise levels were 221 Metabolic equivalent hours/week, comparable with healthy sedentary women. Mean birthweight of babies born >37 weeks gestation was 3.70(0.75) kg.

Conclusions: Gestational weight gain among these severely obese women is within recommended limits. We are currently recruiting ~5 new participants per week and further characterisation and comparison with the control group will aid development of guidelines for women for healthy weight management in pregnancy. Children will be followed long-term to assess growth and the development of obesity and metabolic sequelae. Support: Tommy's the Baby Charity.

1. Catalano PM, *BJOG* 113:1126–1133, 2006.

P-7A-319

Maternal diet-induced obesity leads to hepatic insulin resistance in the offspring

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Objective: As the obesity rates increase around the world, of particular concern is the rise of obesity in pregnant women[1], as this can have detrimental effects on the long term health of their offspring including increased risk of type 2 diabetes, metabolic syndrome and cardiovascular disease[2]. To investigate the mechanisms by which maternal obesity has long term metabolic consequences in the offspring we have employed a murine model of maternal diet-induced obesity. Using this model we have shown that maternal over-nutrition leads to offspring displaying hyperphagia, increased fat mass, hyperleptinaemia and hyperinsulinaemia in adulthood[3]. The aim of the current study was to investigate the effects of maternal diet-induced obesity on expression/phosphorylation of hepatic insulin signaling proteins that may contribute to the development of insulin resistance.
Methods: Obesity was induced in female mice by feeding them an obesogenic diet for 6 weeks prior to pregnancy. This diet was continued during pregnancy and lactation. At 21 days of age offspring of obese dams were weaned onto standard chow and maintained on this diet until 3 months of age. The expression/phosphorylation of hepatic insulin signaling molecules was measured by western blotting.
Results: Expression of the insulin receptor was not altered in the liver of offspring of obese dams. However, expression of IRS1 protein was decreased in both male and female offspring of obese dams (effect of maternal diet $p = 0.001$). There were no differences in either the protein expression or phosphorylation of other insulin signaling molecules including phospho-IRS1 Tyr612, phosphatidyl inositol 3-kinase (PI3 K) p85α regulatory subunit or p110 β-catalytic subunit and phospho-Akt Ser 473. However, the expression of protein kinase ζ (PKC ζ) was significantly increased in male ($p = 0.01$) but not female offspring of obese dams.
Conclusions: Maternal diet-induced obesity leads to indicators of hepatic insulin resistance in the offspring. This is related to a post-receptor defect including reduced signaling through IRS1.

1. ACOG Committee Opinion number 315, Obesity in pregnancy. *Obstet Gynecol.* 106:671–675, 2005.
2. CM Boney *et al.*, *Pediatrics,* 115:e290–e296, 2005.
3. AM Samuelsson *et al.*, *Hypertension,* 51:383–392, 2008.

P-7A-320

PPAR agonists are negative regulators of oxidative and nitrosative stress in fetuses from diabetic rats

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The intra-uterine milieu of a diabetic mother leads to alterations in fetal development and growth, with a consequent diabetogenic tendency in the offspring's adulthood. Oxidative and nitrosative stress have been involved in the etiology of feto-placental anomalies. The peroxisome proliferator activated receptors (PPARs) are ligand activated nuclear receptors that regulate several inflammatory and developmental processes.
Objective: of this work was to evaluate the *in vitro* and *in vivo* putative effects of PPAR agonists as regulators of oxidative and nitrosative stress in fetuses from control and diabetic rats.
Methods: from day 0.5 of pregnancy, control and streptozotocin-induced diabetic rats were fed with a standard diet or with a standard diet supplemented with 6% olive oil (OO) or 6% safflower oil (SO), respectively containing 75% oleic acid and 75% linoleic acid, natural ligands of PPARs. Fetuses were explanted on day 13.5 of gestation. Fetuses from rats fed with the standard diet were cultured for 3 hours in the presence or absence of leukotriene B_4 (LTB_4, 0.1 μM), carbaprostacyclin ($cPGI_2$, 1 μM) or 15deoxydelta ^{12,14}prostaglandin J_2 ($15dPGJ_2$, 2 μM), endogenous ligands for PPARα, PPARβ and PPARγ, respectively. In all the experimental groups, fetal concentration of nitrates/nitrites, stable metabolites of nitric oxide, was quantified by the Griess reaction, whereas, lipid peroxidation was assessed by measuring tiobarbituric acid reactive substances (TBARS). Nitrates/nitrites were expressed as nmol/mg protein and TBARS as nmol/μg protein.
Results: In fetuses from control rats, nitrates/nitrites levels (13 ± 1) were not modified by the addition of LTB_4 (16.2 ± 1.7) or $cPGI_2$ (13.6 ± 1.5), although they were decreased by $15dPGJ_2$ addition (8.7 ± 0.4, $p < 0.01$). In fetuses from diabetic rats, the levels of nitrates/nitrites were increased (30.6 ± 3.3, $p < 0.01$) when compared to controls, and additions of LTB_4 (33.5 ± 2.4), $cPGI_2$ (23.7 ± 2.8) or $15dPGJ_2$ (26.4 ± 3.3) had no effect. Dietary treatments with OO (13.6 ± 0.6) or SO (13 ± 2.9) did not modify the levels of nitrates/nitrites in control fetuses (12.9 ± 1.5), but in the diabetic fetuses (29.8 ± 1.3), they greatly reduced nitrates/nitrites levels (OO (24.4 ± 2.6, $p < 0.05$) and SO (11.5 ± 1.5, $p < 0.001$)). TBARS levels in control fetuses (12 ± 2) were not modified by the addition of LTB_4 (12 ± 2), $cPGI_2$ (13 ± 3) or $15dPGJ_2$ (16 ± 4). In diabetic fetuses the levels of TBARS were elevated (20 ± 8, $p < 0.0001$) when compared to controls, and were reduced by the addition of LTB_4 (9.7 ± 2, $p < 0.0001$), $cPGI_2$ (12.4 ± 3, $p < 0.001$) and $15dPGJ_2$ (7 ± 1, $p < 0.0001$). Dietary treatments with OO (11.2 ± 1.3) and SO (9.3 ± 1.3) did not modify the levels of TBARS in control fetuses (9 ± 1). Differently, in diabetic fetuses, the elevated levels of TBARS (20 ± 2, $p < 0.01$) were reduced by the maternal treatments with OO (13 ± 2, $p < 0.05$) and SO (13.5 ± 0.4, $p < 0.01$).
Conclusion: *In vitro* treatments with PPAR agonists reduced oxidative stress in fetuses from diabetic rats. Maternal treatment with diets supplemented with olive and safflower oils led to a reduction in fetal oxidative and nitrosative stress, possibly acting through PPAR activation. The obtained results highlight the anti-inflammatory properties of PPARs during fetal development, and show that they were more marked in the fetuses from diabetic mothers.

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Maternal birth weight and gestational diabetes: a systematic review of the literature

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Objective: Age, obesity and family history of diabetes are well known risk factors for gestational diabetes mellitus (GDM), however, the role of the woman birth weight is still controversial. The aim of this study was to review the current literature looking for evidence to support the association between the woman own BW and the subsequent development of GDM.

Methods: We carried out a search of the MEDLINE, COCHRANE, LILACS and PAHO databases, and reviewed articles published between 1986 and 2007. Keywords "birth weight," "low birth weight," "small birth weight," "small birth size" and "small for gestational age" were used in combination (*AND*) with "gestational diabetes mellitus" or "pregnancy diabetes mellitus." A complementary search was performed among the references of retrieved papers and at the international guidelines for screening and diagnosis of GDM. We evaluated the methodological quality of the selected papers using the criteria of Downs & Black, adapted for observational studies.

Results: Were retrieved 10 papers, with quality scores (0-23) ranging from 14 to 22 points. Eight studies found statistically significant associations between the woman LBW and GDM, with odds ratios ranging from 1.7 to 4.2. The funnel plot does not suggest the presence of publication bias.

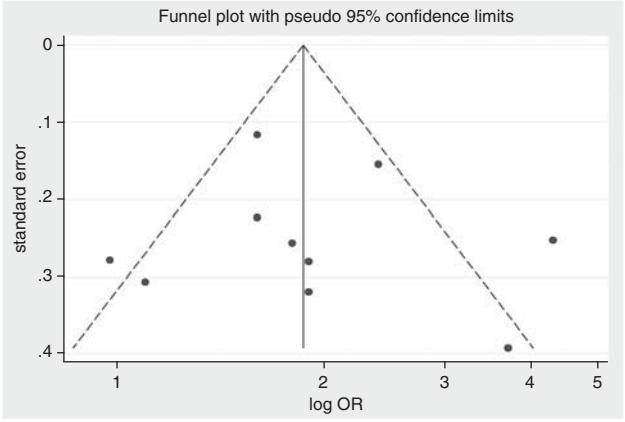

Figure 1-Funnel Plot.

Conclusions: In conclusion, the available literature based on cohort and case control studies indicates the existence of an inverse association between the woman BW and GDM. It also highlights the importance of adjusting BW for family history of DM and especially for the grand-mother history of DM during the pregnancy of the index woman. Although allowing for the woman GA at birth does not seem to change the association between BW and GDM, such adjustment should be considered, given that strong enough evidence for discarding the possibility of confounding is still unavailable. The impossibility of conducting a meta-analysis highlights the need for standardizing measures of BW and GDM as well as cutoff points for GDM screening and diagnosis. Despite of this, the consistency of the results of cohort and case-control studies, using different methods of diagnosing GDM, in several ethnic populations, and the strength of the observed associations indicate the existence of an inverse association between the woman BW and posterior development of GDM.

P-7A-322

Dietary PPAR agonist prevents matrix metalloproteinases overactivity in embryos and decidua from diabetic rats

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Maternal diabetes is a pathology related to embryo malformations and embryo-placental metabolic disorders. Remodelling of the extracellular matrix (ECM) accompanies the changes in embryonic and uterine tissues that occur during development. Alterations in ECM components have been found in placentas and embryonic tissues from diabetic rats and are related to disturbances in the activity of matrix metalloproteinases (MMPs), enzymes involved in ECM degradation. Our previous works have shown that MMP2 and MMP9 are increased in fetuses and placentas in experimental models of diabetes.Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that modulate the expression of genes involved in lipid metabolism, glucose homeostasis and developmental processes. PPAR activators are lipid molecules such as oleic and linoleic acids, present in high concentrations in olive and safflower oils respectively.

Objective: To analyse the capability of dietary supplementation with either 6% olive oil or 6% safflower oil to regulate MMPs activity in embryos and decidua from control and diabetic rats during early organogenesis, a period characterized with a significant remodelling of the decidua and in which most embryo malformations are induced.

Methods: Diabetes was induced by streptozotocin administration (50 mg/kg) to adult rats. From day 0.5 to 10.5 of gestation, control and diabetic rats were fed *ad libitum* with: a standard diet (commercial rat chow) or with a standard diet supplemented with 6% olive oil or with 6% safflower oil. At day 10.5 of gestation decidual tissues and embryos were explanted and used for morphological analysis or stored at −80°C for experimental procedures. Activity of embryonic and decidual MMP2 and MMP9 and their proenzymes (proMMP2 and proMMP9) was evaluated by zymography and the results were expressed as arbitrary units.

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Results: We found that diabetic rats have increased embryo resorptions (35.9%, $P < 0.001$) and malformations (14.2%, $P < 0.001$) when compared to controls (6.5% and 1.9% respectively). Both olive oil and safflower oil-supplemented diets were capable of reducing resorptions (10.3% and 12.1% respectively, $P < 0.001$) and malformations (6.4% and 7.1% respectively, $P < 0.05$) in diabetic rats. Embryos from diabetic rats showed increased activity of MMP9 (4.0 ± 0.6, $P < 0.001$), proMMP2 (5.4 ± 0.6, $P < 0.05$) and MMP2 (3.8 ± 0.6, $P < 0.05$) when compared to embryos from control rats (1.0 ± 0.2, 3.4 ± 0.4 and 2.0 ± 0.5 respectively). Both olive and safflower oil-supplemented diets diminished the overactivity of MMP9 (1.4 ± 0.2 and 1.9 ± 0.4 respectively, $P < 0.05$), proMMP2 (3.5 ± 0.2 and 3.5 ± 0.3 respectively, $P < 0.05$) and MMP2 (2.0 ± 0.2 and 1.7 ± 0.2 respectively, $P < 0.05$) in embryos from diabetic rats. Decidua from diabetic rats showed enhanced activity of MMP9 (2.1 ± 0.2, $P < 0.001$) and proMMP2 (2.9 ± 0.3, $P < 0.001$) when compared to controls (1.0 ± 0.1 and 1.4 ± 0.1 respectively). The olive oil (1.1 ± 0.2, $P < 0.01$) and safflower oil-supplemented diets (1.5 ± 0.1, $P < 0.05$) prevented the MMP9 overactivity in diabetic deciduas.

Conclusions: Maternal diabetes induces increased rates of resorptions and malformations, probably related to the enhanced activation of MMP9 and MMP2 that lead to an abnormal remodeling during embryo and decidua development. Both olive and safflower oil-supplemented diets, enriched in PPAR activators, are able to diminish the resorption and malformation rates and to prevent the overactivation of MMPs induced by maternal diabetes.

P-7A-323

Pre-pregnancy recuperation of effects of maternal obesity and high fat diet on mothers and male offspring in the rat

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Objective: Maternal obesity (MO) in rat pregnancy induced by high fat diets (F) alters offspring body composition and metabolism. We sought to determine whether dietary control before mating would recuperate maternal and offspring effects of MO and F.

Methods: After weaning female albino Wistar rat pups randomly received either control (C) rodent diet 5001 (18CMS-5001–5% fat) or high fat (F −25% fat). One month before breeding 50% of F females were switched to C (maternal recuperation - R) remaining on C diet for the rest of the study. The other 50% of F females remained on F for the study duration. At postnatal day (PND) 120, rats were mated to provide three groups - C in pregnancy and lactation, F in pregnancy and lactation, the R group described above which had been switched from F to C one month before breeding and remained on the C diet during pregnancy and lactation. Litters were adjusted to10 pups/dam. Body and subcutaneous fat tissue weight, serum leptin (RIA) and triglycerides (TG) were averaged in two randomly chosen male pups per litter at weaning (21 PND). Data M ± SEM, analysis ANOVA, n = 5 mothers/group.

Results: Maternal weight at breeding was higher in F vs C mothers (C: 213 ± 9^{a}, F: 262 ± 13^{b} and R: 232 ± 7^{ab} g, $p < 0.01$) but not at weaning. Maternal serum leptin at weaning was higher in F than C and R (C: 0.8 ± 0.1^{a}, F: 3.8 ± 0.1^{b}, R: 1.2 ± 0.1^{a} ng/ml, $p < 0.001$). Male pups showed no differences in weight at birth weight or at weaning among groups. However male pups from F mothers had higher fat tissue content, serum leptin and TG (Fig. 1).

Conclusion: Removal of high dietary fat intake for one month before pregnancy recuperates some of the metabolic changes previously shown by ourselves and others resulting from MO and F.

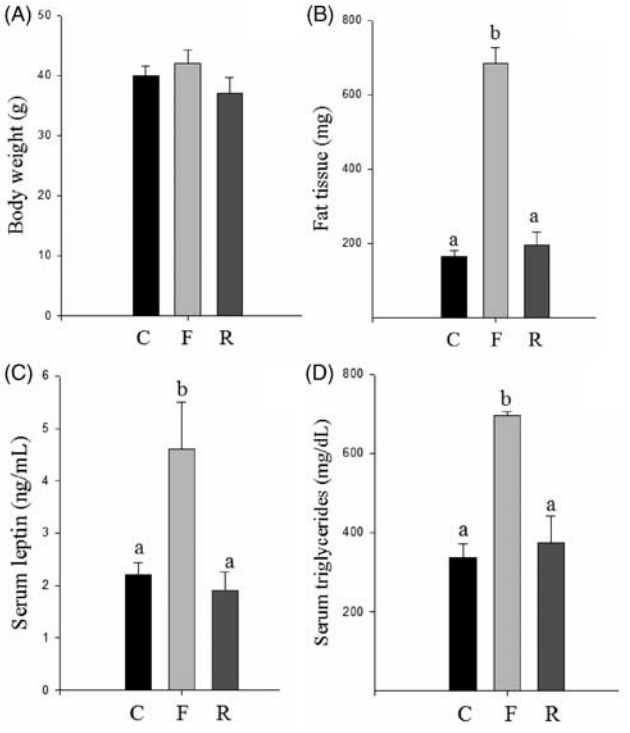

Fig 1. Male offspring at PND 21. Body weight (A), fat tissue content (B), serum leptin (C) and triglycerides levels (D) offspring from mothers fed with control diet (C), high fat diet (F) during pregnancy and lactation or recuperated one month before pregnancy (R). Groups with different letters are significantly different, $p < 0.05$.

P-7C-324

Fetal origin of allergic asthma: insights on mechanistic cues and therapeutic targets arising from a mouse model of prenatal stress challenge

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Objective: Prenatal stress challenge is a pivotal environmental factor which has been proposed to increase the vulnerability of offspring to develop chronic diseases in later life. The aim of our research is to identify biomarkers involved. Such insights would not only allow early detection of individuals at risk, but also open therapeutic targets for primary prevention. In the present study, we analyzed the influence of prenatal exposure to sound stress during late gestation in mice on (i) placental integrity, function and apoptosis and (ii) fetal development. Next, we evaluated if and how prenatal stress challenge affects the risk to develop allergic asthma, a chronic diseases that has experienced an unprecedented increase over the past 5 decades (iii). Further, we tested if progesterone supplementation could abrogate the effect of the prenatal stress challenge (iiii).

Methods: In four subsequent experiments, BALB/c-mated BALB/c mice were exposed to sound stress for 24 hours on gestation day (gd) 12.5 and 14.5 employing a well established device. In one experiment, pregnant mice were sacrificed on gd 16.5, maternal serum was analysed for hormone levels and placentas were morphologically and functionally analyzed. Further, fetal development was scored gender-dependently using the Theiler classification. In the second set of experiments, litters were born and six weeks after birth, allergic asthma was experimentally induced by sensitizing the offspring with Ovalbumin (OVA), followed by nasal OVA-allergen provocation. Sensitized offspring from non-stressed mothers and non-sensitized mice served as controls. Offspring were screened for immune cell frequency and phenotype in lungs, bronchioalveolar fluid (BAL) and lung-draining lymph nodes. In a third set, stress-challenged pregnant females were treated with a progesterone derivative (dydrogesterone), followed by Theiler analyses of the offspring on gd 16.5. Fourth, vulnerability towards asthma was evaluated in adult offspring from pregnancies in which stressed dams were treated with dydrogesterone.

Results: Stress challenge resulted in decreased serum levels of maternal progesterone and testosterone, associated with placental endocrine dysfunction, such as low expression of Proliferin in Trophoblast Giant Cells. Gross placental morphology was not altered by stress challenge. We observed an inverse association between food intake and maternal and fetal weight. Fetal development was impaired upon stress challenge, leading to growth restricted fetuses, especially profound in females. Prenatally stressed adult offspring revealed an increased susceptibility toward asthma, mirrored by an increased airway response and influx of inflammatory cells in the BAL. Further, decreased frequencies of regulatory T cells ($CD3^+CD4^+CD25^+$forkhead box P3$(foxP3)^+$and increased frequencies of $CD11c^+CD4^-CD8^+$ dendritic cells were detectable in prenatally stressed adult offspring. Interesting, progesterone supplementation abrogated the impaired intrauterine development as well as the susceptibility toward asthma in the female offspring.

Conclusion: Our study revealed that prenatal sound stress in mice severely interferes with the intrauterine development, resulting in offspring with an increased vulnerability toward asthma-like symptoms. These effects were particularly profound in female offspring and may account for the increased incidence of chronic immune disorders. Supplementation of progesterone during stress-challenged pregnancies abrogates gender-dependently the increased susceptibility toward asthma. We hypothesize that gender-dependent progesterone receptor density after stress challenge may account for these dramatic gender effects.

P-7C-325

Effect of maternal protein restriction *in utero* on adult offspring lung morphometry

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Objectives: Environmental challenges during early life have been shown to result in greater risk of chronic diseases such as diabetes and coronary disease in later life. Factors such as unbalanced nutrition before birth result in metabolic and structural adaptations that lead to persistent modifications to offspring phenotype. There is evidence that respiratory disease is influenced by the developmental environment. Reduced fetal growth is associated with impaired lung development, increasing risk of developing COPD in later life. We investigated whether exposure to low protein *in utero* affects offspring adult lung morphology.

Methods: Pregnant Wistar rats were allocated to either control (C, 18% casein) or protein restricted (PR, 9% casein) diet. Lungs were collected (225 days after birth) from the offspring (C group, [male] $n = 6$; PR group, [male] $n = 7$). Lungs were fixed for 24 hours in formalin then processed and embedded in paraffin wax before entire lung was sectioned. Sections were H and E stained. Significant differences were determined using T-tests.

Results: There was no significant difference in lung weight and perfused lung volume or between amount of alveolar airspace, airways, airway components (lumen, epithelium and smooth muscle) or vessel components (lumen and smooth muscle) between the two groups, PR and C. However there was a significant increase ($p = 0.046$) in the amount of smooth muscle around the vessels in the PR group compared with controls.

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Conclusions: In this study there is no evidence to suggest that *in utero* exposure to a maternal low protein diet has affected adult lung weight or volume in 225 day old rats. However measured smooth muscle in the pulmonary arteries of the PR group is suggestive of vascular remodelling and pulmonary hypertension in this group.

P-7C-326

Effects of seasonality at birth on asthma and pneumonia in childhood and adult life in a birth cohort in southern Brazil

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Early infancy seems to be a period of particular susceptibility for the development of allergies, as indicated by epidemiological and experimental studies. The season of birth may affect the future development of asthma and respiratory diseases in childhood and in adulthood, but few studies have examined these associations in low and middle income countries.

Objective: To evaluate the effects of seasonality at birth on hospitalizations due to asthma and pneumonia in preschool children and on the diagnosis of asthma in adult life of individuals belonging to the 1982 Pelotas (Brazil).

Methods: Population-based birth cohort study. Individuals born in 1982 in Pelotas (southern Brazil) were visited on a number of occasions from birth to age 23–24y. This cohort included 5,914 live births and 77% were followed up until adulthood. The independent variables evaluated were the trimester of birth and the environmental temperature in the first six months of life (average temperature in tertiles). Information on daily average environmental temperature was obtained from the records of the Research and Meteorological Preview Center from the Federal University of Pelotas (Brazil). The principal outcomes were evaluated at different follow-up: hospitalization due to asthma and pneumonia in childhood (1984 and 1986) and diagnosis of asthma in adulthood (2004–05). All the analyses were adjusted for maternal smoking, weight gain in pregnancy, socioeconomic conditions at birth and age. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Prevalence of hospitalizations due to respiratory diseases in childhood oscillated between 3–10%. The risk of hospitalization due to pneumonia and asthma among children born between April and June (autumn) was 1.31 (CI95% 0.99–1.73) to 2.4 (CI95% 1.11–4.99) times higher than that of children born between January and March (summer). For temperature in the first six months after birth, the risk of hospitalization was 1.64 (CI95% 1.26–2.13) to 3.16 (CI95% 1.63–6.12) times higher for those born in the coldest compared to those born in the hottest tertile. Hospitalizations in poor children were more frequent, but the effects of seasonality on pneumonia were more evident among the wealthiest. Asthma in adulthood (medical and symptomatic diagnosis) was found in ~20% of the cohort members, but the association with seasonality at birth was weak.

Conclusion: The effects of seasonality were stronger in childhood and diminished with age. These results maybe helpful for preventive guidelines directed to reduce the impact and complications of respiratory diseases in early childhood.

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Multi-hits endocrine hypothesis and asthma in pre-adolescence

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Objective: This study was undertaken to determine the association between endocrine changes and composite measures of these changes, and asthma at age 12–13 years. Stress affects multiple endocrine systems and together with obesity and premature adrenarche, is increasingly being recognized as a determinant of late-onset asthma. Our findings will enable a better understanding of the interplay between endocrine system changes and exposure to stress on the development of asthma in pre-adolescence.

Methods: This was a longitudinal follow-up of the nested case-control study of the 1995 SAGE birth cohort in Manitoba, Canada. Children were first assessed for the presence of asthma by a pediatric allergist at age 7–10 years and re-assessed for incident asthma at age 12–13 years. Endocrine levels (cortisol, dihydroepiandrosterone [DHEA], leptin, estradiol, testosterone) were assayed in a fasting blood sample obtained in these children at age 10–11 years. Multiple logistic regression was conducted to determine the association between child asthma at age 12–13 and individual hormone levels, as well as with a composite measure of the hormone levels (named multi-hits), defined by counting the number of hormone levels (hits) in the highest or lowest quartile. Low birth weight status and maternal postpartum distress, defined on the basis of health care or prescription medications for depression or anxiety, were also tested for their association with pre-adolescent asthma. Associations are

reported as the odds ratio (OR) at the 95% level of confidence (CI). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Hormone levels and asthma status were available for 300 children in the SAGE nested case-control study; 31% had asthma at age 12–13 years. By this age, 14.5% of children had 2 hormone levels which fell in the lowest or highest quartile (predominantly testosterone and estradiol); 8.3% of children had 3 hormones and 2.6% of had 4 or more hormones which met this criterion. Few single hormones were associated with asthma. However, an association was found with the multi-hit composite measure, such that for each unit increase in the number of abnormal hormone levels the risk of asthma increased by 31% (OR = 1.31, 95%CI: 1.06–1.62). This association was independent of gender and maternal postpartum distress, of which the latter had a border-line association with asthma (OR = 1.56, 95%CI: 0.95–2.58). Of note, the multi-hits measure was not predicted by low birth weight or maternal distress in the postpartum period, but it did differ by gender.
Conclusions: This is a first report of the association between abnormal levels of multiple, stress-responsive hormones at school-age and pre-adolescent asthma in a general North American population. Our findings provide evidence for a multi-hits endocrine hypothesis for the origins of late-onset asthma, which does not appear to be related to early life exposure to maternal distress.

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Salmon in pregnancy study: the effects of increased oily fish intake during pregnancy on maternal and cord blood mononuclear cell fatty acid composition

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The prevalence of childhood atopic diseases (eczema, asthma, allergies, hay-fever) has increased during the last 30 years[1]. Epidemiological studies link higher fish intake during pregnancy with lower risk of atopy in the offspring[2]. Fish oil supplementation during pregnancy alters maternal and offspring immunity in a way that would be consistent with lowered risk of atopy[3]. Oily fish provide the long chain (LC) n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). There are no studies of oily fish intervention in pregnancy and maternal and infant immunity.
Objective: To examine the hypothesis that increased oily fish consumption during pregnancy by women with high risk of having an atopic offspring will increase maternal and cord blood mononuclear cell (BMC) LC n-3 PUFA status, which may influence the developing foetal immune system in a way that would decrease atopy risk for the offspring.
Methods: Pregnant women (n = 123) with high risk of having atopic offspring and with low habitual intake of oily fish (⩽2/month) were randomised at 20 weeks of pregnancy to either consuming two portions of farmed salmon a week or continuing their habitual diet until the end of their pregnancy. The women attended a clinic at weeks 20 (n = 123 attended) and 34 of pregnancy (n = 111 attended), at which fasting blood samples were collected and a food frequency questionnaire (FFQ) was administered. Cord blood was collected at delivery (n = 101). Maternal and cord BMC fatty acid composition was determined by gas chromatography. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The mean total daily intake of EPA plus DHA based on FFQ increased in the salmon group from 140 mg/day to 410 mg/day ($p < 0.001$), whereas in the control group there was no significant change. In the control group (n = 50) mean maternal BMC EPA decreased from 0.67% to 0.35% of total fatty acids from weeks 20 to 34 ($p < 0.001$), while mean DHA decreased from 2.43% to 2.17% of total fatty acids ($p = 0.021$). In the salmon group (n = 54) mean maternal BMC EPA remained stable between weeks 20 and 34 of pregnancy (0.58% and 0.55% of total fatty acids respectively; $p = 0.738$), whereas mean DHA increased from 2.59% to 2.86% of total fatty acids ($p = 0.005$). In cord BMCs there was a non-statistically significant higher content of both EPA (0.41% vs. 0.34%; $p = 0.339$) and DHA (4.26% vs. 3.84%; $p = 0.109$) in the salmon group compared to the control (n = 40 per group).
Conclusions: Consumption of two portions of oily fish by pregnant women with a low habitual intake of oily fish prevents the pregnancy-associated decrease in maternal immune cell EPA and DHA status, and increases maternal BMC DHA status. This is associated with a higher cord BMC LC n-3 PUFA status. Increasing maternal and cord blood immune cell EPA and DHA status may affect the immune system of the mother and foetus in a way that would decrease atopy risk for the offspring. Support: The European Commission under Framework 6 and forms part of the AquaMax project (FOOD-CT-2006-016249-2). The authors thank staff at the Princess Anne Hospital, Southampton, and the Medical Research Council Epidemiology Resource Centre.

1. T.K. Ninan, G. Russell. *BMJ*, 304:873–875, 1992.
2. S. Willers *et al.*, *Thorax*, 62:773–779, 2007.
3. S. Krauss-Etschmann *et al.*, *J Allergy Clin Immunol*, 121:464–470, 2008.

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Effect of maternal protein restriction *in utero* on F_1 and F_2 offspring lung physiology

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Objectives: Environmental challenges during early life have been shown to result in greater risk of chronic diseases such as diabetes and coronary disease in later life. Environmental factors such as unbalanced nutrition before birth result in metabolic and structural adaptations that lead to persistent modifications to offspring phenotype. There is evidence that respiratory disease is also influenced by developmental environment. Reduced fetal growth is associated with impaired lung development and increased risk of asthma in childhood. We investigated whether maternal exposure to low protein during pregnancy affects offspring lung size and volume.

Methods: Pregnant Wistar rats were allocated to either control (C, 18% casein) or protein restricted (PR, 9% casein) diet for the duration of the pregnancy. Lung tissue was harvested from F_1 (28 days) and F_2 (22 day) male offspring (C group, F_1 $n = 5$, F_2 $n = 18$; PR group, F_1 $n = 7$, F_2 $n = 16$). Lungs were fixed by tracheal instillation of formalin and volumes were calculated by volume displacement. Significant differences were determined using Mann-Whitney U tests in SPSS v15.

Results: In this study we found a significant reduction in body and lung weight in F_2 PR offspring. However, this reduction in lung weight does not remain significant after adjusting for body wt. Although statistically non-significant, ancestral PR rats have a smaller median lung volume index, equating to a median reduction of 23% (F_1) and 20% (F_2) compared to C animals.

Lung measurements (Median (Range))	F_1Control	F_1 PR	F_2 Control	F_2 PR
Body weight (g)	96 (87–101)	81 (72–101)	76 (61–88)	*66 (60–73)
Total lung weight (g)	0.93 (0.68–1.51)	0.82 (0.52–1.51)	0.85 (0.58–1.19)	†0.78 (0.50–1.06)
Lung volume (ml)	0.70 (0.41–0.93)	0.46 (0.32–0.97)	0.61 (0.32–1.15)	0.47 (0.32–1.40)
Lung weight index (% of lung wt/body wt)	0.92 (0.70–1.24)	1.03 (0.69–1.57)	1.11 (0.78–1.83)	1.17 (0.78–1.57)
Lung volume index (ml/kg of body wt)	6.96 (4.70–9.65)	5.36 (3.2–13.47)	8.89 (4.47–14.59)	7.09 (4.62–22.30)

*2−tailed P value = 0.0004; †2 tailed P value = 0.044.

Conclusions: This study has shown a reduction of body and lung weight at 22 days of age in F_2 PR rats which is not seen in 28day old F_1 animals. However, the reduction in lung weight is in proportion with body weight. Therefore, it is likely that there is a period of catch-up growth that occurs in PR rats up to 22 days of age and by day 28 they are similar in weight to controls. During this growth period the lungs appear to remain in proportion to body size. The apparent reduction of the lung volume index in both generations is suggestive of decreased internal lung surface area induced by programmed changes to lung maturation that could affect alveoli number or size and may be controlled by epigenetic mechanisms. These hypotheses will be confirmed by analysis of lung stereology, gene expression and DNA methylation.

P-7C-330

Maternal dietary polyunsaturated fatty acids in the early development of the immune system

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Objective: Polyunsaturated fatty acids (PUFAs) are important immune modulating elements. Prostaglandins and leukotrienes, which play an important role in the immune response, are derived from PUFAs. Furthermore, PUFAs can alter immune cell function via various mechanisms, including the activation of transcription factors[1]. The development of the immune system occurs mostly perinatally and since most PUFAs are acquired from the diet, the maternal diet may influence fetal and neonatal PUFA status. During the last decades there has been a significant increase in the prevalence of allergic disorders which coincides with a marked change in dietary fatty acid intake[2,3]. Therefore, we investigated the effect of maternal dietary ω-3 and ω-6 PUFAs on the development of the imuune system in the offspring.

Methods: Pregnant and/or lactating BALB/c mice were fed diets varying in C18:3ω-3/C18:2ω-6 ratio. After weaning, pups were transferred to a Western-style diet and the effects of maternal PUFA-diet were examined using the ovalbumin-induced allergic asthma model.

Results: Significant differences in the acute allergic skin response were observed between different diet groups and between different feeding periods; all PUFA-diets lowered the acute allergic skin response compared to control diet, but the high C18:3ω-3 diet was most effective when fed during lactation while the high C18:2ω-6 diet diminished the allergic skin response most when fed to pregnant dams.

Conclusions: Both the maternal ω3 and ω6-PUFA-diets lowered the allergic skin response in the adult offspring, indicating a long lasting effect of the maternal diet. Because each diet has the strongest effect when given in a different feeding period, the mechanism by which these PUFAs lower the allergic response might also differ.

1. U. Enke *et al. Br J Nutr*, 100:1158–68, 2008.
2. P.C. Calder, *Clin Exp Allergy*, 33:412–5, 2003.
3. A.P. Simopoulos, *Biomed Pharmacother*, 56:365–79, 2002.

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Chronic hypoxia in-utero alters the role of superoxide anions in peripheral chemoreceptors in the anaesthetised adult rat

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Objective: In the rat, chronic hypoxia *in-utero* reduces baseline ventilation in the adult offspring (CHU) breathing room air and, in carotid bodies isolated from these rats, the responses to acute hypoxia are also blunted[1]. We have presented evidence of increased oxidative stress in the hindlimb vasculature of CHU rats [in press], and also of changes in the oxidative profile of the hindlimb muscles [2]. We have now examined the role of superoxide dismutase (SOD; a major contributor to oxidative stress) on baseline ventilation and the ventilatory response to acute hypoxia.

Methods: Pregnant Wistar rats were exposed to 12% O_2 from day 11–21 of pregnancy and CHU offspring were born into, and reared in room air. At 8–9 weeks of age, normal (N) and CHU rats were anaesthetised and the trachea, brachial arteries and femoral vein cannulated. Respiratory frequency (R_F) and ventilatory tidal volume (V_T) were recorded during air breathing and 10 minute periods of breathing 8% O_2, before and during infusion of the cell permeant SOD inhibitor Diethyldithiocarbamate (DETC). Arterial blood gasses were sampled in normoxia and in the 10th minute of hypoxia.

Results: When breathing room air, CHU rats had significantly lower P_aO_2, but normal P_aCO_2, and minute ventilation tended to be depressed relative to N rats as a result of a lower baseline R_F. In response to 8% O_2, both N and CHU rats showed similar increases in R_F but V_T increased more in CHU such that minute ventilation was similar in both groups. In normoxia, DETC caused a reduction in P_aO_2, but no change in P_aCO_2 or respiratory variables in both N and CHU rats. In hypoxia, P_aO_2 and P_aCO_2 were similar in N and CHU rats, however, DETC blunted the increase in R_F recorded at the first minute of acute hypoxia in N rats, but abolished it in CHU rats.

Conclusions: These results suggest that the tonic drive to respiration from peripheral chemoreceptors is reduced in CHU relative to N rats. They also raise the possibility that superoxide makes a larger contribution to the ventilatory drive induced by peripheral chemoreceptor stimulation during air breathing and acute hypoxia in CHU than N rats.

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1. Coney A., Marshall J.M., *Early Human Dev*, 83:S69, 2007.
2. Rook, W.H.R., *et al. Proc Physiol Soc*. 2008.

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Is there association of preterm birth and intrauterine growth restriction with asthma in young adults?

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Objective: To verify the association between preterm birth and markers of impaired intrauterine growth and asthma in early adult life.

Methods: Data from 2063 young adults (23–25 years old) from a large population-based birth cohort from Ribeirão Preto, São Paulo State, Brazil, was studied. At birth, the following variables were collected: birth weight, preterm birth (gestational age <37 complete weeks), newborn gender, maternal smoking during pregnancy, maternal schooling and age, occupation of the family head and mode of delivery. The concept of intrauterine growth restriction (IUGR) was based on the ratio between the newborn's weight and the mean weight for gestational age of the sex-specific reference curve. A birth weight ratio (BWR) $\geqslant 0.85$ was defined as no IUGR, and a BWR < 0.85 was defined as IUGR. Newborns were classified as large for gestational age, appropriate for gestational age, and small for gestational age when their birth weight was, respectively, above the 90th percentile, between the 90th and 10th percentiles, and less than the 10th percentile of the weight for gestational age from the curve of Williams *et al*. At 23–25 years old, the following data were collected: participant's body mass index (BMI), schooling, smoking habit and asthma, and current occupation of the family head. Two aspects of asthma diagnosis were considered: bronchial hiperresponsiveness to metacholine challenge test and symptoms. Four multiple logistic regression models were used to evaluate factors associated to asthma in adult life, one of each explanatory variable (preterm birth, IUGR, birth weight and adequacy of birth weight for gestational age). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: From the original 2063 individuals, 1862 performed the metacholine test and 201 (10.9%) were considered asthmatic. In the adjusted analysis, in the four models the individuals of lower schooling level, those belonging to manual working classes and those who were ex-smokers had higher risk of asthma at 23–25 years old ($p < 0.05$), whilst male gender showed a protective effect ($p < 0.001$). Preterm birth ($p = 0.958$), birth weight ($p = 0.867$), IUGR

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($p = 0.954$) and adequacy of birth weight for gestational age ($p = 0.648$) were not associated to asthma.

Conclusions: There was no association between preterm births and markers of IUGR and asthma in early adult life. Conversely, there was an association between asthma and socioeconomic factors and participant's smoking habit, with ex-smokers being more prone to have asthma. Support: CNPq, FAPESP and FAEPA.

P-7C-333

Gestational chronic hypoxia programs developmental origins of pulmonary hypotension in adulthood which is nitric oxide-independent

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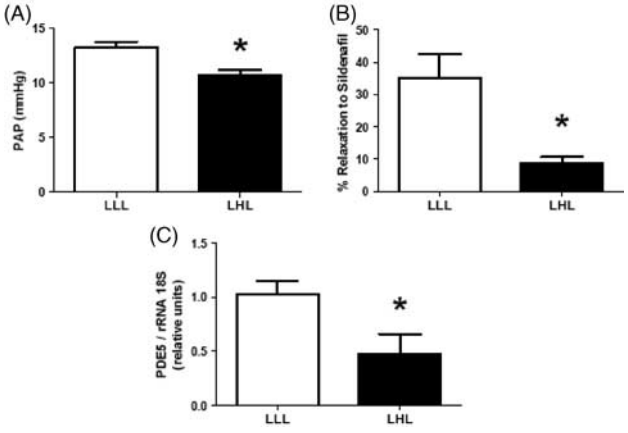
Neonatal lambs whose pregnancy took place partially in high altitude have pulmonary arterial hypertension. In contrast, when these neonates turn into young adult sheep they show pulmonary artery hypotension compared to low altitude controls.

Objective: To investigate whether the pulmonary artery hypotension in these young adult sheep was the result of enhanced pulmonary NO function.

Methods: Thirteen pregnant ewes were divided into two groups: conception, pregnancy, delivery and postnatal period at lowland (Santiago, 580 m, LLL, $n = 7$) and conception at lowland, taken to high altitude (Putre, 3,600 m) from 30% of gestation until delivery, return to lowland at postnatal day 1 (LHL, $n = 6$) and kept in lowland until adulthood. Under general anesthesia, the young adult sheep (15 to 18 months) were instrumented with pulmonary catheters. Pulmonary arterial pressure (PAP), cardiac output (CO) and pulmonary vascular resistance (PVR) was determined *in vivo* during basal and acute hypoxic conditions before and after treatment with L-NAME. *Ex vivo* wire myography was performed on isolated small pulmonary arteries and the dilator responses to the NO donor, sodium nitroprusside (SNP) and to sildenafil were evaluated. Finally, we determined eNOS, sGC and PDE5 protein expression in pulmonary tissue. The University of Chile Ethics Committee approved all experimental procedures.

Results: Basal values for PAP (Figure A) and PVR (0.108 ± 0.007 vs. 0.068 ± 0.006 torr $\times$ ml^{-1} $\times$ kg $\times$ min; $p < 0.05$) were significantly lower in LHL vs. LLL. During acute hypoxia plus L-NAME there was a lesser increase in PAP in LHL (27.4 ± 1.9 vs. 32.03 ± 2 torr; $p = 0.078$) with no differences in PVR and CO between both groups. The small pulmonary arteries showed no differences in the relaxation to SNP. In contrast, there was a diminished relaxation to sildenafil in LHL compared to LLL (Figure B). We found no differences in eNOS and sGC protein expression between the two groups of sheep. However, PDE5 protein expression was significantly less in LHL sheep compared to LLL sheep (Figure C).

Conclusions: Partial exposure to high altitude during pregnancy resulted in lower pulmonary arterial pressure in young sheep. This hypotension is not NO dependant. However, the decreased protein expression and function of PDE5, could result in higher cGMP function and may explain the reduced pulmonary arterial resistance in young adult sheep that underwent high altitude hypoxia during its pregnancy. Support: FONDECYT 1050479, 1090355.



P-7C-334

Effects of antenatal steroid exposure on pulmonary function and aerobic fitness in adolescents born prematurely with very low birth weight

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Objective: Persons born prematurely with very low birth weight (VLBW) have been show to have reduced pulmonary function, lower fitness, and increased risk for cardiovascular and other chronic diseases. Women with preterm labor are often given glucocorticoid injections to facilitate lung maturation and survival of the infant. While the immediate effects are beneficial in the infant, the long-term effects are not known. Consequently, the objective of this study was to examine the effects of antenatal glucocorticoid exposure (ANS) on pulmonary function and aerobic fitness in a cohort

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of adolescents born prematurely with very low birth weight (VLBW), 50% of whom were exposed to antenatal corticosteroid therapy.

Methods: Fourteen year-old adolescents born preterm with VLBW (between 1992–1995) were recruited using information from a computerized neonatal database, and volunteers were asked to perform pulmonary function and exercise testing. Forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV_1) were assessed via standard spirometery, and aerobic fitness (VO_{2peak}) was assessed via analysis of expired gases collected during progressive exercise testing on a cycle ergometer. Independent t-tests were used to make between group comparisons based on ANS exposure (exposed ANS+ v. unexposed ANS-). Chi-squared analysis was used to compare proportions. Data are expressed as means and standard deviations or percentages. Appropriate institutional ethics committee clearance and participants' assent and guardians' written informed consent were obtained.

Results: To date, 100 14 year-old adolescents (43% male, 50% Caucasian) have been enrolled. Thirty-nine children were exposed and 61 were not exposed to ANS. Mean FVC, expressed as % of predicted, was significantly *($p = 0.02$)* higher in ANS+ (104 + 13%) than ANS- (97 + 16%). In contrast, the mean FEV_1 did not differ between ANS+ and ANS− groups (89 + 12% and 85 + 16%, respectively. However, a significantly greater percentage of the ANS- group (33%) had an FEV1 < 80% of predicted compared to the ANS+ group (15%) ($X^2 = 3.93$, $p < .05$). VO_{2peak} (% of predicted) did not differ between the two groups (85 + 19% v. 82 + 21% for ANS+ and ANS-, respectively). Thirty-nine% of the ANS+ and 51% of the ANS− had reduced fitness (<80% of predicted), but the proportions were not significantly different.

Conclusion: These data support a long-term beneficial effect of antenatal steroid exposure on pulmonary function, but not on aerobic fitness, in adolescents born prematurely with VLBW. The reduced fitness observed in both groups may contribute to the increased risk of developing cardiovascular as well as other chronic diseases in adulthood. Support : The General Clinical Research Center of Wake Forest University Baptist Medical Center MO1-RR07122 and NIH Grant number PO1HD047584.

P-8A-335

Associations between lactase persistence and the metabolic syndrome: a Mendelian randomization study in the Canary Islands

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Objective: The single nucleotide polymorphism (SNP) LCT −13910 C > T associated with genetically determined phenotypes of lactase persistence (LP) or non-persistence (LNP) was studied in relation to the metabolic syndrome (MetS) by Mendelian randomization (MR). Mendelian randomization is a new approach for studying modifiable causes of disease in genetic epidemiology. The metabolic syndrome is a concept that assembles risk factors for cardiovascular disease, type II diabetes and stroke into a defined clinical phenotype. The aim of the study was to identify a possible intermediate phenotype for MetS that is associated with LP or LNP that has been shown to modulate milk and dairy product consumption.

Methods: A representative sample of adults belonging to the Canary Islands Nutrition Survey (ENCA) in Spain aged 18–75 years (n = 551) was genotyped for the LCT −13910 C > T polymorphism. Mendelian randomization was used to assess, if milk consumption is correlated to the metabolic syndrome. Adult Treatment Panel III (ATP III) was used to define MetS. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The studied population shows 60% of subjects with LP and 40% with LNP. In the total population, 84 of the subjects with LP (27.3%) and 41 (19.7%) subjects with LNP met the definition for MetS ($p = 0.047$). Women with LP showed, after adjustment for environmental and nutritional variables, an odds ratio (OR) for MetS of 2.13; 95% CI: 1.09–4.17 ($p = 0.032$). Women with LP also displayed a higher prevalence of triglyceridemia (≥150 mg/dl) ($p = 0.021$). Men, aged 45–75, with LP had significantly greater waist circumferences ($p = 0.049$).

Conclusions: The T allele of the SNP, LCT–13910 C > T, might constitute a nutrigenetic factor increasing the susceptibility of LP subjects, especially women, to develop MetS. Support: The Spanish Canarian Health Service supported the Canary Islands Nutrition Survey (ENCA). The present work was also supported by the Örebro County Council, Sweden.

P-8A-336

Long-term inflammatory consequences of maternal smoking: findings from young adults belonging to a Brazilian birth cohort

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Objective: Smoking during pregnancy is associated with low birthweight and a number of other poor health outcomes in offspring, but the impact of maternal smoking on inflammatory outcomes in offspring has not been investigated. We examined the effects of smoking during pregnancy on elevated C-reactive protein (CRP) levels in young adults belonging to a Brazilian birth cohort.

Methods: The 1982 Pelotas birth cohort has followed-up 5914 individuals since birth, most recently in 2004 when the entire cohort was sought and levels of serum CRP were measured. Sociodemographic and behavioral variables were collected at every follow-up. Three sex-stratified logistic regression models with robust variance estimates were constructed to assess the impact of maternal smoking on elevated CRP levels (>10 mg/L) at age 23 years. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: During pregnancy, 64% of mothers were non-smokers whereas 27% smoked 1–14 cigarettes daily (light) and 9% smoked 15 or more (heavy). In 2004, 4297 cohort members were followed-up (77.4% follow-up rate including known deaths) and 3827 had CRP levels measured. Four percent of men and 9% of women had elevated CRP levels. In unadjusted analyses, a significant trend between maternal smoking and elevated CRP levels was observed in men only ($p = 0.05$). After adjusting for confounding factors (model 1: socioeconomic indicators at birth; mother's skin color; birthweight; gestational age; breastfeeding), odds ratios (95% CI) for elevated CRP levels were 1.92 (1.09, 3.40) for light smokers and 2.29 (1.02, 5.15) for heavy smokers during pregnancy (p for trend = 0.007). Further adjustment for concurrent socioeconomic factors (model 2: family income and offspring's attained education) weakened point estimates slightly and a significant trend persisted. Behavioral variables known to impact CRP levels were then added (model 3: body mass index, smoking, and alcohol intake) but did not notably impact associations. All associations were apparent only in men.

Conclusions: These findings suggest that smoking during pregnancy may have long-term consequences for the developing immune system, resulting in impaired regulation of the complement cascade or other inflammatory pathways. Further research on the associated pathways is called for.

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Table. Odds Ratio (95% CI) of elevated C-reactive protein levels (>10 mg/L) by categories of maternal smoking (daily cigarette consumption in # of cigarettes). The 1982 Pelotas birth cohort study (1982 and 2004).

	Crude	Model 1	Model 2	Model 3
Male (N)	1919	1485	1485	1482
0 cigarettes/d	(ref)	(ref)	(ref)	(ref)
1–14	1.32 (0.79,2.21)	1.92 (1.09,3.40)	1.88 (1.06,3.32)	1.79 (1.02,3.17)
15+	1.94 (1.01,3.75)	2.29 (1.02,5.15)	2.15 (0.96,4.84)	2.06 (0.92,4.63)
P for trend	0.05	0.007	0.01	0.02
Female (N)	1370	1060	1060	1059
0 cigarettes/d	(ref)	(ref)	(ref)	(ref)
1–14	1.32 (0.87,2.00)	1.42 (0.85,2.37)	1.39 (0.83,2.32)	1.33 (0.79,2.24)
15+	1.19 (0.60,2.38)	0.87 (0.28,2.65)	0.81 (0.26,2.53)	0.73 (0.22,2.39)
P for trend	0.3	0.5	0.6	0.8

*Pregnant women and women using oral contraceptive therapies excluded.

P-8A-337

Blood pressure in 8-and 10-year old singleton intra cytoplasmic sperm injection children compared to spontaneously conceived children

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Objective: Accumulating evidence suggests that the pre-implantation environment has an influence on postnatal development and cardio-metabolic health later in life. To evaluate if the *in vitro* procedure in humans has long term consequences on the cardiovascular functioning, we compared blood pressure at the age of 8 and 10 years in children born after intra cytoplasmic sperm injection (ICSI) with children born after spontaneous conception (SC).

Methods: Longitudinal questionnaire data and parameters collected at physical examination including longitudinal blood pressure measurements of 8-year-old ICSI children were compared with those of peers born after SC. At the age of 10 years, 108 of the initially recruited 150 ICSI children were re-examined and 93 out of the 147 SC children. All children were singletons born after 32 weeks gestation. Blood pressure measurements were taken using a portable manual sphygmometer. The measurements were taken by a qualified investigator on the non-dominant arm in the sitting position after the child had been seated for at least five minutes.

Results: Blood pressure measurements were available at 8 years in 100 ICSI and 90 SC children and at years in 104 ICSI and 92 SC children. Birth weight, birth weight SDS, birth length and gestational age did not differ between the ICSI population and the SC group. Anthropometric findings such as height and weight in ICSI and SC children were comparable, at the age of 8 as well at the age of 10 years. Systolic and diastolic blood pressure were higher in ICSI children than in SC children at the age of 8 years (98 mmHg versus 94 mmHg; $p < 0.001$; and 59 mmHg versus 55 mmHg; $p = 0.001$ respectively). The difference remained after correcting for birth characteristics (weight, gestational

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age), maternal factors (age, nulliparity, level of education) and current physical characteristics (age, BMI, pubertal score). Ten-year old ICSI children had a comparable systolic but a lower diastolic blood pressure compared to their spontaneous conceived peers (99 mmHg versus 99 mmHg; $p = 0.5$ and 65 mmHg versus 68 mmHg; $p = 0.002$ respectively), although the effect of lower diastolic blood pressure in 10-year-old children attenuated after adjusting for confounders. ***Conclusion:*** ICSI conception is associated with a 5 mmHg increase in blood pressure at 8 years, but this finding could not be confirmed at age 10. Blood pressure levels in childhood are known to be predictive of those in later life. Our findings warrant long-term follow-up of ICSI conceived individuals to assess possible effects of periconception events on cardiovascular health in later life. Currently, ICSI children are being examined at the age of 14–15 years.

P-8A-338

Effects of teratogenic agents on zebrafish (*Danio rerio*) nervous system development

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Objectives: We are studying the effects of environmentally induced birth defects in the nervous system using zebrafish (*Danio rerio*) as a model system. Specifically, we are using three teratogenic agents: Alcohol, Triazol and Ivermectin and analyzing their affects on early development. Alcohol is a very common teratogenic agent; when consumed during pregnancy in humans it can cause craniofacial malformations in the offspring as well as a wide spectrum of neurological and behavioral disorders, collectively known as Fetal Alcohol syndrome (FAS)[1]. It is well accepted that Alcohol affects the development of craniofacial structures[2] and these structures arise from cranial neural crest (CNC) cells during early development. A less commonly known teratogen is the fungicide Triazol, which is also known to cause craniofacial malformations[3]. Finally, Ivermectin, an antiparasitic widely used in humans, pets, cattle and controversially used in aquiculture, may have a teratogenic effect on CNC. CNC share a common border with the cells that will give rise to the olfactory placode (OP) during development of the zebrafish[4], thus we propose these teratogenic agents might also affect the development of the olfactory system. We are analyzing the effects of Alcohol, Triazol and Ivermectin on OP and CNC progenitor migration and gene expression.
Methods: 3–4 hours post fertilization (hpf) zebrafish embryos were incubated with different ethanol/ pisco (100 to 400 mM), Triazol (0,12 g/L to 12 g/L) and Ivermectin (10 μg/L to 500 μg/L) concentrations until they reached a desired embryonic stage. The embryos were processed for *in situ* hybridizations using chemokines probes (receptor and ligand), members of a signaling pathway recently shown to be fundamental for olfactory placode development[5]. For *in vivo* analysis of cell migration we used embryos expressing Green Fluorescent Protein in developing CNC. For morphological analysis, we analyzed structural malformations and cell death. ***Results:*** Our results suggest that alcohol affects OP progenitor organization, CNC migration, and enhances cell death. Also, alcohol produces craniofacial abnormalities very similar to the malformations seen in FAS patients. Triazol affects CNC migration and produces craniofacial malformations (Figure 1). Ivermectin effects on CNC are more subtle, but a total lack of spontaneous movements was observed with higher concentrations.

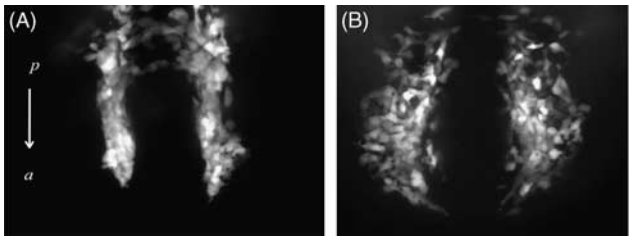

Figure 1: *in vivo* CNC Migration. 16–18 somites *sox10-GFP* embryos incubated with 1,2g/L Triazol. A & B. Fronto-dorsal images of CNC (in white) Maximun intensity projection of Z stacks taken with a confocal microscope. A. Control: Migrating CNC. *a:* anterior, *p:* posterior, B. 1,2g/L Triazol. The cells are less aggregated compared with the control, which might indicate a disrupted migration (A).

Conclusions: This research will allow us to better understand the mechanisms by which teratogenic effects converge on the given developmental events thus creating common phenotypes in affected individuals.

1. K. Sulik, *Exp Biol Med*, 230:366–75, 2005.
2. L. Hoffman *et al.*, *Int J Dev Biol,* 43:167–74, 1999.
3. E. Menegola *et al.*, *In vitro Cell Dev Bio,* 36:88–95, 2009.
4. K. Whitlock, *Brain Behav Evol,* 64:126–140, 2004.
5. N. Miyasaka *et al. Development,* 134:2459–2468, 2007.

P-8A-339

Derangements in global methylation in very young children with nonisolated congenital heart disease

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Objective: To investigate associations between the global methylation status in blood and congenital heart defects (CHDs). We hypothesize that the global methylation status in children with CHD is altered due to a derangement in

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intrauterine metabolic programming as a consequence of periconceptional exposure to maternal hyperhomocysteinemia and concomitant global hypomethylation. The observations that derangements in DNA methylation due to alterations in the global methylation status in blood and other tissues during pregnancy and post-weaning can modify embryonic, fetal and metabolic development are in line with the developmental origin of health and disease hypothesis[1–4].

Methods: In the Western part of the Netherlands a case-control study was performed in 143 case children with CHD and 186 children without a congenital malformation at the age of 17 months. The case group comprised isolated (n = 119) and non-isolated CHD (n = 24), including 16 syndromal CHD. The primary outcomes were the biomarkers of the global methylation status determined in blood, i.e., S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), total homocysteine (tHcy), folate, and vitamin B12, and the methylenetetrahydrofolate reductase polymorphisms, i.e., MTHFR; 677C > T and 1298A > C. Comparisons were made between cases and controls with and without adjustment for age, medication, vitamin use, and family history of CHD. Odds ratios (OR) with 95% confidence interval (95%CI) estimating the risk of CHD were calculated.

Results: In the overall CHD group the median concentrations of SAM ($P = .01$), serum folate ($P = .02$) and red blood cell folate ($P = .03$) were significantly higher than in controls. In particular in nonisolated CHD significantly higher median concentrations of SAM ($P < .001$), SAH ($P = .01$), and serum folate ($P = .01$) were established independent of carriership of the MTHFR polymorphisms. The highest concentrations of SAM, SAH and serum folate were observed in nonisolated syndromic CHD. High SAM and serum folate both increased the overall CHD risk, OR (95% CI) 1.71 (0.96–3.07) and 1.72 (0.96–3.09), respectively.

Conclusion: A status of global hypermethylation in very young children is associated with CHD, in particular nonisolated syndromic CHD. Research is needed to unravel whether the increased biomarker concentration of methylation biomarkers and folate reflect a metabolic derangement induced in early pregnancy or is a consequence of CHD.

Support: The Netherlands Heart Foundation (2006B083 & 2002B027) and the Corporate Development International (2005).

1. R. Jaenisch, A. Bird. *Nat Genet*, 33S:245–54, 2003.
2. P. Bateson *et al.*, *Nature*, 430:419–21, 2004.
3. G.C. Burdge *et al.*, *Br J Nutr*, 97:1036–46, 2007.
4. P.D. Gluckman *et al.*, *N Engl J Med*, 359:61–73, 2008.

P-8A-340

Maternal protein restriction induces tissue-specific global downregulation of gene expression in microswine offspring

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Objective: Malnutrition in pregnant animals produces lasting changes in offspring physiology and metabolism. However, molecular mechanisms by which the intrauterine environment conveys fetal programming remain to be defined. We hypothesized that one of the primary effects of dietary restrictions is a decrease in availability of substrates for synthesis of RNA and other molecules. Therefore, limited nutrients may globally slow down transcription rate and decrease per cell transcript levels. We further postulated that, along the fetal blood flow route, downstream (post-heart) tissues receiving less flow at lower pO2 (kidneys) will be more affected than those located upstream (pre-heart) receiving oxygenated placental blood (liver). If true, such an effect may have a fundamental lasting impact on organism function.

Methods: To explore the effect of dietary limitations on fetal gene expression, we used a microswine model of maternal protein restriction (MPR) where low protein diet (1% v 14% NP) was applied during the last trimester of pregnancy and for 2 weeks after birth. We used several approaches to estimate per cell RNA levels in kidney cortex and livers from animals during MPR (2 days before birth; 2 weeks after birth) and in juveniles (3–5 months). First, as cell number in a tissue fragment is proportional to the DNA content, we extracted total nucleic acids (DNA + RNA) from tissues and estimated per cell RNA content by measuring RNA/DNA ratios. Second, we measured levels of ten different individual mRNAs (RT qPCR) and total mRNA (biotinylated cDNA synthesized with oligo-dT primer (poly(A)+ RNA)) per 1 μg of each RNA sample, that were normalized to corresponding RNA/DNA ratios. In addition, ChIP assay with antibodies to different histone modifications was used to define the stage of transcription that was primarily targeted by MPR.

Results: We show that total per cell RNA content is lower by ~ 20% in LoP vs NP kidney cortex from near-term and 2 week old animals ($p < 0.05$, $n > 7$ per group). The level of all individual housekeeping transcripts per cell was also decreased in in the same tissues by 40% on average ($p < 0.005$). Furthermore, the level of total Poly(A)+ mRNA was substantially lower in the same LoP kidneys (35% difference, $p < 0.05$). Remarkably, livers from the same LoP animals were not significantly affected, suggesting that the effect of MPR on gene expression in the offspring is tissue-specific. ChIP analysis of downregulated genes revealed universally decreased levels of histone modification H3K36m3 ($p < 0.001$), a marker of transcription elongation rate. Following transition to normal diet, in juvenile offspring, per cell mRNA levels and histone modifications recovered to near-control levels indicating that transcription downregulation is a direct effect of ongoing MPR.

Conclusions: Findings demonstrate that perinatal MPR triggers tissue-specific global downregulation of transcription and define transcription elongation as the most sensitive

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stage; they suggest that MPR-induced transcriptional slowdown is caused by decreased availability of metabolic substrates for RNA synthesis. Standard normalization of specific mRNA to total RNA masks this profound metabolic change and may confound interpretation of biologic impact. Support: Gates Foundation.

P-8A-341

DNA methylation of hepatic glucocorticoid receptor in adult sheep

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Objective: In humans and animals environmental constraints during pre- and postnatal life result in phenotypic changes that can be associated with altered cardiovascular and metabolic disease risk in later life[1,2]. The liver is a key organ in glucose and lipid metabolism and altered maternal diet or body composition are associated with changes in fetal liver blood flow[3]. In rats, maternal gestational low protein diet alters adult hepatic gene expression in the offspring, with different effects between liver lobes[4]. In addition, promoter methylation of the glucocorticoid receptor (GR), a gene associated with disturbances in metabolic control, is reduced in this model[5]. In contrast, we have previously shown in adult sheep liver that early life nutrition does not alter GR mRNA expression but differences did exist between the lobes and sexes[6]. This study investigated whether these findings in sheep liver were associated with changes in methylation status of the GR promoter.
Methods: Welsh Mountain ewes received 100% (C, $n = 36$) or 50% of total nutrient requirements (U, $n = 39$) from 1-31 days of gestation, and 100% thereafter. Offspring were fed *ad libitum* (CC, $n = 20$; UC, $n = 19$) or to reduce body weight to 85% of individual target weight from 12 to 25 weeks postnatal age and *ad libitum* thereafter (CU, $n = 17$; UU, $n = 21$). Each group contained approximately equal numbers of males and females and the ratio of twins to singletons was ~2:1. Offspring were killed at 2.5 years; the segments from the left and right liver lobes were frozen in liquid nitrogen. Methylation within the ovine GR promoter region was determined by methylation-sensitive PCR. All data were analysed by ANOVA.
Results: There was no effect of lobe, sex, nutrition or number of offspring on GR gene methylation within the DNA promoter region analysed.
Conclusions: This is the first study to investigate the methylation status of GR in the adult sheep liver. Early life nutrition did not affect hepatic GR methylation, in agreement with the absence of changes in mRNA expression of this gene. However, lobar and sex differences in hepatic GR mRNA expression found previously[6] were not accompanied by changes in DNA methylation within the promoter region selected. Further analysis of additional areas within the promoter region will be required in order to confirm this. Support: BHF, BBSRC, Gerald Kerkut Trust & The Foundation of Research, Science and Technology, New Zealand.

1. D.J. Barker *et al.*, *Int J Epidemiol,* 31:1235–1239, 2002.
2. K.R. Poore *et al.*, *Am J Physiol,* 292:E32–E39, 2007.
3. G. Haugen *et al.*, *Circ Res,* 96:12–14, 2005.
4. J. Zhang, C.D. Byrne, *Am J Physiol* 278:G128–G136, 2000.
5. K.A. Lillycrop *et al.*, *Br J Nutr,* 97:1064–1073, 2007.
6. L. Hollis *et al.*, *Proc Physiol Soc,* PC4:2007.

P-8A-342

Different regimens for maternal dietary restriction during pregnancy induce changes in common gene ontologies in rat embryos via differential effects on specific genes

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Objectives: Induction of an altered phenotype in the offspring by maternal dietary constraint during pregnancy has been suggested to reflect adaptive responses which predict the future environment [1]. This implies that correct prediction involves changes to metabolic pathways which reflect the nature of the environmental cue. We investigated the effect of two regimens for maternal nutrient constraint during pregnancy on the expression of transcriptome of the gastrula (E8) in rats.
Methods: Rats were time mated and then fed either a control (18% (w/w) casein), PR (9% (w/w) casein) or the control diet at 70% of *ad libitum* (UN). RNA was extracted from embryos on collected at E8 and expression analysed by RNA microarray in pooled samples (n = 6 embryos per group). The results of the microarray analysis were confirmed for specific genes by real time RTPCR[2].
Results: Transcriptome analysis of E8 embryos showed a similar number of genes were altered in expression by more than 2 fold in PR (3,182) and UN (3,489) embryos compared to controls, of which 11% were changed in PR embryos were also altered in the UN embryos. Of these, 54 genes were down-regulated and 289 up-regulated in PR embryos, while all of these genes were up-regulated in embryos from UN dams. There was no significant association

between the difference in expression from controls between PR and UN embryos for genes down-regulated in PR embryos. However, the difference from controls for up-regulated genes in PR embryos predicted 49% of the variation in the change in expression of these genes in UN embryos ($P < 0.0001$). Ontology analysis showed significant changes (Z score >2) in the same gene pathways in PR and UN embryos: developmental process, homeostatic process, stress response, chromatin modification and DNA methylation. However, different genes were altered within each ontology. For example, in the chromatin modification ontology, histone deacetylase (HDAC) 5 and HDAC11 are up-regulated in PR embryos, while HDAC2 and HDAC8 are up-regulated in UN embryos, but sirtuin 1 is up-regulated in embryos from both groups of dams.

Conclusions: These results suggest that induction of an altered phenotype in embryos involves changes in the expression of specific genes and in gene ontologies which respond to nutritional constraint. However, there appear to be a subset of genes which are differentially expressed contingent on the nature of the maternal dietary constraint and so may facilitate adaptations which predict specific future environmental challenges. Support: The Wessex Medical Trust, University of Southampton and Biotechnology and Biological Sciences Research Council. MAH receives salary support from the British Heart Foundation.

1. P.D. Gluckman, M.A. Hanson *Science,* 305:1733–1736, 2004.
2. K.A. Lillycrop *et al. Br J. Nutr*, 97:1064–1073, 2007.

P-8A-343

Protein restriction and under-nutrition during pregnancy induces hypomethylation of the glucocorticoid receptor promoter in the developing embryo

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Objectives: Epigenetic processes including DNA methylation have been proposed as a central mechanism in the induction by maternal under-nutrition during pregnancy of an altered phenotype in the offspring [1]. For example, feeding pregnant rats a protein-restricted (PR) diet induces hypomethylation of glucocorticoid receptor (GR) and peroxisome proliferator activated receptor (PPAR) α promoters in the liver of juvenile and adult offspring[2,3] However, there is no direct evidence that maternal macronutrient or total nutrient restriction induces altered DNA methylation during the development of the embryo. Therefore, we investigated the effect of two regimens of maternal under-nutrition during pregnancy on the methylation status of a GR promoter in the gastrula (E8) and whole embryo (E14).

Methods: Rats were time mated and then fed either a control (18% (w/w) casein), PR (9% (w/w) casein) or the control diet at 70% of ad libitum (UN). RNA and DNA was extracted from embryos on collection at E8 and E14. Methylation of the $GR1_{10}$ promoter was assessed by methylation-sensitive PCR[2]. mRNA expression of the $GR1_{10}$ promoter was measured by real time RTPCR[3]. Statistical comparisons were by Student's unpaired t-test (values are mean ± SEM, $n = 6$ embryos per time point per maternal dietary group). Results: GR methylation was significantly reduced in E8 embryos in the UN group (34%, $P < 0.05$) but not in the PR group. GR mRNA expression did not differ from controls in E8 embryos from PR or UN dams. GR methylation was significantly lower in E14 embryos from PR (18%, $P < 0.05$) and UN (11%, $P < 0.001$) dams. This was accompanied by a significantly greater expression in PR embryos (140 ± 14%, $P < 0.05$) and a trend towards higher expression in UN embryos (222 ± 52%, $P = 0.1$). Conclusion: Our findings show that maternal under-nutrition induces hypomethylation of the GR promoter, and that the timing and extent of the change in methylation is dependent upon the maternal diet. The association between the difference in GR methylation and expression at E14, but not at E8, suggests that appropriate transcription factors and other regulatory proteins are required in order for changes in methylation to be reflected in altered transcriptional activity. Together these findings show that maternal under nutrition induces changes in DNA methylation in embryos comparable to those found is adult tissues and so supports the hypothesis that induction of altered epigenetic regulation during development underlies variation in phenotype[1]. Support: The Wessex Medical Trust, University of Southampton and Biotechnology and Biological Sciences Research Council. MAH receives salary support from the British Heart Foundation.

1. G.C. Burdge *et al. Br. J. Nutr.* 97:1036–1046, 2007.
2. K.A. Lillycrop *et al. J. Nutr.* 135:1382–1386, 2005.
3. G.C. Burdge *et al. Br. J. Nutr.* 97:435–439, 2007.

P-8A-344

Maternal protein undernutrition in mouse alters DNA methyltransferase expression

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Objective: While the adverse effect of a sub-optimal maternal diet on the postnatal health and disease risk of offspring has

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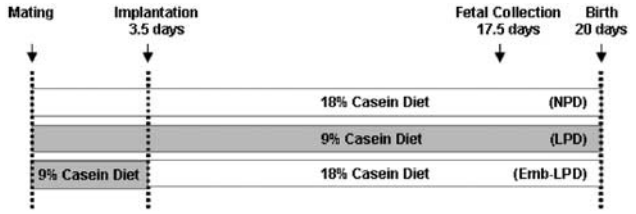
been reported extensively, the precise mechanisms underlying the establishment of a programmed response during early development are as yet poorly understood. One possible candidate is altered epigenetic modifications, which may lead to inappropriate gene expression. The most studied epigenetic modification, DNA methylation, has been shown previously to be altered in rat offspring in response to low protein maternal diet[1], likely due to altered methyl cycle parameters[2]. The aims of this study were to examine DNA methyltransferase (DNMT) expression in the low protein diet *mouse* model, both in embryonic, extra-embryonic and adult tissues, to determine whether epigenetic changes may be associated with the development of the reported postnatal phenotypes[3].

Methods: Virgin MF-1 female mice were naturally mated with MF-1 males then randomly assigned to receive one of the following dietary regimes:



For adult tissues, offspring from dams fed as above were weaned onto a standard chow diet, and sacrificed for tissue collection at 28 weeks of age. All animal procedures were carried out in compliance with the Animals (Scientific Procedures) Act 1986, under UK Home Office Project Licence. Relative expression of gene transcripts was examined by RT-qPCR using intron-spanning primer sets, and normalised to reference gene expression levels using geNorm[4].

Results: Tissue- and sex-specific differences in DNMT transcript expression were observed in response to maternal low protein diet, both in adult tissues and in tissues from day 17.5 conceptuses. For example, in adult kidney the maintenance methyltransferase, DNMT1, was upregulated in LPD females ($P < 0.05$), and at day 17.5 both DNMT1 and the *de novo* methyltransferase, DNMT3b, were downregulated in LPD placenta ($P < 0.05$).

Conclusions: Epigenetic changes during early development are an attractive candidate mechanism for establishing the long-term effects of sub-optimal maternal diet in offspring. DNA methyltransferases are responsible for the establishment and maintenance of DNA methylation patterns within the genome. The changes we see in DNMT expression may lead to downstream changes in gene expression in response to the low protein diet, resulting in the reported phenotypic consequences of maternal protein undernutrition. Support: BBSRC Grant Reference BBF007450.

1. K.A. Lillycrop, *et al.*, *J Nutr*, 135:1382–1386, 2005.
2. L. Petrie, *et al.*, *Br J Nutr*, 88:471–477, 2002.
3. A.J. Watkins, *et al.*, *Biol Reprod*, 78:299–306, 2008.
4. J. Vandesompele, *et al.*, *Genome Biol*, 3, 2002.

P-8A-345

DNA methylation: a possible common mechanism for tissue-specific gene expression changes associated with maternal folate depletion

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Objectives: The developmental origins of health and disease hypothesis proposes that adverse exposures during early life, including poor nutrition, may contribute to increased risk of disease in adulthood but the molecular mechanisms mediating these programming events are not well-understood. One attractive candidate mechanism to explain developmental programming is the modification of epigenetic markings, such as CpG methylation of DNA and covalent histone modifications. These marks are established during development and constitute a rich information source layered on top of the DNA sequence, contributing to cell-specific gene expression, which is essential for cell differentiation. We aim to investigate the mechanisms by which nutritional insults influence gene programming during development. Our focus is upon folate depletion, which may influence DNA methylation through effects on the supply of the methyl donor *S*-adenosylmethionine.

Methods: Pairs of female C57BL/J6 mice were assigned randomly to a folate-adequate (2 mg folic acid/kg) or folate-deplete (0.4 mg folic acid/ kg) diet 5 weeks prior to timed mating with a C57BL/J6 male. Dams remained on allocated diets until day 17.5 gestation, when fetuses and placentas were removed and weighed. Fetal livers and placentas were snap frozen, and DNA and RNA were extracted. To identify genes regulated by maternal folate depletion, hepatic and placental RNA from male fetuses was hybridised to NuGO Affymetrix mouse whole genome arrays. We have combined bioinformatic and pyrosequencing approaches to explore gene promoters to investigate if changes in DNA methylation could be responsible for the observed changes in gene expression.

Results: Based on a fold change of $+/-1.2$ and $p < 0.05$, 680 genes were expressed differentially in fetal livers (321 up-regulated, 359 down-regulated), and in placental tissue 612 genes were expressed differentially (341 up-regulated, 271 down-regulated). These changes appeared to be tissue-specific since only 22 genes were expressed differentially in both tissues, 12 of which had the same directional change in both tissues. Bioinformatic analysis identified a CpG island (CGI) in the promoter regions of 9 of the 12 commonly regulated genes, identifying them as candidates for regulation by DNA methylation. Furthermore, transcription factor binding sites containing CpG dinucleotides were found in these CGIs, consistent with our hypothesis that DNA methylation may be a mechanism of gene regulation in this model.

Conclusions: Effects of folate depletion *in utero* on gene methylation may underlie tissue-specific changes in gene expression in this mouse model. We are testing this hypothesis by determining if genes with promoter CGI are over-represented statistically among differentially expressed genes in placenta and liver in response to folate depletion, and by quantifying promoter DNA methylation in genes differentially expressed in the same direction in both tissues. Data generated from this study will be presented and discussed. Support: NuGO and the BBSRC (BB/G007993/1).

P-8A-346

Tissue specific effects of maternal nutrient restriction on the mid-gestation fetal non-human primate transcriptome

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Objectives: There is currently much interest in mechanisms whereby maternal challenges such as maternal nutrient restriction (MNR) can alter the trajectory of fetal and placental development and produce persistent effects on phenotype that may alter life long health. While there is a considerable literature on physiological responses in individual fetal tissues to hypoxia[1] and gene and protein changes to MNR in rodents[2] and sheep[3], we know of no global transcriptome studies that address normal organ development and effects of NR during pregnancy in any species. Our first aim in the present study was to evaluate and compare biological pathways that are active in multiple organs in a well-studied nonhuman primate model of pregnancy[4]. Our second aim was to use data from normal tissues to provide a framework for assessment of the impact of 30% global maternal MNR on each tissue at mid-gestation.

Methods: We have performed transcriptome profiling (Affymetrix gene chips) for mid-gestation (0.5 gestation - G; term 180 days) placenta and fetal frontal cortex, liver, kidney, lung and adrenal. We defined normal gene expression and normal pathways of gene expression for key fetal organs and compared profiles and pathways between organs and compared effects of MNR among organs using pathway and cluster analyses (GeneSifter, IPA software).

Results: MNR affected gene expression and biological pathway activity in all organs examined. The impact of MNR on pathways such as apoptosis, ubiquitin mediated proteolysis and vascular endothelial growth factor signaling, were seen in several organs. For other pathways such as axonigenesis, the impact of MNR on activity was limited to a specific organ – in this case the frontal cortex. Interestingly, Wnt signaling was activated in right liver as well as kidney.

Conclusions: Our results indicate that this moderate degree of MNR impacts all organs studied at the molecular level and that while some pathways are common among organs, each organ is also impacted in a unique way. Importantly, same pathways exhibiting nutrient sensitivity in multiple organs, such as Wnt signaling in kidney and liver, show organ specific patterns of differential gene expression that may assist in interpreting organ-specific phenotypic changes associated with maternal NR in the primate.

1. H.E. Cohn *et al.*, *Am. J. Obstet. Gynecol.* 120:817–824, 1974.
2. M. Desai *et al.*, *Am. J. Obstet. Gynecol.* 193:1224–1232, 2005.
3. D. Sharkey *et al.*, *FASEB J.* 23:1314–1324, 2009.
4. L.A. Cox *et al.*, *J Physiol.* 572: 67–85, 2006.

P-8A-347

Maternal high fat diet during pregnancy and lactation alters hepatic expression of insulin like growth factor-2 and key microRNAs in the adult offspring

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Objective: miRNAs play important roles in the regulation of gene functions. Maternal dietary modifications during pregnancy and gestation have long-term effects on the offspring, but it is not known whether a maternal high fat (HF) diet during pregnancy and lactation alters expression of key miRNAs in the offspring.

Methods: We studied the effects of maternal HF diet on the adult offspring by feeding mice with either a HF or a chow diet prior to conception, during pregnancy and lactation. Both maternal HF and chow fed offspring were weaned onto the same chow diet until adulthood (HF/C and C/C).

Results: There was no significant difference in body weight, fasting plasma triglyceride, cholesterol and glucose concentrations between HF/C and C/C mice. Hepatic mRNA levels of peroxisome proliferator activated receptor-alpha (ppar-alpha) and carnitine palmitoyl transferase-1a (cpt-1a) were increased in HF/C mice compared to C/C mice (HF/C v C/C: 97445 ± 11712 v 61873 ± 7638, $p < 0.05$ for ppar-alpha and 126777 ± 23720 v 34193 ± 4420, $p < 0.01$ for cpt-1a, mean ± S.E.). Interestingly, mRNA levels of insulin like growth factor-2 (Igf-2), an early growth factor, were also

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increased in HF/C mice (HF/C v C/C: 1404 ± 266 v 520 ± 70, $p < 0.01$). In Igf-2 knockout mice, a HF diet induced up-regulation of ppar-alpha and cpt-1a expression was suppressed. Furthermore, hepatic expression of let-7c, a key miRNA regulating development timing, was also reduced in maternal HF fed offspring. Among 579 miRNAs measured with microarray, ~23 miRNA levels were reduced by ~1.5–4.9-fold. Reduced expression of miR-709 (the most abundantly expressed in the liver), miR-122, miR-192, miR-194, miR-26a, let-7a, let7b and let-7c, miR-494 and miR-483* (reduced by ~4.9 fold) was validated by qPCR. The predicted targets for these altered miRNAs include genes regulating epigenetics and fat metabolism, such as methyl-CpG binding domain protein 6 and methyl-CpG binding protein 2 are predicted targets of miR-709; DOT1-like histone H3 methyltransferease, chromodomain helicase 4 and hypermethylated in cancer 2 are predicted targets of let-7c; and Igf1 receptor and citrate synthase are predicted targets of miR-122 and miR-494.

Conclusions: Maternal HFP feeding during pregnancy and lactation induced co-ordinated and long-lasting changes in expression of Igf-2, fat metabolic genes and several important miRNAs in the adult offspring. These data suggest that HF/C will have a differential response to postnatal diet despite having no significant difference in metabolic parameters.

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P-8A-348

The liver X-receptor gene promoter is hypermethylated in a mouse model of prenatal protein restriction

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Prenatal nutrition, e.g., nutritional status of the mother, has been epidemiologically identified as a determinant of adult disease. Feeding low-protein diets during pregnancy in rodents is a well-established model to induce ''programming'' events in offspring. One proposed underlying mechanism for fetal programming is altered DNA methylation. The consequences for lipid metabolism and hence predisposition to atherosclerosis have not yet been studied.

Objectives: We hypothesized that protein restriction would induce epigenetic adaptations that would interfere with lipid metabolism.

Methods: C57BL/6 mice were fed a protein restricted diet during pregnancy. At day 19.5 of pregnancy, dams and fetuses were sacrificed. CpG island methylation microarrays were performed on fetal liver DNA.

Results: 204 gene promoter regions were found to be differentially methylated upon protein restriction. *Hyper*-methylation and *hypo*methylation were found in comparable numbers. The liver X-receptor (Lxr) alpha promoter was hypermethylated in protein-restricted pups. Lxr alpha is a nuclear receptor critically involved in control of cholesterol and fatty acid metabolism. The mRNA level of *Lxra* was reduced by 32% in fetal liver upon maternal protein restriction, whereas expression of the Lxr target genes *Abcg5/Abcg8* was reduced by 56% and 51%, respectively. In parallel, expression of lipogenic genes was reduced in the low protein group. *In vitro* methylation of a mouse Lxra-promoter/luciferase expression cassette resulted in a 24-fold transcriptional Lxr repression.

Conclusions: Our study demonstrates that, in mice, protein restriction during pregnancy interferes with DNA methylation in fetal liver. As hypermethylated and hypomethylated regions are comparable in numbers, it is unlikely that only a shortage of methyl groups by protein restriction is the underlying cause. Lxra was identified as a new target of differential methylation. Furthermore, Lxra transcription is dependent on DNA methylation. It is tempting to speculate that perinatal nutrition influences adult lipid metabolism by DNA methylation. This could contribute to the epidemiological relationship between perinatal/neonatal nutrition and adult disease.

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P-8A-349

Prevention of fetal programming of vascular dysfunction by a histone deacetylase inhibitor

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Objective: Studies in humans and experimental animal models indicate that fetal insults predispose to cardiovascular and metabolic diseases later in life. We have recently shown in mice that offspring of restrictive diet pregnancies display pulmonary vascular dysfunction. Male offspring transmit this vascular dysfunction to the next generation, but the underlying mechanisms are unknown. Epigenetic alterations (histone deacetylation and DNA methylation) may result in stable changes of gene expression that are transmitted throughout generations. We hypothesized that the vascular dysfunction in offspring of restrictive diet pregnancies is related to an epigenetic mechanism.

Methods: To test for this possibility, in C57/BL6 mice, we examined the effects of the histone deacetylase inhibitor Butyrate (2 mg/kg/day, i.p for 2 weeks) on global DNA methylation and pulmonary vascular function in male

offspring of restrictive diet pregnancies (65% of the normal caloric intake) and in their progeniture.

Results: The major new findings were that restrictive diet impaired DNA methylation in lung tissue, as shown by greater uptake of radioactive methyl groups compared to controls (1104 ± 89 vs. 729 ± 84 CPMA/ng genomic DNA, $P = 0.02$). In offspring of restrictive diet pregnancies Butyrate treatment normalized pulmonary DNA methylation (706 ± 103 CPMA/ng genomic DNA, $P = 0.046$). This beneficial effect on DNA methylation was associated with normalization of pulmonary endothelium-dependent vasodilation in vitro ($P < 0.0001$, Butyrate vs. vehicle) and pulmonary-artery pressure response to hypoxia in vivo (32.9 ± 2.3 vs. 36.2 ± 4.4 mmHg, $P = 0.05$). Finally, Butyrate treatment of the offspring prevented the transmission of vascular dysfunction to the next generation ($P < 0.0001$, Butyrate vs. vehicle).

Conclusions: The histone deacetylase inhibitor Butyrate restored pulmonary endothelial function in male offspring of restrictive diet pregnancies in mice, and prevented the transmission of this diet-induced vascular dysfunction to the next generation. These findings suggest, for the very first time, that epigenetic mechanisms play an important role in the pathogenesis of fetal programming of vascular dysfunction.

P-8A-350

Variations in DNA methylation in a large cohort of children differs between different CpG sites within the promoters of candidate genes

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Objective: Based on concepts developed for imprinted genes, CpG groups of non-imprinted genes have traditionally been viewed as being either methylated or unmethylated in particular cell types and tissues. Experimental studies have, however, shown that the methylation state of different CpGs in non-imprinted genes exhibits substantial variation, and recent evidence shows that environmental factors such as maternal stress and nutritional state influence epigenetic state in the offspring. As yet there is little evidence as to whether there are substantial variations in the methylation state of CpGs in non-imprinted genes in human infants.

Methods: We extracted DNA from stored umbilical cord from 384 neonates in the Southampton Women's Survey, a large population based cohort study. We used pyrosequencing to measure the DNA methylation status of specific CpGs within the promoters of candidate genes. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The methylation measured varied greatly at particular CpG sites, with almost no variation at other sites. For example, the methylation of one CpG site within the lipoprotein lipase (LPL) promoter ranged from 4.3% to 20.7% (median 9.7%), while methylation at an adjacent CpG site ranged from 23.6% to 54.3% (median 35.6%). The correlation of methylation levels at these adjacent CpG sites was no more than modest ($r_p = 0.19$). Similar findings were observed in other candidate genes, including endothelial nitric oxide synthase, with the range in methylation at several CpG sites being less than 10%. The CpG sites in these genes were not located within any known single nucleotide polymorphisms (SNPs) that could explain the variation found, nor were any SNPs identified during the measurement of this data.

Conclusions: The methylation status of the promoters in candidate non-imprinted genes in DNA extracted from umbilical cord varies across a normal population of children. These sites could prove to be important in controlling expression of these genes and contribute to the variation in vulnerability to later chronic non-communicable disease in the population that cannot be explained by genomic variation. Support: The National Institute of Health Research (UK), the UK Medical Research Council and the EpiGen consortium.

P-8A-351

Periconceptional maternal folic acid use increases methylation of the IGF2 gene in the very young child

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Objective: Countries worldwide recommend women planning pregnancy to use daily 400 μg of synthetic folic acid in the periconceptional period to prevent birth defects in children. The underlying mechanisms are not clear, however, epigenetic modulation of growth processes by folic acid is hypothesized. Here, we investigated whether periconceptional maternal folic acid use and markers of global DNA methylation potential (S-adenosylmethionine and S-adenosylhomocysteine blood levels) in mothers and children affect

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methylation of the insulin-like growth factor 2 gene differentially methylation region (IGF2 DMR) in the child. Moreover, we tested whether the methylation of the IGF2 DMR was independently associated with birth weight.

Methods: IGF2 DMR methylation in 120 children aged 17 months (SD 0.3) of whom 86 mothers had used and 34 had not used folic acid periconceptionally were studied. Methylation was measured of 5 CpG dinucleotides covering the DMR using a mass spectrometry-based method. Appropriate institutional ethics committee clearance and participants informed consent were obtained.

Results: Children of mother who used folic acid had a 4.5% higher methylation of the IGF2 DMR than children who were not exposed to folic acid (49.5% vs. 47.4%; $p = 0.014$). IGF2 DMR methylation of the children also was associated with the S-adenosylmethionine blood level of the mother but not of the child (+1.7% methylation per SD S-adenosylmethionine; $p = 0.037$). Finally, we observed an inverse independent association between IGF2 DMR methylation and birth weight (−1.7% methylation per SD birthweight; $p = 0.034$).

Conclusions: Periconceptional folic acid use is associated with epigenetic changes in IGF2 in the child that may affect intrauterine programming of growth and development with consequences for health and disease throughout life. These results indicate plasticity of IGF2 methylation by periconceptional folic acid use.

P-8A-352

Methylation of glucocorticoid receptor promoter in humans after prenatal exposure to the Dutch famine

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Objective: Poor nutrition during fetal development can permanently alter growth, cardiovascular physiology and metabolic function. The Dutch famine birth cohort study has provided the first evidence in humans that maternal undernutrition during gestation leads to a striking 2-fold increase in cardiovascular disease in the offspring. Animal studies have recently started to unravel the underlying molecular mechanisms. Poor intrauterine nutrition leads to persistent alterations in the epigenetic regulation of specific genes, which give rise to altered expression of a range of genes which may raise cardiovascular risk. There is evidence of epigenetic alterations of imprinted genes in humans after prenatal undernutrition. The aim of this study was to assess whether prenatal exposure to the Dutch famine alters GR promoter methylation.

Methods: Methylation status of the non imprinted glucocortiocoid receptor promoter was investigated in DNA isolated from peripheral blood samples of 58 year old subjects born as term singletons in the Wilhelmina Gasthuis in Amsterdam, The Netherlands around the time of the 1944–45 Dutch famine. We compared methylation levels of participants exposed to famine during late ($n = 128$), mid ($n = 107$), or early gestation ($n = 69$) to levels of participants not exposed to famine during gestation ($n = 416$). The methylation status of the GR 1C promoter was assessed using a methylation sensitive PCR assay[1]. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Undernutrition in early gestation was associated with increased methylation of the GR promoter compared to non-exposed time controls, but the association was not statistically significant (20.7%, 95%CI −4.3 to 52.2). A similar association was found when adjusted for sex, maternal age, SES and parity (21.5%, 95% CI −4.0 to 53.4) and also after adjustment for birth weight (18.9%, 95% CI −6.0 to 50.5). Exposure in late or mid gestation was not associated with altered GR methylation ($p > 0.1$). Regardless of exposure status, people with high birth weight had increased GR methylation; an increase of 26.7% (95% CI 9.6 to 46.4) per kilogram increase in birth weight adjusted for sex, maternal age, SES and parity.

Conclusion: Famine exposure in early gestation seems to be associated with an increased level of methylation of the GR promoter. Independent of this, higher birth weights are associated with hypermethylation of the GR promoter.

1. K. Lillycrop *et al.*, *Nutr J.*, 135:1382–1386, 2005.

P-8A-353

Early life infection predisposes to epigenetic transference of anxiety

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We have previously established that neonatal exposure to a bacterial stimulus, lipopolysaccharide (LPS), produces an increase in anxiety-like behaviour in rats when exposed to a subsequent psychological stressor in adulthood. Furthermore,

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these behavioural changes appear to be associated with perturbations in the neuroendocrine-neuroimmune interface.
Objective: The current study assessed the potential transgenerational implications of early life infection on anxiety behaviours. Specifically, we were interested in whether neonatal exposure to LPS alters the quality of maternal care of second generation offspring, and whether those offspring exhibit increased anxiety-like behaviour in adulthood despite not being directly exposed to any immunological stimulus themselves.
Methods: 86 Wistar rats were exposed to either LPS (0.05 mg/kg, *Salmonella enteritidis*) or non-pyrogenic saline (equivolume) on days 3 and 5 of life. In adulthood, animals were exposed to either restraint and isolation stress or no stress, and subsequently examined for anxiety-like behaviours on the Elevated Plus Maze (EPM), Acoustic Startle Response (ASR), and Holeboard Apparatus. Blood was collected to examine the corticosterone response to restraint stress. Animals were then bred with naive counterparts of the opposite sex, and maternal care of the second generation was monitored over the first week of life. The second generation were left completely undisturbed until adulthood, at which time they underwent identical testing procedures as the parental (F1) generation. Hippocampal tissue from all animals was snap frozen for analysis of methylation of the glucocorticoid receptor (GR) gene promoter.
Results: As previously demonstrated animals in the first generation exposed to LPS in early life exhibited increased anxiety-like behaviours on all behavioural measures compared to saline controls ($p < .05$ for all). These animals also displayed perturbations in their corticosterone response to stress. Females exposed to LPS during the neonatal period were similarly found to provide significantly lower quality maternal care on all measures, including licking and grooming, arched back nursing, and nest building ($p < .05$ for all). No difference in maternal care was observed for naïve females mated with LPS or saline-treated males. Testing of the second generation of both males and females in adulthood, revealed that offspring of animals exposed to LPS during their neonatal life, exhibited increased anxiety-like behaviours in regards to risk assessment hypervigilance during behavioural testing on the EPM, Holeboard, and ASR ($p < .05$). Furthermore, second generation offspring of LPS-treated parents hypersecreted corticosterone in response to stress compared to offspring of saline-treated parents ($p < .05$). Methylation of the GR gene promoter will be assessed.
Conclusions: This study demonstrates a clear epigenetic transference of anxiety-like behaviour via exposure to an immunological stimulus. That is, exposure to bacteria during the neonatal period results in increased anxiety symptomology in adulthood, as well as poor maternal care of second generation offspring. Furthermore, subsequent generations also appear predisposed to adult onset anxiety despite non-exposure to the infectious stimulus themselves. Such behavioural changes appear to be associated with perturbations to the HPA axis. This study may indicate a possible mechanism through which psychopathology appears to precipitate down familial lines.

P-8A-354

Elevated offspring growth induced by maternal protein undernutrition exclusive during preimplantation development is associated with altered signalling through the mammalian target of rapamycin pathway in mice

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Objectives: The mammalian target of rapamycin (mTOR) is a serine/threonine kinase which coordinates cell growth (enhanced mass and size) and cell cycle progression in response to nutrients (amino acids), energy (ATP:ADP) and growth factors via the phosphorylation of down stream targets including 4EBP1 and the 70S ribosomal protein S6. Phosphorylation of S6 results in ribosome biogenesis, enhanced protein translation, and increased cell mass, whilst the phosphorylation of 4EBP1 results in increased translation of key growth-promoting proteins such as c-Myc and cyclin D1[1]. We have demonstrated previously that maternal low protein diet (LPD) given exclusively during the preimplantation stage of mouse development results in significantly elevated weight at birth, which is maintained for up to 28 weeks[2]. Here we investigate whether altered signalling patterns through the mTOR pathway may associate with the enhanced growth phenotype observed in these mice.
Methods: Following mating, female MF-1 mice were assigned to one of the following dietary treatments shown opposite. Embryonic, fetal and adult tissues were collected at the times indicated. Levels of total and phosphorylated 4EBP1 and S6 were determined using quantitative western blotting. Animal procedures were conducted using protocols approved by UK Home Office and local ethics committee.
Results: Levels of phosphorylated S6 and the ratio of phosphorylated:total S6 were lower in blastocysts from Emb-LPD mothers, but in the adult liver, levels of total S6 were elevated in Emb-LPD mice when compared to controls. In heart tissue from adult LPD mice, decreased levels of phosphorylated S6 were observed, whilst reduced levels of phosphorylated 4EBP-1 were observed in LPD and Emb-LPD adult liver tissue when compared to controls.

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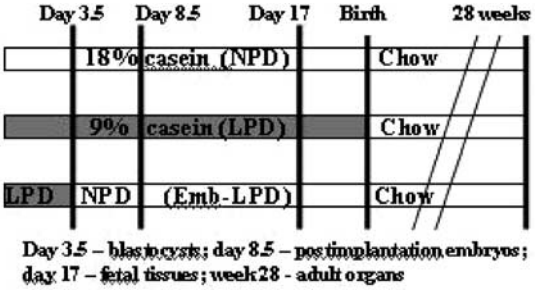

Conclusions: These data demonstrate that maternal protein undernutrition given during discrete windows of gestation result in significant changes in the levels of key down-stream mediators of mTOR signalling. These changes appear to initiate in the preimplantation embryo, and perpetuate into adult life. However, not all tissues and pathways were equally affected suggesting developmental and tissue-specific responses in the regulation of protein synthesis, cell division and translational control. Support: BBSRC [BBF007450], National Institutes of Health [grant number U01 HD04435] and the Gerald Kerkut Charitable Trust.

1. M. Hwang *et al., Curr Med Chem.* 15:1192–1208, 2008.
2. A.J. Watkins *et al.*, *Biol Reprod*. 78:299–306, 2008.

P-8A-355

The environmentally induced alterations in gene expression within the nervous system

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Objective: We are examining the effects of the environment on gene expression sing the olfactory epithelium of the zebrafish as a model system[1]. We are testing the hypothesis that in addition to intrinsic (lineage restricted) mechanisms, there are also extrinsic (environmentally determined) mechanisms controlling the differentiation of the olfactory sensory neurons in the developing zebrafish.
Methods: To explore whether the Immediate Early Genes (IEGs; transcription factors that are rapidly up-regulated in response to sensory stimuli) are involved in the signaling pathway that effects gene expression changes in the olfactory sensory system we cloned the IEGs *egr1, c-jun,* and *c-fos* from zebrafish and generated digoxigenin (DIG) labeled mRNA probes that recognize these genes. We analyzed the expression of these IEGs via whole-mount *in situ* hybridization of the olfactory epithelia of developing zebrafish embryos. We used two odorants shown to be behaviorally significant to fishes: the hormone prostaglandin (PGF2α) which modulates reproductive behaviors in adult goldfish, and taurocholic acid, an odorant we have shown previously to elicit a behavioral response in juvenile and adult zebrafish. We are using a genomic approach to identify down-stream targets of the transcription factor *otx2*, which we have previously shown to responsive to changes in the environment[2].
Results: We have previously shown that zebrafish are able to form and retain olfactory memories of the odor phenyl ethyl alcohol (PEA) experienced as juveniles, and that these memories are correlated with long-term changes in expression of the transcription factor *otx2* within the olfactory sensory epithelium (2,3). We show that both *c-jun* and *c-fos* are expressed in the developing olfactory epithelium and *egr1* is expressed outside the olfactory epithelium in several tissues including the differentiating olfactory bulb. Because of the restricted pattern of expression of *c-fos* we used this gene to determine whether odorant exposure modulates expression of *c-fos* in the olfactory epithelia. After exposing juvenile zebrafish to various odorants for 48 hours of development we found that there is a significant decrease in the number of *c-fos* cells when fish are exposed to taurocholic acid and a significant increase after exposure to Prostaglandin F_{2a}. Whether these changes in *c-fos* expression are related to neuronal activity, differentiation or both remains yet to be determined.
Conclusions: We have shown that the expression of *otx2* and *c-fos* is altered in the peripheral olfactory system in response to the odorant environment experienced during development. Our data are essential to the understanding of the plasticity of the developing nervous system and its ability to respond to chemical changes (both natural and man-made) in the environment. Support: FONDECYT 1071071, CGC/ICM-P06-039F, NIH/NICHD R01HD050820.

1. Whitlock KE. *Brain Res Bull.* 75:340–347, 2008.
2. Harden MV *et al. J Neurobiol*, 66:1452–1466, 2006.
3. Whitlock KE. *Zebrafish*. 3:203–213, 2006.

P-8A-356

Parity affects epigenetic status at birth

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Objective: Clinical data indicates that suboptimal fetal development as reflected in birth size affects later life health

outcomes and there is increasing evidence for epigenetic processes being involved. It has been suggested that "maternal constraint", that is the mechanism by which fetal growth is limited by maternal physiology, is an important factor in determining later life outcomes. Maternal constraint is greater in first born children and there is evidence that first born children are at greater risk of later obesity and other measures of adverse health outcomes[1]. In the present study we investigated the promoter methylation state of candidate genes (which have been implicated in the regulation of growth and/or the postnatal determination of health related outcomes) in umbilical cord tissue collected at birth. Specifically, we tested whether variance in methylation state correlates with variance in birth weight, gestational age and the mother's parity, clinical measures typically associated with sub-optimal fetal growth.

Methods: We measured the methylation state of the upstream promoter regions of NOS3, NR3C1, RXRA, SOD1, PI3KDP and H19 genes in singleton infants with either low birth weight (LBW, <2500g) or considered to have experienced optimal fetal development (OBW) – that is subjects of normal birth weight (3200–3600 g) with no clinical factors likely to impair fetal development. DNA from umbilical cord tissue was analysed using the Sequenom EpiTyper platform to quantify methylation in regions surrounding the proximal promoter regions. A total of 43 subjects were analyzed. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Maximum likelihood statistical methods were used to relate promoter CpG methylation to birth weight, gestational age and the mother's parity. While neither birth weight nor gestational age were related to the methylation state of the selected candidate genes, the methylation state of 24 specific CpG dinucleotides in the H19 gene locus showed strong associations with the mother's parity ($P < 0.003$).

Conclusions: Our data show, although not related to birth weight per se, the epigenetic state of an imprinted gene implicated in the regulation of fetal growth and maternal-fetal nutrient partitioning is strongly influenced by the mother's parity, possibly reflecting the degree of maternal constraint. Although preliminary, our findings suggest that epigenetic mechanisms may contribute to the manifestation of birth rank effects on later health and illustrate that in human infants imprinted genes may be subject to variable methylation due to maternal factors. Support: A*STAR, NHG(NHG/SIG-07072) and NRCGD. We thank Yen Ling Low for statistical analysis.

1. N. Stettler, *et al. Am. J. Clin. Nutr.* 72:378–83, 2000.

P-8A-357

Changes in gene expression and associated chromatin states induced by maternal protein restriction in microswine offspring

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Objective: Fetal growth and development is dependent on the nutrients provided by the mother. Even minor dietary alterations in pregnant animals can produce lasting changes in the offspring's physiology and metabolism. In animals and humans, maternal undernutrition slows fetal growth, reduces birth weight and increases risk of developing hypertension and other chronic diseases later in life. Following perinatal maternal protein restriction (MPR) in microswine, still-normotensive Juvenile low protein (LoP) offspring exhibit increased vascular reactivity to pressors. Hyperactive stress responses are described in normotensive children born small, and also associated with increased risk of later hypertension in unselected adolescents and young adults. We thus believe that microswine model system reproduces key patterns of dysfunction operative in the generation of developmental hypertension in humans.Molecular mechanisms by which the intrauterine environment conveys future risk for disease remain to be defined. We hypothesized that maternal diet induces alterations in epigenetic marks along disease-related genes that persist after birth to maintain changed postnatal expression of these genes in offspring.

Methods: To explore contribution of epigenetic mechanisms in fetal programming, we used a microswine model of maternal protein restriction (MPR) where low protein diet was applied during the last trimester of pregnancy and early lactation (2 weeks after birth). We used microarrays and RT qPCR to examine gene expression levels, and ChIP assay to explore changes in histone modification and RNA polymerase densities along dysregulated genes in kidneys from animals before and after birth. Because of globally reduced total RNA and mRNA per cell in LoP offspring during MPR (see separate abstract), we report results referenced to total RNA and also after adjustment to per cell RNA content.

Results: Microarray and RT qPCR analyses, when referenced to total RNA, revealed increased transcript levels of two pro-hypertensive genes genes in LoP kidneys from near-term and two week old animals: Angiotensin II type 1 receptor (AT1R) and a cytochrome P450 ω-hydroxylase (CYP4a24), a monoxygenase capable of formation of the vasoconstrictor 20-HETE in response to AngII. However, on a per-cell basis, AT1R and CYP4a24 mRNA levels were equal to those in the control animal group, suggesting relative protection of key survival/growth pathways in the face of global transcriptional downregulation. In juvenile kidneys, AT1R levels normalised, whereas Cyp4a24 remained upregulated. Using ChIP assay, we found increased recruitment of RNA polymerase II and elevated transcription-conducive histone modifications along Cyp4a24 in all LoP animal age groups, an observation that implicates transcription as a stage primarily targeted by MPR.

Conclusions: Results of these studies demonstrate that, while maternal nutrient limitation in microswine induces global

reduction of gene expression in kidneys (see separate abstract), some genes escape such downregulation, including Cyp4a24 and AT1R, both implicated in blood pressure control. Associated changes in histone marks along these genes, some of which persist after birth, provide an avenue to examine the role of epigenetic factors in fetal programming. Support: Gates Foundation.

P-8A-358

Abstract to communicate as Oral Presentation (see O-6C-55)

P-8B-359

Newborn weight is decreased after synthetic glucocorticoid treatment in women at risk of preterm birth

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Objectives: Administration of antenatal Betamethasone (BET) to women who are at high risk of giving birth prematurely reduces neonatal mortality. Multiple courses of antenatal corticosteroids, however, may have adverse effects and long-term consequences.
Methods: Pregnancy and birth data collected from 1996–2008 at Charité Campus Virchow hospital were analyzed retrospectively. The effects of maternal BET on neonatal anthropometrics (birth weight, head circumference, overall length) and placenta weight and on cord blood gases, Apgar scores and ponderal index were analysed. Data were also analysed according to neonatal sex. Standard curves and percentiles for each parameter were calculated according to Royston *et al.* (1). BET exposed women who delivered between 23 + 5 and 42 + 0 weeks of gestation (wks) were compared to gestational age-matched controls: control males (n = 22,351), BET males (n = 1,305), control females (n = 20,997), BET females (n = 1,133). Three different dosage regimes were compared: group I (2 × 8 mg BET every 10 days until birth), group II (2 × 8 mg BET once) and group III (2 × 12 mg BET once). Statistical analysis was performed using SPSS® with significance accepted for $p < 0.05$.
Results: BET exposed newborns had a significantly lower birth weight and weight percentiles compared to controls between 35–37 and 38–40wks in males (−219g and −151g, respectively) and between 32–34, 35–37, 37–40 and >40wks in females (−168 g, −230, −224 g and −115 g, respectively). In the different gestational age groups, significant differences were also present for body lengths, head circumference and ponderal index. BET-induced differences in neonatal outcomes were more common in females than in males. One minute Apgar scores were significantly higher in BET-exposed male neonates born between 23–34 wks compared to controls and in BET-exposed females born between 23–25 and 29–31 wks. Five minute Apgar scores were significantly higher in BET exposed males born between 23–25 and 32–34 wks and in BET-exposed females born between 23–25 wks. Ten minute Apgar scores were significantly higher in BET exposed males born between 23–25 and 32–34 wks and in BET-exposed females between 23–31 wks. Placental weight and cord blood gases were independent of BET treatment. There was no difference in BET-induced neonatal effects between the three dosage regimes.
Conclusion: It appears that in this cohort, BET administration to women at risk of preterm delivery significantly decreased neonatal birth weight, body length and head circumference even in women that received as little as only one BET dose. Intriguingly, BET administration was associated with significantly higher Apgar scores in neonates born between 23–34 wks compared to controls, but less so in neonates born later in gestation. Further studies are required to provide a better understanding of the mechanisms underpinning prenatal glucocorticod treatment effects on fetal growth and development.

1. Royston P, Wright EM. *Ultrasound Obstet Gynecol*, 11:30–38, 1998.

P-8B-360

Maternal cortisol is influenced by fetal gender

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Objective: It is known that enhanced cortisol levels have a negative impact on pregnancy outcomes and results in late onset disease in adulthood (eg cardiovascular disease and diabetes), with gender specific effects. However, it is unknown whether fetal gender influences maternal cortisol levels. The present study explored the influence of fetal gender on maternal total cortisol levels in early pregnancy.
Methods: Data were derived from the Amsterdam Born Children and their Development (ABCD) multiethnic cohort-study (The Netherlands). Pregnant women completed a questionnaire and donated blood during their first antenatal

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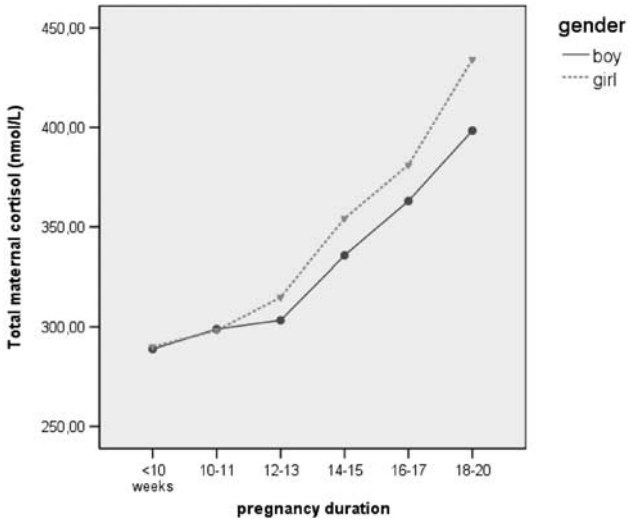
visit to asses total serum cortisol levels (n = 4252). Gestational age at blood sampling varied between 5 and 20 weeks (median 13; IQR 12–15 weeks) gestational age. Women with pre-existent diabetes mellitus, steroid medication, multiple pregnancy and no information on time at blood sampling were excluded; final inclusion of 3049 women. Main outcome measure was total serum cortisol levels (nmol/L). Gender effects were determined by linear regression and adjusted for maternal age (continuous), pre-pregnancy-BMI (continuous), smoking during pregnancy (yes/no), parity (primiparous vs. multiparous) and time at blood sampling. All first order interactions were tested. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Total cortisol levels increased during pregnancy with an average levels of 390 ± 22 nmol/L at 5th week to 589 ± 15 nmol/L at the 20th week of pregnancy, respectively. The presence of a female foetus was associated with higher maternal cortisol levels, however depending on pregnancy duration (see figure). The difference was not significant before 11th week, at 12th week the difference was 15 (SE 7) nmol/L which increased to 45 (22) nmol/L at 20th week (p interaction = 0.05). Maternal cortisol was negatively associated with maternal age, pre-pregnancy-BMI, smoking and parity, the last one also increasing with pregnancy duration.

Conclusion: Maternal total cortisol levels are influenced by foetal gender which becomes significant after first trimester pregnancy. A potential mechanism might be in alterations of placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) activity or in generation of specific sex hormones by the foetal adrenal gland that will be converted subsequently by aromatases expressed in large quantities in the placenta. The consequences of the gender differences on maternal cortisol levels for mother and child needs further research. Support: The Netherlands Organisation for Health Research and Development (ZonMw).



P-8B-361

The maternal cortisol awakening response in human pregnancy is associated with the length of gestation

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Objective: The state of pregnancy produces progressive endocrine alterations including increased cortisol secretion and attenuated responsiveness to challenge. The aim of this study was to examine the association between intra-individual changes in physiological (cortisol) responsiveness over gestation and the length of human gestation.

Methods: Using a prospective, longitudinal design, pregnancy-related changes in the cortisol awakening response (CAR) were assessed as a measure of hypothalamic-pituitary-adrenal (HPA) axis responsiveness. Saliva samples were collected immediately and +30, +45 and +60 min post awakening in 101 pregnant women at 16.8 ± 1.4 and 31.4 ± 1.3 weeks (±SD) gestation. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: As predicted, the CAR was significant in pregnancy (P's < 0.001) and progressively attenuated over the course of gestation (P < 0.01). Analyses using multi-level hierarchical linear models indicate that a larger CAR in late pregnancy and reduced attenuation of the CAR from early to late gestation were significantly associated with shorter gestational length (p < 0.05). The magnitude of this difference in the attenuation of the CAR response was approximately 12% for each week of shortened length of gestation.

Conclusions: The findings are the first to suggest that cortisol responses to awakening as well as the degree of attenuation of the CAR over the course of gestation are associated with the length of human gestation, and may represent markers of underlying biological vulnerability for earlier birth. Support: in part by US PHS (NIH) grants HD-33506 and HD-041696 to PDW.

P-8B-362

Postnatal maternal interaction style moderates the influences of prenatal maternal stress on child behaviour

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Objective: Children of women with emotional complaints during pregnancy, show more difficulties in motor, cognitive,

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and especially socio-emotional development from infancy to adolescence[1]. A potential moderator in this relation might be postnatal maternal interaction style. Animal studies have found evidence for this moderating role of the postnatal rearing style[2]. However only a few human studies have studied this relationship[3,4]. We aimed to provide additional support for the potential moderating role of maternal postnatal interaction style. In earlier studies sex differences in the associations between prenatal maternal stress and child behavioural problems have been found[5], therefore we peformed analyses separetely for boys and girls.

Methods: Healthy pregnant, Dutch Caucasian women (N = 132, *M* age = 30.9 years, *sd* = 3.8) completed questionnaires of anxiety and depression in week 12, 24, and 36. When the children were between 23–60 months of age (*M* age = 39.89 months, *sd* = 9.2) both parents completed the Child Behaviour Questionnaire (CBCL $1\frac{1}{2}$–5) to assess behavioural problems of their children. During a home visit, mothers and their children were asked to perform several tasks together to measure mother-child interaction with the Emotional Availability Scales (EAS)[6]. The four maternal scales of the EAS were standardized and summed to create a total maternal interaction score. Based on the cut off scores of the prenatal measures, the children were divided into a prenatally exposed to maternal emotional complaints group (N = 66) and a non-exposed group (N = 66). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Multiple regression analyses were performed with group (exposed or non-exposed) and maternal interaction as predictor variables, parental current emotional complaints as confounder variables, and CBCL scores as dependent variables. A product term between group and maternal interaction style was entered to assess a potential interaction effect. Separate regression analyses were performed for maternal and paternal reports and for boys and girls. A significant interaction effect between prenatal group status and maternal postnatal interaction style was found on CBCL Total and Internalizing behavioural problems as reported by the fathers in the analyses for girls ($t = -2.24$, $p < .05$; $t = -3.05$, $p < .01$). Prenatally exposed girls show more total and internalizing behavioural problems when their mothers show postnatally a less optimal interaction style. In contrast, prenatally exposed girls show less behavioural problems when their mothers show a more optimal interaction style postnatally. No significant results are found in the analyses for boys.

Conclusion: The postnatal environment can modify the effects caused by prenatal maternal emotional complaints on child behaviour, especially for girls.

1. B.R.H. van den Bergh *et al.*, *Neurosci Biobehav Rev*, 29:237–258, 2005.
2. C. Caldji *et al.*, *Proc Natl Acad Sci*, 95:5335–5340, 1998.
3. L.A. Kaplan *et al.*, *Early Hum Dev*, 84:249–256, 2008.
4. K. Bergman *et al.*, *J Child Psychol Psychiatry*, 49:1089–1098, 2008.
5. T.C.E. de Bruijn *et al.*, *Early Hum Dev,* 85:319–324, 2009.
6. Z. Biringen, *Am J Orthopsychiatry,* 70:104–114, 1998.

P-8B-363

Pre-pregnancy serum hemoglobin and inflammatory status may influence birth weight: prospective study with mothers and children residing in Rio de Janeiro, Brazil

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Objective: To investigate determinant factors of birth weight in a cohort of Brazilian women.

Methods: Prospective study with 195 pairs of women monitored during pregnancy and their off-springs in Rio de Janeiro, Brazil. The dependent variable was birth weight (BW), and the independent were socio-demographic (income, schooling, maternal age) reproductive (parity, age at menarche), anthropometric (pre-pregnancy body mass index, gestational weight gain), and biochemical variables (serum levels of insulin, leptin, C-reative protein, interleukin-6, hematocrit, hemoglobin, glucose, triglycerides, cholesterol and its fractions) measured in the first gestational trimester. Interleukin-6 was measured by ELISA and hemoglobin using an automatic counter. The statistical analysis was performed by means of multivariate linear regression. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The mean (SD) BW was 3,253 g (±525). Results of the multivariate model showed that pre-pregnancy body mass index (BMI) ($\beta = 32.8$; $p = 0.001$), gestational weight gain (GWG) ($\beta = 36.6$; $p = 0.003$), gestational age at birth ($\beta = 82.4$; $p = 0.031$), hemoglobin serum levels ($\beta = 94.4$; $p = 0.015$) and interleukin-6 serum levels ($\beta = 14.3$; $p = 0.026$) remained significantly associated to the birth weight.

Conclusions: The present study corroborates others[1,2] that revealed the effect of well-known variables like GWG, pre-pregnancy BMI and gestational age at birth on the BW. Additionally, the serum levels of interleukin-6 and hemoglobin remained associated with increased values of birth weight. This finding indicates that the iron-nutritional and the inflammatory status in the beginning of pregnancy also play an important role in the definition of birth weight. Support: Coordination Support of Higher Education (CAPES), National Council for Scientific and Technological Development (CNPq).

Variable	β coefficient	95%Confidence Interval	P value*
Body mass index (kg/m^2)	32.8	13.6–52,0	0.001
Gestational weight gain (kg)	36.6	13.1–60.1	0.003
Gestational age at birth (weeks)	82.4	8.0–156.9	0.031
Hemoglobin (mg/dl)	94.4	19.0–169.9	0.015
Interleukin-6 (pg/ml)	14.3	1.8–26.9	0.026

*p-value refers to Wald's test.

1. N.E. Stotland. *Obstet Gynecol.*, 108:635–643, 2006.
2. I.O. Frederick. *Matern Child Health J.*, 12:557–567, 2008.

P-8B-364

Maternal cortisol and offspring birthweight: results from a large prospective cohort study

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Objective: Maternal depression during pregnancy may affect fetal growth through elevated maternal cortisol levels. This hypothesis is however hardly examined and the results are inconsistent. This large prospective cohort study examined 1) the association of maternal cortisol levels with offspring birthweight and small for gestational age (SGA) risk, and 2) the mediating role of maternal cortisol on the relation between maternal depression and fetal growth.
Methods: From January 2003 till March 2004, all pregnant women in Amsterdam were approached during their first prenatal visit (±13 weeks of gestation); 8266 women (response rate 67%) filled out a questionnaire covering sociodemographic data, lifestyle and psychosocial health; 4389 women also provided a blood sample for biomarker analyses. Total cortisol level in serum was determined. To measure depressive symptoms the CES-D scale was administered. For this study, only women were included who delivered a singleton at term, who provided a blood sample ⩽20 weeks of gestation, and who had complete data available on all relevant variables (n = 2810). Maternal cortisol levels were standardized for the time of day and the pregnancy duration at blood collection. Separate analyses were performed for a subsample of women who provided a blood sample ⩽9.00 a.m. (n = 94) to explore the hypothesis that fetal growth is mainly affected by early morning cortisol level. Multiple linear and logistic regression analyses were performed for respectively the outcome variables birthweight and SGA. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Maternal cortisol level was negatively related to birthweight (B = −0.35, $P < 0.001$) and positively to SGA (OR = 1.00, $P = 0.027$), however, these effects disappeared after adjustment for covariates (gestational age at birth, infant gender, ethnicity, maternal age, parity, BMI, and smoking). Among the subsample of early morning cortisol, maternal cortisol levels remained significantly related to birthweight (B = −0.94, $P = 0.025$) and a higher SGA risk (OR = 1.01, $P = 0.032$) after adjustment for covariates. A mediation effect of maternal cortisol on the relation between maternal depressive symptoms and birthweight was not observed, by lack of a significant association between maternal depressive symptoms and maternal cortisol level (B = 0.36, $P = 0.139$).
Conclusions: Offspring of women with high morning maternal cortisol levels in early pregnancy had lower birthweights and a higher SGA risk compared to offspring of women with low morning cortisol levels. The hypothesis that maternal depression during pregnancy affects fetal growth through elevated maternal cortisol levels could however not be supported.

P-8B-365

Maternal antenatal mood and the HPA-axis in adolescence: findings from the ALSPAC cohort at age 15

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Objective: Several human studies document links between maternal antenatal mood and children's behavioral and biological development in infancy and early childhood, but few studies have examined such effects beyond childhood. Adolescence is a period marked by increasing rates of psychopathology, particularly in females, and maturation of the HPA axis in both sexes. In this study we sought to determine the long-term effects of maternal antenatal mood on hypothalamic-pituitary-adrenal (HPA) axis function in adolescence.
Method: Participants were drawn from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort. This prospectively designed study has psychometric and other data on mother and child from pregnancy through to adolescence. HPA-axis function was indexed by salivary cortisol in a subgroup of n = 918 adolescents (416 male, 502 female, mean age 15 years) who provided samples over three school days at waking, 30 minutes, 8 and 12 hours post waking.
Results: Participants demonstrated a marked cortisol awakening response (CAR) followed by declining levels across the day ($F_{(3,\ 3809)} = 1464$, $p < .0001$, n = 918). Females had

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significantly higher CAR ($t = -9.07$, $p < .001$). Raised maternal antenatal symptoms of depression at 18 weeks gestation predicted a blunted CAR in females (ΔCort: $t = 2.355$, $p = .02$) but not in males. This finding remained after multivariate analyses including depression later in pregnancy or postnatally, maternal social class, smoking and education.

Conclusions: We describe a marked CAR in both sexes at this age, greater in females than males. Maternal antenatal depression predicted a blunted CAR in females. Our result suggests a contribution of the *in utero* environment to diurnal cortisol in later adolescence, giving some support to the fetal programming hypothesis. The blunted CAR we describe has been observed to be associated with PTSD, chronic fatigue and other psychopathology. The sex specific nature of this finding is of interest given the higher rates of psychopathology in females at this developmental stage. Future work will determine whether this blunted CAR is associated with specific symptoms in this cohort, and explore other determinants of the cortisol diurnal profile in male and female adolescents.

P-8B-366

Maternal stress and functional brain development in fetal sheep

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Stress during pregnancy seems to predispose for behavioral and cognitive disorders in later life.[1] We have shown in fetal sheep that a single course of betamethasone (BM) at the dose used clinically to induce fetal lung maturation is a profound stimulator of maturation of cortical neuronal activity at the expense of persisting glucocorticoid receptor resistance and sleep state fragmentation.[2] Sleep state fragmentation persists until adulthood after prenatal stress in rats.[3] Hypercortisolism and sleep state fragmentation are major symptoms of neurobehavioral disorders and depression in postnatal life.

Objective: To examine if maternal stress - similar to prenatal BM treatment - affects fetal functional brain development.

Methods: Fourteen pregnant German Longwool Merino ewes underwent repeated isolation stress between 30 and 100 dGA (days gestational age, term 150 days) in a soundproofed, temperated (18°C), bright illuminated room thrice weekly for 3 hours at random times. This procedure resulted in reproducible stress responses without habituation (S. Rupprecht *et al.*, DOHaD meeting, 2009). Three days after chronic instrumentation fetal electrocorticogram (ECoG) was recorded in non-stressed controls ($n = 7$) between 109 and 136 dGA equivalent to 0.7 to 0.9 gestation. Since stressed fetuses were more vulnerable and had difficulties to survive the long recording period fetal ECoG was recorded in a first set of seven stressed fetuses between 109 and 120 dGA and in a second set of seven fetuses between 120 and 136 dGA. Artifact-free one hour ECoG epochs from each day were analyzed using spectral analysis.

Results: Cycling ECoG activity started to develop with maturation of the NREM sleep ECoG at 114 dGA followed by the REM sleep ECoG at 130 dGA (Fig. 1). Thus, maturation of subcortical thalamic pacemaker circuits that are active in NREM sleep precedes maturation of complex cortical neuronal interactions in REM sleep. Maternal stress did not affected maturation of cyclic ECoG activity (Fig. 1).

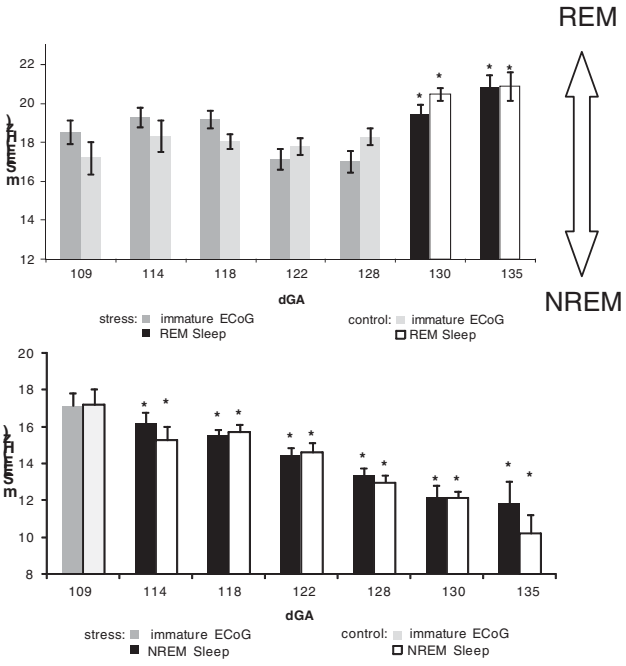

Fig. 1. Intrauterine maturation of cycling ECoG activity as measure of sleep state development in control and stressed fetuses. Mean+SEM, *$p < 0.05$ *vs.* 109 dGA. An increase of the mean spectral edge frequency (mSEF) reflects an increase of high frequency and a decrease of low frequency patterns in the ECoG signal that is typical for the development of the REM sleep ECoG (top). A decrease of the mSEF reflects an increase of low frequency and a decrease of high frequency patterns typically for the development of the NREM sleep (bottom).

Conclusions: Contrary to BM treatment, our maternal stress model applied between 0.2 and 0.67 gestation did not affect functional brain and sleep state development. This might be due to the stress exposure during the first and second trimester compared to the BM treatment at the beginning of the third trimester or the lower GC levels reaching the foetus during maternal stress than after BM administration.

1. A.C. Huizink *et al.*, *Psychol Bull.* 130:115–42, 2004.
2. K. Schwab *et al.*, *Early Human Dev.* 83:S129, 2007.
3. C. Dugovic *et al.*, *J Neurosci*,19:8656–8664, 1999.

P-8B-367

Prenatal stress and risk of behavioural morbidity from age two to 14 years: the influence of the number, type and timing of stressful life events

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Objective: The maternal experience of stressful events during pregnancy has been associated with a number of adverse consequences for behavioural development in offspring. However, the measurement and interpretation of prenatal stress varies among reported studies. Further, little was understood about whether and how the number; type; and timing of stress events might influence subsequent child behavioural development between two- and 14-years of age.
Methods: The Western Australian Pregnancy Cohort (Raine) Study recruited 2900 pregnant women and recorded life stress events experienced at 18 and 34 weeks gestation along with extensive sociodemographic data. The mother's exposure to life stress events were again collected when the children were followed-up in conjunction with behavioural assessments at ages two, five, eight, ten and 14 years using the Child Behaviour Checklist (CBCL). Logistic regression models with generalized estimating equations were used to assess the relationships between the maternal experience of life stress events and child behaviour between age 2- and 14-years. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: The maternal experience of increasing numbers of stressful events during pregnancy was associated with a higher risk of behavioural problems for offspring compared with those who were exposed to none. Events that were defined as acute (e.g. death of a relative, job loss) and more pervasive chronic stress experience (e.g. financial problems, marital problems) were both significantly associated with greater mental health morbidity between age two- and 14-years. Exposure to stressful events in the first 18 weeks of pregnancy showed stronger associations with total and externalizing morbidity than events occurring between 18 and 34 weeks gestation, although both were significantly predictive of behavioural morbidity. These results were independent of other pre- and postnatal influences, including postnatal stress exposure and socioeconomic factors.
Conclusions: The maternal exposure to life stress events during pregnancy has long-lasting consequences for mental health of offspring during childhood and adolescence, independent of later stress exposure. The mothers' experience of multiple stressful events during pregnancy was associated with higher behavioural morbidity for offspring later in childhood and adolescence, compared with no experience of stressful events. Both acute and chronic stressors were associated with increased behavioural problems. Experiencing multiple stressful events during pregnancy was also associated with: i) an increased likelihood for the mother to be further exposed to stressful events after birth, and; ii) indicators of general social disadvantage. Our findings show that the effect of stress events in pregnancy remained a significant predictor of behavioural problems in children and adolescents independent of pre- and postnatal confounding variables, including stressful events which mothers experienced later in life, indicating a role for fetal programming. Improved support for women with chronic stress exposure during pregnancy, particularly women with social disadvantage, is likely to improve the mental health of their offspring in later life.

P-8B-368

Emotional stress during pregnancy and negative temperamental reactivity in infancy and toddlerhood

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Objective: There is good evidence that alterations of neurodevelopment underlying behavioral problems and psychopathology have their origins in prenatal life. We prospectively studied the relationship between maternal anxiety and depression during pregnancy and individual differences in negative reactivity in infancy and toddlerhood.
Method: In a prospective-longitudinal study maternal anxiety was measured at 5–14, 15–27 and 28–40 weeks of pregnancy with the State Trait Anxiety Inventory (STAI), Pregnancy Anxiety Questionnaire (PRAQ-R) and Edinburgh Depression Inventory (EDI), in 155 pregnant women. Infant negative reactivity was measured with standardized temperament questionnaires at two times. Between 4 and 8 months, with the Infant Behavior Questionnaire-Revised (IBQ-R; mean age = 6 months); between 15 and 36 months with the Early Childhood Behaviour Questionnaire (ECBQ; mean age = 32 months). Data were analysed with hierarchical regression analyses (SPSS 17.0). Parity, maternal educational level and postnatal maternal State anxiety, birth weight and sex of the baby were added as covariates if they were significantly correlated with infant reactivity. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: State anxiety in first trimester (STAI; Mean = 35.96; SD = 9.92) was significantly associated with offspring negative reactivity; it explained 8% of the variance at 4–8 months (IBQ-R; $F(2, 102) = 5.95$; $p = .004$) and 6.5% of the variance at 15–36 months (ECBQ; $F(2, 125) = 7.23$; $p = .001$). Of specific pregnancy related anxieties (PRAQ-R) only third trimester fear for changes (e.g. of physical appearance) was significantly associated with infant reactivity at 4–8 months,

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explaining 7% of the variance (IBQ-R; F (2,102) = 7.151; p = .001). Postnatal maternal anxiety was in all analyses significantly associated with infant reactivity but it did not erase the influence of prenatal anxiety. State or trait anxiety in second and third trimester and depressive symptoms were not independently from either covariates or anxiety in first trimester significantly associated with offspring negative reactivity.

Conclusions: Our results provide evidence for an association between maternal anxiety during pregnancy and negative reactivity in infancy and toddlerhood that is independent of key sociodemographic and obstetric factors and concurrent postnatal maternal anxiety. They suggest that negative reactivity may have prenatal environmental origins. Fetal programming of the HPA-axis as been suggested as a possible underlying mechanism. To investigate this mechanism data on infant cortisol reactivity and regulation (gathered during and after inoculation at 4–6 months) will be combined with maternal psychological and physiological (i.e., cortisol awakening response) during pregnancy and preliminary data presented.

P-8B-369

Maternal depressive symptoms, serum folate status and birth weight: Results of the ABCD birth cohort study

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Objective: Low birth weight is considered a reflection of an adverse fetal environment, and is associated with disease in later life. An adverse fetal environment might be the result of maternal stress, including depressive symptoms and nutritional constraints, such as an unfavourable folate status of the mother. Empirical data on the combined potentially detrimental effect of maternal depressive symptoms and serum folate status on birth weight are scarce. In the present study we explored whether different levels of depressive symptoms and differences in serum folate status of pregnant women were associated with differences in birth weight in the offspring. A possible synergistic effect of depressive symptoms and low folate status on birth weight as well as possible fetal gender differences were additionally explored.

Methods: Data were derived from a subsample of women with life born singleton infants (N = 4044) from the Amsterdam Born Children and their Development (ABCD) study, a prospective multiethnic birth cohort study in the Netherlands. Depressive symptoms, measured by the CES-D scale, were assessed with a questionnaire around the 16th (interquartile range [IQR] 14th–18th) week of pregnancy. Three CES-D categories were defined (<16 not depressed (72%), 16–23 possibly depressed (16%), 23 = < probably depressed (12%)). Serum folate status was determined around the 14th (IQR 12th–15th) pregnancy week, standardized for pregnancy duration at the time of blood collection and divided into quintiles. Birth weight, fetal gender and pregnancy duration, were available from Youth Health Care Registration. Potential confounders (maternal age, pre-pregnancy BMI, educational level, parity, ethnicity, smoking, alcohol consumption and hypertensive disorders) were obtained from the pregnancy questionnaire and the Dutch Perinatal Registration Analyses were conducted using SPSS 17.0 (SPSS Inc.). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Univariately the highest category of depressive symptoms was associated with lower birth weight (−157 g in boys, $p < 0.001$ and −81 g in girls, $p < 0.05$). This association decreased after adding gestational age to the model, indicating a mediating effect. After the addition of all potential confounders, the association became insignificant in girls, but remained significant in boys (−65 g, $p = 0.05$). Univariately low serum folate status was associated with lower birth weight (−88 g in boys, $p < 0.05$ and −101 g in girls, $p < 0.01$). The effect of serum folate status fully disappeared after adding gestational age, again indicating a mediating effect. There was no significant interaction between depressive symptoms and serum folate status.

Conclusions: Both depressive symptoms and serum folate status during pregnancy were associated with birth weight, which was mainly due to a shorter gestational period. Furthermore, depressive symptoms more strongly seem to affect male than female fetuses. Support: The Netherlands Organisation for Health Research and Development (ZonMw), Netherlands Heart Foundation and Nutricia Research BV.

P-8B-370

Long term outcome of pregnancies complicated by hyperemesis gravidarum. A systematic review

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Objective: There is evidence that HG is associated with a predominance of female fetuses, lower birth weight and

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shorter gestational age, but studies report conflicting results. As the adverse effects of prematurity and low birth weight on disease risk in later life have become clear, the repercussions of HG might not be limited to pregnancy alone. The aim of this review was to summarize the available evidence on fetal and neonatal outcome of pregnancies complicated by HG and long term health effects of the offspring.

Methods: A literature search was conducted in the electronic databases PubMed and Embase (February 2008). Studies were included that reported on the fetal, neonatal and long term outcome of pregnancies complicated by HG. Furthermore, we tracked references.

Results: The search resulted in 203 studies, of these, twenty-three suitable studies were identified. The quality of reporting of most studies was limited. All studies confirmed the higher female/male ratio in pregnancies complicated by HG (OR female fetus 1.28 [1.22, 1.35]). Results on birth weight and gestational age were too heterogeneous for meta analysis. No studies reported on long term health effects.

Conclusion: HG is associated with a higher female/male ratio of the offspring. No studies reported on reported on long term health effects.

P-8B-371

Stress during lactation induces changes in the liver endocannabinoid system and related lipogenic factors in adult mice

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Several reports have proposed that environmental events occurring during fetal/neonatal periods may have permanent adverse health effects in adulthood[1]. Thus, it has been previously shown that stress during lactation induce metabolic disturbances and overweight in adult mice[2]. Moreover, we found that all these metabolic alterations may be treated with a type 1 cannabinoid/endocannabinoid receptor (CB_1ER) antagonist, suggesting a role for the endocannabinoid system (ECS) in such effects. In fact, it was recently demonstrated that liver CB_1ER play a crucial role in hepatic lipogenesis *de novo* and systemic metabolic perturbations associated to diet-induced obesity[3]. The ECS mainly includes the endocannabinoids anandamide (ADA) and 2-arachydonoylglycerol (2-AG), their receptors (CB_1ER/CB_2ER) and synthesizing or degrading enzymes such as the fatty acid amido hydrolase (FAAH).

Objective: To evaluate the effects of stress during lactation on adult liver FAAH expression and activity, CB_1ER expression and related lipogenic factors such as the sterol regulatory element binding protein-1c (SREBP-1c), fatty acid synthase (FAS) and acetyl coenzyme A carboxilase (ACC1).

Methods: Twelve hours after birth, male CD-1 mice pups were selected and randomly distributed for maternal cross-fostering. During lactation (21 days) mice were stressed or not with a daily subcutaneous injection of saline solution in the back (1 μl/g body weight). Adult animals (130 days old) were subsequently sacrificed and the liver extracted to evaluate mRNA expression of CB_1ER, FAAH, SREBP-1c, FAS and ACC1 by RT-PCR. Protein expression of CB_1ER and FAAH was assessed by Western blot analysis controlled with respective blocking peptides. Additionally, FAAH enzyme activity was determined by measuring liver protein extract ability to hydrolyze ^{3}H-[ADA] to ^{3}H-arachidonic acid and ethanolamine at 37°C.

Results: In our experimental conditions, stress during lactation significantly increases body weight (7%) and epididymal fat (29%) in adult mice. Significant increases in levels of leptin (19.7 ± 2.1 vs 11.1 ± 2.2 ng/ml), corticosterone (35.5 ± 11 vs 8.1 ± 2.95 ng/ml), and triglycerides (TG; 115.8 ± 10.3 vs 89.6 ± 9.9 mg/dl) were also observed in stressed animals in comparison to controls. Messenger RNA expressions of liver CB_1ER, FAAH, SREBP-1c and FAS were similar in both groups of animals; however, ACC1 expression was higher in stressed than control mice. Western blot analysis revealed that CB_1ER protein expression was not different in both groups, while FAAH protein expression was decreased by 40% in stressed animals, a finding consistent with same decreased amount in enzymatic activity.

Conclusions: Stress during lactation decreases protein expression and activity of adult liver FAAH, a fact that may result in sustained availability of ADA able to stimulate CB_1ER. Enhanced ACC1 mRNA expression could be dependent or independent of the CB_1ER/SREBP-1c pathway. In any case, it is suggested to be the first step for liver fatty acid and TG de novo synthesis with consequences in elevated circulating TG levels and availability for accumulation in adipose tissue during adulthood. Support: FONDECYT 1070663.

1. C. Gicquel *et al., Best Pract Res Clin Endocrinol Metab.,* 22:1–16, 2008.
2. A. Loizzo *et al., Pediatr Res.,* 59:111–115, 2006.
3. D. Osei-Hyiaman *et al., J Clin Invest.,* 118:3160–3169, 2008.

P-8B-372

Higher acculturation and higher pregnancy-specific distress are correlated with the incidence of pregnancy complications in latinas

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High levels of stress during the pre-natal period have been documented as having a negative impact on birth outcomes, infant complications and the immune system specifically, on cytokine production.

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Objective: Our study focuses in the psychosocial factors that affect the distress levels and the inflammatory response, and the negative consequences of these interactions upon pregnancy.
Methods: We evaluated social support, acculturation levels, stress and distress in a sample of 30 Latina-pregnant women that were recruited and consented at Denver Health Medical Center in the Woman's Care Clinic in Denver CO, during one of their regular appointments following the procedures approved by the Colorado Multiple Review Board. Subjects were asked to the Denver Maternal Health Assessment (DMHA), the Revised Pregnancy Distress Questionnaire (NUPDQ), Prenatal Social Support Instrument and the Acculturation Survey, once every trimester of the pregnancy. Data regarding pregnancy outcomes were obtained through chart extraction. The study collected blood samples twice: early and late pregnancy periods. Two extra blood samples were collected in addition to the ones required at Denver Health for the participant's regular pre-natal care appointments. First, a non-heparinized tube was used to extract serum and analyze Cytokines levels; specifically IL-6, IL-10 and TNF-α. And a second heparinized tube, for isolation of peripheral blood mononuclear cells.
Results: Data analyses showed a positive correlation between high levels of distress specific of pregnancy early in pregnancy and lower birth weight. Distress specific of pregnancy early in pregnancy was significantly correlated with lower gestational age at birth. Higher acculturation levels were associated with higher social support during early pregnancy. Higher acculturation was also correlated with lower levels of IL-10 during early and late trimester of pregnancy.
Conclusions: These findings support the idea that distress specific of pregnancy during early pregnancy is negatively affecting birth outcomes. Previous studies have shown more negative consequences of stress when occurs early on pregnancy, due to the development process occurring in the uterus. Surprisingly, higher levels of acculturation were associated with higher social support, but lower levels of IL-10 during early and late periods of pregnancy. This suggests that acculturation and stress during the pre-natal period have a strong influence on the cytokine production. Cytokines are involved on the inflammatory process during pregnancy and are related to negative birth outcomes and/or pregnancy complications, such as pre-term delivery. Finally, acculturation seems to have an important role to understand how ethnicity influences stress during pregnancy and we are evaluating how higher acculturation levels can be changing eating habits and diet in Latinas. In this ongoing study, we want to start looking at some measures to evaluate eating habits and iron deficiency.

P-8B-373

Prenatal dexamethasone exposure results in gender specific responses in plasma ACTH, cortisol and β-endorphin to postnatal stress in lambs

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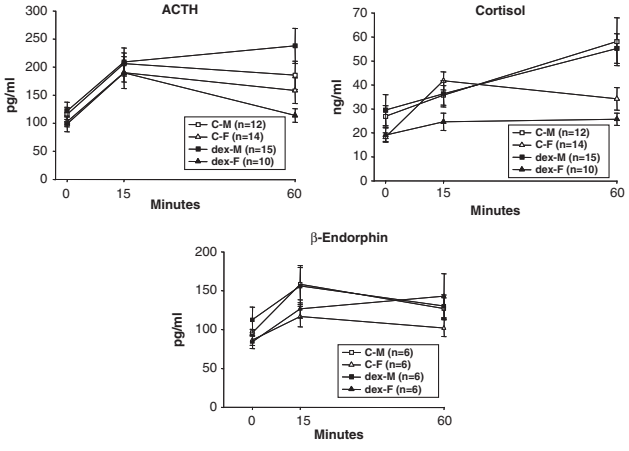
Objective: To evaluate the effects of prenatal dexamethasone exposure on responses to two procedures that are conducted routinely as standard agricultural procedures in young Australian sheep. These procedures are placement of a tight rubber ligature around the base of the tail and, in males, a similar procedure and castration by applying a ring above the testicles.
Method: Pregnant ewes carrying singleton fetuses were randomized to control (2 ml saline/ewe) or dexamethasone (dex) treatment (0.14 mg/kg ewe weight) consisting of four intramuscular injections at 12-hourly intervals over 48 hours on days 40–41 (term 150 days). At 31 days of postnatal age, a rubber ring was placed around the base of the tail of each lamb, and in males, a ring was also placed around the scrotum to cause castration. Blood samples were taken from the jugular vein before commencement of the first procedure (0 minute) and 15 and 60 minutes after completion of the ring placements. ACTH, cortisol and β-endorphin were measured by ^{125}I radioimmunoassay.
Results: ACTH levels at 60 minutes in treatment females (dex-F) were significantly lower than in treatment males (dex-M) ($P = 0.006$). Cortisol levels at 15 minutes in dex-F were significantly lower than in control females (C-F) ($P = 0.005$). At 60 minutes, cortisol levels in dex-F were lower than in control males (C-M) ($P = 0.01$) and treated males (dex-M) ($P = 0.002$). Cortisol levels in C-F were lower than in C-M ($P = 0.029$). β-Endorphin levels at 15 minutes in C-M and dex-M were significantly higher than C-F and dex-F ($P = 0.03$), possible reflecting an additional response to castration. At 60 minutes, β-endorphin levels in C-M, C-F and dex-M were lower, but the level in dex-F was consistently higher than at 0 minutes ($P = 0.02$).



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Conclusions: Exposure of fetal sheep at 40 days gestation to maternal dexamethasone treatment results in a decreased hypothalamic-pituitary-adrenal response after birth in females but not males. 60 minutes after tail docking, in females but not males, prior prenatal dexamethasone exposure was associated with lower levels of cortisol and higher levels of β-endorphin when compared with controls. These finding suggests that the response to dexamethasone in early pregnancy is sexually diergic with suppression in females of development of the hypothalamic-pituitary-adrenal axis, but not of the pathways associated with endorphin production.

P-8B-374

Isolation is a reliable stressor of the pregnant ewe

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In epidemiological studies, stress during pregnancy programs predisposition for diseases in later life such as behavioral and cognitive disorders[1]. To examine the mechanisms it would be useful to have a stress model in pregnant sheep as an important model of fetal physiology. Isolation has been found a major stressor in gregarious animals resulting in immediate and sustained activation of the HPA axis[2].

Objective: To examine if repeated isolation is a reliable stressor to which the pregnant ewe doesn't habituate.

Methods: Eighteen two-year old pregnant German Longwool Merino ewes were isolated between 30 and 100 dGA (days gestational age, term 150 days) in a soundproofed, temperated (18° C), bright illuminated room thrice weekly for 3 hours at random times. Plasma cortisol response profiles were determined at 30, 44, 59, 72, 89 dGA. Blood samples were taken from the external jugular vein 15 min prior to isolation, at 15 min, 60 min and 120 min of isolation and 60 min after isolation.

Results: Repeated isolation results in a profound and sustained cortisol response at each examined isolation bout ($p < 0.001$, Fig. 1). Baseline cortisol level were elevated prior to the first but not prior to the following isolation bouts ($p < 0.05$, Fig. 1) revealing the novelty stress in response to first blood sampling during the protocol. Low baseline cortisol levels prior to the following isolation bouts reflect adaptation to handling. Though the absolute peak cortisol response was higher at the first than at the following isolation bouts ($p < 0.05$, Fig. 1) the cortisol response relative to baseline did not differ from the following isolation bouts (Fig. 1). There was no difference in the peak cortisol response between the following isolation bouts (Fig. 1).

Conclusions: While the pregnant ewes habituate to handling, isolation is a reliable stressor for pregnant sheep to which the ewe doesn't habituate.

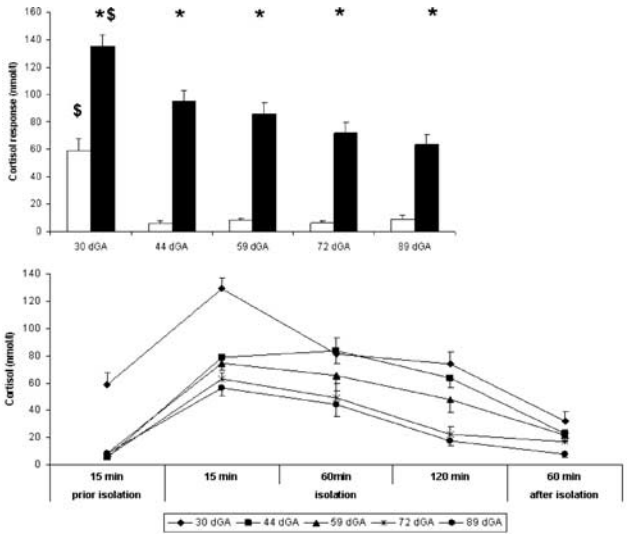

Fig. 1. Baseline and peak serum cortisol (Top) and cortisol response profiles (Bottom) in pregnant sheep exposed to repeated isolation stress for three hours thrice weekly between 30 dGA and 100 dGA. in pregnant sheep exposed to repeated isolation stress for three hours thrice weekly between 30 dGA and 100 dGA. White bars reflect baseline cortisol levels 15 min prior to isolation and black bars peak cortisol response during isolation. Mean ± SEM, $^{\$}p < 0.05$ compared to the older ages, * $p < 0.001$ compared to baseline.

1. B.R. van den Bergh *et al.*, *Neurosci Biobehav Rev.* 29: 237–258, 2005.
2. J.E. Minton, F. Blecha, *J Anim Sci.* 68:3145–3151, 1990.

P-8B-375

Maternal psychosocial stress during pregnancy and the placenta: evidence from a population-based cohort study

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Objective: Maternal stress during pregnancy may have short- and long-term effects on health in the offspring. Animal data suggest that the placenta is involved in this stress-related fetal programming but human evidence is lacking. Our primary objective was to study the association between maternal psychosocial stress during pregnancy and placental growth.

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Methods: In 78017 singleton pregnancies, we studied the associations of maternal stress during pregnancy with the average rate of placental growth (indicated by gestational age- and sex-specific z-scores [ZS] of placental weight; estimates reported in 10^{-3}), and with the absolute placental weight at birth. We conducted multiple regression analyses adjusted for potential confounders. We used two *a priori* defined types of psychosocial stress, life stress in terms of perceived burdens in major areas of life (e.g. partnership, work) and emotional stress in terms of self-reported emotional disturbances (e.g. anxiety, nervousness). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Life stress (per increase in stress score by 1, range: 0–18) during pregnancy was associated with an increased average placental growth rate (ZS; B = 14.33, CI: 10.12–18.54), and increased absolute placental weight at birth (g; B = 1.67, CI: 1.06–2.28). Emotional stress during pregnancy, in contrast to life stress, was not associated with average placental growth rate and absolute placental weight at birth.
Conclusions: Maternal life stress but not emotional stress during pregnancy was associated with increased average placental growth and increased absolute placental weight at birth, but the estimates of effect were rather small in both cases. Our results are the first to show in humans that maternal psychosocial stress may affect the placenta. They may contribute to a better understanding of the role of the placenta in the regulation of intrauterine processes in response to maternal stress. Support source: The Danish National Research Foundation has established the Danish Epidemiology Science Centre that initiated and created the Danish National Birth Cohort. The cohort is furthermore a result of a major grant from this Foundation. Additional support for the Danish National Birth Cohort is obtained from the Pharmacy Foundation, the Egmont Foundation, the March of Dimes Birth Defects Foundation, the Augustinus Foundation, and the Health Foundation. Support: German National Academic Foundation (PhD scholarship to MT), and the Swiss National Science Foundation (SNSF), project no.51A240–104890, (to GM).

P-8B-376

Variations in family composition alter reproduction, stress and behavioural responses in adulthood: effects of litter-overlapping in rats

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Early-life environment experienced by the pups strongly influences their development and several behavioural and neuroendocrine responses during adulthood. Commonly, the rat pups' environment is determined by the mother and littermates in the nest. However, this familiar unity could differ if mating at postpartum oestrus occurs, a common situation for rodents in nature. If older offspring remain in the maternal burrow following the birth of the new litter conceived in postpartum oestrus, temporal overlapping of successive litters will occur. In the laboratory, dams' maternal behaviour toward newborn pups is affected by the presence of older siblings. When rearing overlapping litters (two juveniles from the former litter and the newborns from the second one), mothers licked the newborn pups less than mothers with single litters. Interestingly, both male and female juveniles developed maternal-like behaviours toward their newborn siblings and overrode the deficit in maternal licking behaviour received by newborns of the second litter.
Objective: Determine de effects of being reared in overlapped litters on anxiety-like behaviour, stress response and reproductive functions.
Methods: Adult male and female newborn pups from single litters (SR) and from overlapped litters (OR) were assessed at adulthood (>90 days-old). SR and OR male and females' anxiety-like behaviours were tested in the open field test (1 m^2 arena/5 min) and their plasmatic levels of corticosterone were quantified following a 20-min restrain period. Spermatogenesis (sperm count), ovulation (oocytes in oestrus morning) and sexual behaviour of SR and OR males and females were determined.
Results: Adult OR animals increased the time (s) spent in central quadrants of the open field [♀: SR: 9.9 ± 2.4, OR: 20.3 ± 5.1; ♂: SR: 9.1 ± 1.9, OR: 20.3 ± 4.3, means $\pm$ SE, $p < 0.05$, Student t test]. While the stress response (augmented corticosterone) did not differ between SR and OR males [$F(1,50) = 4.45$, $p = ns$], only SR females exhibited enhanced corticosterone levels following stress [$F(1,50) = 4.97$, $p < 0.05$]. The frequency and latencies of mounts and intromissions as well as the sperm count, the daily sperm production and the transit time through epididymis, did not differ between the SR and OR males. By contrast, OR females showed reduced sexual behaviour if compared to SR ones [lordosis quotient, OR: 0.6 ± 0.1, SR: 0.4 ± 0.1, means $\pm$ SE, $p < 0.05$, Student t test], without changes in the number of oocytes.
Conclusions: The early-life experience in overlapping litters reduced the anxiety-like behaviour of males and females, and diminished the endocrine stress response and reproductive behaviour of female offspring during adulthood. These long-term sex dimorphic effects could be attributed to a more complex precocious rearing environment than the one provided in single litters, which probably results in an altered quality/quantity of stimulation from mothers and/or siblings. The present model of overlapping-litters in rats could be a useful tool to study how variations in family composition affect the development of individuals. Support: ANII, Proyecto FCE2007 302, SNI.

P-8B-377

Long term effect of stress in pregnancy on HPA function at awakening in adolescence

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Stressful life events reported by the mother in pregnancy appear associated with compromised physical and mental health of the newborn child across the life course. Examples are an increased risk for metabolic syndrome and depression, both complex diseases possibly related to adverse development and life long atypical activity of the stress-sensitive hypothalamic-pituitary-adrenal (HPA) axis. We hypothesize that early life stress has long-term effects on the development and maturation of the HPA axis during childhood and adolescence.

Objective: We aimed to investigate the relationship between maternal stress experienced during pregnancy and its effect on non-stimulated morning adolescent HPA-activity as an outcome measure of HPA development during the perinatal and childhood period of life.

Methods: This study was part of the 17-year follow up of the Western Australian Pregnancy (Raine) Cohort Study, a longitudinal study on child health and development. Fasting morning blood (n = 1200) and saliva upon awakening on 3 consecutive days (n = 1077) was collected under non-stressful circumstances at home for analysis of ACTH, total circulating cortisol and free salivary cortisol at mid-adolescence. Previously, maternal stress was reported at 18 and 34 weeks of gestation by confirmation of any of 10 selected items of the life stress inventory by Tennant and Andrews[1]. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Using General Linear Models with adjustment for potential confounders including maternal age, education and smoking, family income and gender, gestational age, birth weight and APGAR scores of the newborn, we found that increasing levels of stress experienced up to 18 weeks of gestation was associated with lower levels of awakening free cortisol in 17-year-old adolescents ($\beta = -.027$, 95%CI $-.050$ to $-.005$, $p = 0.017$). However, stressful life events experienced between 18–34 weeks of gestation were not related to a reduction in free cortisol at 17 years of age ($\beta = -.015$, 95%CI $-.040$ to .011, $p = 0.252$). At no time during pregnancy were stressful life events associated with 17-year total cortisol, ACTH, the percentage of bound cortisol in the circulation or a total cortisol/ ACTH ratio as an index of the earlier HPA cascade of neuroendocrine events.

Conclusion: Maternal stressful life events during the first half of pregnancy appear to be associated years later with morning day-to-day HPA activity in the adolescent child. Specifically, the number of stressful life event exposures earlier in pregnancy was inversely related to adolescent awakening free cortisol. Our data support the view that early life stress may have a fetal programming effect on HPA development. Yet, to our knowledge, no study so far has reported a negative association between the number and timing of stressful life events in pregnancy and teenage HPA regulation under resting conditions. A reduced ability to produce sufficient biologically active cortisol at the start of the day may increase vulnerability to stress-related disease development.

1. Tennant C *et al* . *Aust NZ J Psychiatry,* 10:27–32, 1976.

P-8B-377B

Diurnal cortisol rhythms and birth weight in the Cebu Study

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Objective: Maternal stress and stress physiology are important influences on birth outcomes. Intrauterine exposure to stress hormones can also program metabolic and physiologic function in offspring. Here we describe maternal diurnal cortisol rhythms and explore relationships with offspring birth weight recorded in reproductive histories collected among female birth cohort members enrolled in the Cebu Study (age 20.5–22.5 years), a large longitudinal birth cohort that has followed several thousand young men and women since their mothers were pregnant with them in 1983–4.

Methods: Salivary cortisol, reflecting the unbound, bioavailable fraction of the hormone, was assayed in three samples designed to characterize the key parameters of diurnal change: one collected just prior to bed, the second collected immediately after waking the following morning, and a third collected 30 minutes after waking as an index of the cortisol awakening response. Birth weights were obtained retrospectively from birth records and, when not available, from maternal recall. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: We find evidence for relationships between multiple cortisol measures and offspring birth weight, with relationships varying by sex of offspring. There was a strong inverse relationship ($p < 0.01$) between offspring birth weight and maternal bedtime cortisol levels ($p < 0.01$). There was a significant interaction between maternal cortisol and offspring sex as a predictor of birth weight, with the effect stronger in males (interaction $p < 0.05$). Thus, birth weights are lower among women with higher bedtime cortisol, and

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the normal male excess in birth weight is also reduced, leading to a reduction in sexual dimorphism in offspring birth weight. Adjustment for recalled gestational timing did not modify these relationships significantly, suggesting an impact on fetal growth rate.

Conclusions: Our analyses highlight the importance of stress physiology as a predictor of birth weight in a population characterized by low mean birth weight, and suggest important sex differences in these effects.

P-8C-378

Exercise imposed in early life is associated with altered bone tissue responses to later training

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Objective: Exercise beginning soon after birth might affect the size of joints, and larger joints may sustain lower mechanical stress and possibly a reduced likelihood of onset or progression of chondro-osseous diseases in later life. High volumetric bone mineral density (BMD_v) has been associated with osteoarthritis, the single biggest musculoskeletal disease in both humans and horses. We hypothesized that the size and BMD_v of the distal third metacarpal epiphysis of horses exercised early in life would be different than in those which had not.

Methods: 32 Thoroughbred foals allowed either spontaneous exercise (PASTEX group) or also subjected to 1080m of exercise (CONDEX) 5 days/week from 10 days to 18 months of age were scanned 5 times (peripheral quantitative computed tomography) during this period. Bone area (BA), bone mineral content (BMC), and BMD_v were not significantly different between groups at any scan time or over time (pooled data). Nineteen of the horses were trained for two year old and three year old racing, separated by several months pasture rest from training, and were scanned before two year old training (Pre2), after two year old training ceased (Post2), after several months at pasture and just before three year old training began (Pre3), and immediately after three year old training ended (Post3). The mean workload and mean group bodyweights of the two groups during training were not significantly different in either racing campaign. For each epiphyseal bone parameter, mean group values for each scan time and as pooled values over time were compared (significance level $p < 0.05$). The study was approved by two institutional animal ethics committees.

Results: The conditioning exercise resulted in no negative effects. Bone parameters were not different for CONDEX and PASTEX groups at any of the 4 scan times except trabecular BMD_v at Post2, which was ~7.5% greater in the PASTEX group. Group values pooled over time showed highly significant differences between the two groups in BA ($p = 0.003$–0.016) and BMD_v ($p = 0.000$–0.023), but not BMC ($p = 0.053$–0.655); BA was higher in CONDEX than in the PASTEX group, and BMC less in the CONDEX group until Post3. The BA changed hardly at all over the 4 scans in PASTEX, but increased in the CONDEX group. At Post3, the site sustaining highest loads had different bone density distributions, the smaller PASTEX joint having a significantly higher proportion of very dense voxels.

Conclusions: The group exposed to early conditioning exercise increased Mc3 joint size when later exposed to athletic challenge, whereas the PASTEX group did not. BMD_v of the epiphysis of the PASTEX group was higher, and the distribution of density values was different in the two groups, which might have implications in the initiation of joint disease. There was no significant difference between the two groups in any bone parameter on completion of conditioning exercise, but the response to race training was significantly different. We conclude that early exercise alters the sensitivity of mechanosensing and/or metabolic responsiveness of joint tissues.

P-8C-379

Maternal high fat diet – deleterious effects on offspring bone structure

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Objective: Epidemiological studies suggest skeletal growth is programmed during intrauterine and early postnatal life. We hypothesize that development of optimal peak in bone mass has, in part, a fetal origin and investigated this using a mouse model of maternal dietary fat excess.

Methods: Offspring from mouse dams fed either standard chow (C) or lifetime high fat diet (HF) were maintained on a HF diet to adulthood. Three offspring groups were studied: maternal control diet/offspring control diet (C/C), maternal control diet/offspring high fat diet (C/HF), maternal high fat diet/offspring high fat diet (HF/HF), n = 4–6 for each sex/diet group. Femur samples were taken at 30 weeks of age and bone structure, adiposity and strength analysed.

Results: Our study allowed us to examine whether any predictive adaptive responses (PAR) occur for the skeleton. For male offspring, feeding a high fat diet (C/HF compared to C/C diet) increased mass and reduced femur length

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($p = 0.004$ and 0.04). However, with a prior maternal high fat diet (HF/HF compared to C/HF diet) there were no additional alterations in bone structure. Hence, any PAR cue from the mother does not appear to be utilized in male offspring. In contrast, whilst feeding a high fat diet in female offspring (C/HF group compared to C/C group) increased mass ($p = 0.002$), increased bone volume ($p = 0.03$), increased maximum midshaft load ($p = 0.06$), and increased midshaft cross-sectional area ($p = 0.02$), with the addition of a maternal high fat diet (HF/HF compared to C/HF diet) the response was to reduce mass (but not to the level of controls, $p = 0.002$)), reduce femur length ($p = 0.008$), reduce bone volume (to control levels, $p = 0.06$)), and lower trabecular thickness ($p = 0.03$). These data are consistent with the operation of a PAR in terms of bone structure in the female offspring. This sexual dimorphism may reflect the differing life-course strategies in developmental response to a challenge; males may prioritise body growth to promote reproductive success, while females prioritise body composition in terms of fat deposition and skeletal structure in order to sustain pregnancy.

Conclusions: These studies demonstrate effects of high fat maternal diet during pregnancy, with or without a high fat diet in offspring post weaning, on the bone quality and quantity of those offspring. The skeleton is subject to the processes of developmental plasticity, responding to environmental cues within a non-pathological range at critical points in the life course, as do other body components. Whilst such adaptive responses may theoretically confer adaptive advantage, for example in terms of reproductive fitness, they may confer greater risk of chronic disease such as osteoporosis in human populations exposed to increasingly rich diets and with greater longevity. These studies indicate the importance of early life interventions to promote the health of subsequent generations.

P-8C-380

Alteration of fetal bone structure by a maternal low protein diet

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Objective: Epidemiological studies suggest skeletal growth is programmed during intrauterine and early postnatal life. We hypothesize that the age-related decrease in bone mass has, in part, a fetal origin and have investigated this using a microswine model of maternal protein insufficiency.

Methods: The right femur and L1-L3 vertebrae from near-term (gestational age 113 of 115) piglets were removed from 7 offspring of mothers fed a control diet (14% protein) and 7 offspring of mothers fed a low (1%) protein diet during pregnancy. The femur and L2 vertebra were analysed to measure differences in bone structure, density and strength.

Results: The proximal femur of offspring from mothers fed a low protein diet were found to have increased bone density and increased porosity ($p = 0.04$) compared to controls. Although there was no difference in trabecular structure in the proximal femur, when strength tested the low protein group trabeculae failed at a lower load ($p = 0.02$), required less energy to break ($p = 0.04$), and was less stiff than controls ($p = 0.07$). In addition, the cartilage from the femoral head in the restricted group was less stiff than controls i.e. the cartilage would compress more with the same load than controls ($p = 0.003$). No differences were found in bone density or structure at the midshaft of the femur. The distal femur showed a higher prevalence of weaker rod-like trabeculae ($p = 0.02$), but these were more connected than controls ($p = 0.01$). However, the distal femur showed no differences in strength parameters or bone density between the two diet groups. No differences were found in the bone density of the vertebra, however, vertebra from fetal offspring from low protein fed mothers showed increased porosity ($p = 0.04$), but a stronger more-plate like trabecular structure ($p = 0.05$). Load testing of the vertebral body showed the restricted group failed at a lower load ($p = 0.02$).

Conclusions: Fetal offspring from mothers fed a restricted protein diet during pregnancy displayed significant differences in bone structure, density and strength in the femur and vertebra and altered cartilage properties in the femoral head. These differences result in altered bone characteristics indicative of significantly altered bone turnover producing weaker bone structures in critical area such as the proximal femur and the vertebral body. These results from a microswine model further support the need to understand the key role of the nutritional environment in early development on programming of skeletal development and consequences in later life.

P-8C-381

A lifecourse study of bone resorption in men at age 49–51 years: The Newcastle Thousand Families Study

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It has been suggested that bone health in adulthood is programmed by development in utero. Most previous investigations addressing this topic have focussed on bone mineral density or bone mineral content, rather than other indicators of bone health, such as biochemical markers of bone turnover.

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Objective: The objective of this study was to investigate whether potential predictors, from different stages of life, including early growth and socio-economic disadvantage, influence bone resorption in men in the Newcastle Thousand Families birth cohort.
Methods: The Newcastle Thousand Families Study is a prospective study initiated in 1947 when all 1142 children born to mothers resident in the city of Newcastle upon Tyne in northern England were recruited. Detailed information on many aspects of their lives was collected prospectively during childhood, including early growth, illnesses and socio-economic conditions. At age 49–51, 574 study members returned detailed self-completion questionnaires and 412 attended for clinical examination, including 172 men in whom bone resorption was assessed by measurement of serum β C-telopeptide of type 1 collagen (CTX). This was analysed, using multiple linear regression, in relation to a range of variables at different stages of life, including birth weight (standardised for gestational age and sex), duration breast fed, position in family (calculated from the number of older surviving siblings, including half-siblings, at the time of the individual's birth), cigarette smoking history, alcohol consumption, percent body fat, physical activity levels, dietary intake of certain nutrients previously found to be significant predictors of either bone mineral density or bone area in a previous analysis of this cohort[1], and socio-economic status both at birth and in adulthood. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: A significant trend was seen between increasingly disadvantaged socio-economic status at birth and increased bone resorption ($p = 0.04$, $r^2 = 2.6\%$). However, birth weight, standardised for sex and gestational age, was not associated with serum CTX ($p = 0.77$, $r^2 = 0.05\%$). Significant trends were also seen between increasing dietary intake of saturated fat ($p = 0.02$, $r^2 = 2.6\%$), protein ($p = 0.04$, $r^2 = 2.5\%$) and sodium ($p = 0.04$, $r^2 = 2.4\%$) and higher serum CTX. However, in adjusted analyses, only dietary intake of saturated fat showed a significant trend with serum CTX ($p = 0.03$).
Conclusion: Our findings suggest that early socio-economic disadvantage and later dietary factors may be associated with increased bone resorption in middle aged men. However, as little of the variance in serum CTX was explained by the variables included within this investigation, further longitudinal studies are required to assess the lifecourse predictors of bone resorption in adulthood and their relative impacts.

1. M.S. Pearce *et al.*, *J Epidemiol Comm Health*, 59:475–480, 2005.

P-8C-382

Calcium supplementation from adolescence increases adult bone mineral density in female rats born small, but not in males or in offspring of normal birth weight

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Objective: It is established that low birth weight and poor childhood growth program a variety of adult diseases. The developmental programming of bone disorders, such as osteoporosis, has only recently been investigated. We have previously reported that offspring born small, as a result of uteroplacental insufficiency, have shorter femurs, lower bone mineral content and a bone strength deficit as adults[1]. The aim of this study was to determine the effects of calcium supplementation from adolescence on growth restricted offspring which have a programmed bone deficit.
Methods: Bilateral uterine vessel ligation (Restricted) or sham surgery (Control), was performed on gestational day 18 in WKY rats to induce uteroplacental insufficiency and growth restriction. Pups remained with their mothers until weaning at postnatal day 35. At 2 months pups were allocated to either a normal (0.46%) or high (2.4%) calcium diet, on which they were maintained until 6 months of age ($n = 10$ male and female pups per group from 10 litters). After post-mortem at 6 months, femur length was measured and both dual energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT; for true volumetric trabecular and cortical mineral content, density, dimensions, and stress strain index) were performed on the right femur. Analysis of biochemical markers of bone turnover at 4 months is ongoing.
Results: Male and female Restricted offspring were born 14% lighter compared to Controls ($p < 0.05$). By 6 months, female, but not male, Restricted offspring remained smaller compared to Controls. Femur length was reduced in both sexes at 6 months ($p < 0.05$). In males, no differences were observed between Control and Restricted groups for either trabecular or cortical content and density regardless of diet. Trabecular content in females was not different between Control and Restricted groups. After body weight correction, Restricted females consuming a high calcium diet had an increased trabecular density by 10.5% when compared to the normal calcium diet group ($p < 0.05$). This trend was also observed for cortical density whereby Restricted females consuming the high calcium diet had a 7% greater cortical density compared to Restricted females consuming a normal calcium diet. No changes in cortical thickness were observed, however absolute periosteal and endosteal circumferences were lower in Restricted male and female offspring compared to Controls with no effect of diet. Importantly, the stress strain index of bone bending strength was lower in male and female Restricted offspring, regardless of diet by up to 10.9% and 9.3%, respectively. DXA results were similar to pQCT results.
Conclusions: Being born small, due to uteroplacental insufficiency, programs reduced adult femur length, dimensions and stress strain index. Supplementation with a high

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calcium diet from adolescence can increase adult trabecular and cortical bone density in females, not males, who were born small. This increase in bone density was not sufficient to rescue the bone dimension and strength deficits which were programmed *in utero*, suggesting that the early life environment is critical for bone programming. Support: The Gardiner Foundation.

1. T. Romano *et al.*, *Bone*, 45: 132–141, 2009.

P-8C-383

Caries reduction in rural school-children exposed to fluorides through a Milk-Fluoridation Program in Araucania, Chile

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Objectives: The aim of this study was to assess the effectiveness of a dental caries prevention program for the permanent dentition of Chilean rural schoolchildren using fluoridated powdered milk and milk derivatives.
Methods: The fluoridated products were delivered to 35,000 schoolchildren in the rural areas of the Ninth Chilean Region using the standard School Feeding Program (PAE), which has been operating for the last 39 years. The daily fluoride dose from milk fluoridated products was estimated at 0.65 mg/day. Cross-sectional samples of schoolchildren aged 6, 9 and 12 years from study communities and from positive control communities (ongoing APF-gel program) were examined at the start of the study in 1999 and after 36 months (follow-up).
Result:. No significant difference was found for the DMFT and dmft indices in the 6-year-old group in the study and positive control groups either at baseline or 36 months later. Significant reductions (range 24–27%) were observed in the DMFT index in the 9 and 12-year-old groups in the study communities when data were compared from baseline and after 36 months of receiving fluoridated milk products. At the follow-up examination the DMFT indices of schoolchildren aged 9 and 12 year old receiving fluoridated milk were not significantly different than those of the positive control group.
Conclusions: Taking into account the relative costs and technical difficulties involved in both caries preventive programs, it appears that under Chilean rural conditions fluoridation of powdered milk and milk derivatives is an effective caries prevention alternative for areas where either water fluoridation or other community delivered programs are difficult to apply. Support: The Borrow Foundation, UK.

P-8C-384

Improvements in birth weight over time in one remote Australian Aboriginal community

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Objective: To describe birth weights (BW) over time in one remote Australian Aboriginal community. Historically, BWs have been low in remote Australian Aboriginals. Evidence in the Aboriginal Australian setting and in other populations demonstrates that lower BWs are associated with chronic disease morbidity and mortality[1].
Methods: We analysed readily available BWs of people born between1955–1994 (n = 1325) from one discrete remote community. BWs were taken from community clinics and hospital registers. Of these, a sub-sample born more recently have recorded GA (n = 334). Low birth weight (LBW) was considered <2.5 kg and pre-term birth <37 weeks gestation. All data were analysed in birth decade specific groups. Appropriate institutional ethics committee clearance and patients consent were obtained.
Results: Aggregate data (Table) show a significant increase in mean BW and a significant decrease in the prevalence of LBW per birth decade. Of the 334 with recorded GA, 23.1% were LBW and 14.1% were preterm birth: more than half (52%) of the LBW babies were not preterm, indicating their LBW was due to intrauterine growth restriction (IUGR).

		All birth weights (n = 1,325)			
Decade of birth	n	BW (kg)	95%CI	%LBW	95%CI
1955–1964	288	2.65	2.58–2.70	39.6	34.1–45.3
1965–1974	355	2.69	2.63–2.74	33.2	28.5–38.3
1975–1984	394	2.83	2.76–2.88	24.1	20.1–28.6
1985–1994	288	2.94	2.88–3.00	20.1	15.9–25.1
p		0.0001		0.0001	

Conclusions: Birth weight has increased and the prevalence of LBW has fallen over time. Much of the LBW is associated with IUGR, as has been demonstrated in remote-living NT Aborigines more broadly[2].The increase in BW probably results, in part, from better maternal health status of young women and better care during pregnancy. Although BW is increasing with time, mean BW remains lower than the national average (3.38 kg) while the prevalence of LBW remains higher than the national prevalence (6.1%)[3]. Indeed, it is yet to be determined what an ideal BW may be for this population. Regardless, priority should be given to optimise maternal health and the intrauterine environment. Our findings are likely to be generalizeable to other developing populations experiencing improvements in maternal and perinatal care. In this population with traditionally low BWs. Infant mortality has fallen dramatically due to improved care of sick newborns and infants from within this community. Thus, LBW infants are now surviving to adult life,

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potentially, at high risk for chronic disease development. Ironically, the high rates of observed chronic disease morbidity and mortality now seen in the Australian Aboriginal setting may be associated with previous improvements in maternal and perinatal care. It is yet to be determined what an ideal birth weight may be for this population. However, in this community and Aboriginal Australians generally, mean birth weight remains lower than that of the national average (3.38 kg) while the prevalence of LBW too remains higher than the national prevalence (6.1%). It was not the focus of this study to identify the effects of either intra uterine growth retardation (IUGR) and/or pre term delivery on subsequent birth weight, however, previous studies have given differing reports on the predominant cause of LBW.

1. M. McCormick. *New Engl J Med.,* 312:82–90, 1985.
2. S. Sayers, J. Powers. *Aust N Z J Obstet Gynaecol.*, 37:131–132, 1997.
3. Australia's Health 2008. The eleventh biennial report of the Australian Institute of Health and Welfare. AIHW Cat no Aus 99, Canberra 2008.

P-9A-385

Maternal copper deficiency perpetuates altered vascular function across two generations of Sprague-Dawley rat offspring

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Objective: Several studies provide evidence of impaired macronutrient availability on future disease and highlight the tissue-specificity and timing of the nutritional insult on adult phenotype, though less is known about the long-term effects of micronutrient deficiencies. Further, little is known about the consequences of maternal copper (Cu) deficiency on the vascular function of offspring or on perpetuation of vascular effects to a second generation. We examined vascular functional responses in mesenteric arteries from copper deficient Sprague-Dawley rat dams and from offspring directly exposed to maternal copper deficiency during development and lactation and perpetuation of the effects in a second generation of offspring.
Methods: Dams were fed an AIN-93 G diet formulated with $CuSO_4 \cdot 5H_20$ to contain either marginal (1 mg Cu/kg) or adequate (6 mg Cu/kg) Cu 3 weeks prior to conception and throughout pregnancy and lactation periods. Half of the first generation (F1) litters were cross-fostered. F1 pairs were bred within groups resulting in second generation (F2) offspring. Offspring were fed a diet adequate in copper after weaning. Hepatic Cu concentrations were analyzed by atomic absorption spectroscopy in dams and F1 male and female pups at weaning and repeated at 9 weeks in F1 offspring. Plasma ceruloplasmin activity was determined in dams and offspring at time of hepatic Cu measurement. Mesenteric artery (200 μm) isometric tension was determined in response to vasoconstrictors and vasorelaxants using a small wire myograph at weaning in dams and at nine weeks of age in offspring. Approval obtained from the Animal Care and Use Committee of the GFHNRC in accordance with National Research Council Guidelines.
Results: At weaning, dams fed the Cu deficient diet had significantly lower liver Cu concentrations and ceruloplasmin activities compared to dams consuming Cu adequate diet. Hepatic Cu concentrations were significantly lower on in F1 offspring postnatal day 21, the effect limited to those born to dams on the Cu deficient diet. At 9 weeks of age, no differences in F1 Cu status were evident. Cu deficiency did not alter vascular function in dams. In F1 offspring, increased responsiveness to potassium chloride in male offspring was due to direct exposure to maternal copper deficiency in the birth mother, while enhanced endothelial dependent and independent relaxation responses in female offspring resulted from postnatal and combined in-utero and postnatal exposure to maternal copper deficiency, respectively. Altered relaxation responses were perpetuated to a second generation of male offspring, consistent with the maternal F1 phenotype.
Conclusions: Low hepatic Cu concentrations in offspring of dams fed Cu-deficient diet were established prenatally and were not readily reversed by allowing the offspring to suckle dams that were fed Cu-adequate diet. As F1 dams and sires were copper replete at the time of conception and F2 offspring were not subjected to dietary intervention, alterations in F2 vascular function represent the influence of the persistence of F1 exposure to prenatal and/or postnatal maternal Cu deficiency. These data indicate that exposure to maternal Cu deficiency during critical windows of development alter vascular function which are propagated to a second generation of offspring.

P-9A-386

Paraoxonase activity and oxidative stress status in patients with active pulmonary tuberculosis; risk factor for the developing of cardiovascular disease

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Objective: Tuberculosis is a major cause of death around the world despite the fact that the causative organisms discovered more than hundred years ago and the highly effective drugs available making tuberculosis a preventable and curable disease. There are some findings that can be accepted as clues for the possible involvement of *Mycobacterium tuberculosis* in

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atherosclerosis eventually causing cardiovascular disease. Therefore, the aim of our present study was to investigate effect of tuberculosis infection on paraoxonase-1 (PON1) activity and oxidative status in patients with pulmonary tuberculosis (PTB).

Methods: A total of 77 (40 newly diagnosed active PTB and 37 healthy control) subjects were recruited for this study with the mean age of 37.31 ± 1.72 years. Anthropometric variables, total peroxide and PON1 activities were determined in control and PTB subjects. Anthropometric variable was measured by standard method. Total peroxide was determined by FOX2 reagent and paraoxonase enzymatic activity was measured by UV double beam spectrophotometer using phenyl acetate and paraoxonase as a substrate. Serum lipid profile, glucose, urea, total protein, albumin, globulin and uric acid levels were also determined in the participants by using fully automated biochemistry analyzer.

Results: Significant difference in BMI, SBP and DBP was observed between PTB and control subjects ($p < 0.001$, $p < 0.01$ and $p < 0.01$ respectively). Total protein, albumin, TC and HDL were significantly lower in PTB subjects ($p < 0.05$, $p < 0.001$, $p < 0.01$ and $p < 0.01$ respectively). TG and LDL levels were also decreased though not statistically significant. However, the level of uric acid and globulin were significantly increased ($p < 0.01$, $p < 0.001$ respectively). Serum PON1 activities against arylesterase and paraoxon were significantly lowers in PTB patients than control subjects giving p value 0.01 and 0.001, respectively. The value of total peroxide level was significantly higher ($p < 0.001$) in PTB patients while compare with control and total peroxide level was significantly correlated with PON1 activity against paraoxon substrate ($r = -0.390$, $p < 0.001$).

Conclusions: Patients with active PTB are exposed to potent oxidative stress and they have decreased PON1 activity. These predisposal factors may play a role in the pathogenesis of atherosclerosis in PTB.

P-9A-387

Long-term statin treatment in hypercholesterolemic pregnant mice reduces cardiovascular and metabolic risk in their offspring also fed a high fat diet post-weaning

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Objective: We have shown that long-term maternal high-fat (HF) feeding during pregnancy and lactation predisposes offspring to hypertension, raised plasma lipids and fatty liver in mice[1]. Recently, we have also demonstrated that pharmacological intervention with statin in late pregnancy in HF fed dams reduces cardiovascular (CV) and metabolic risk factors in their offspring[2]. In this study, we examined the effects of long-term statin administration to HF-fed female mice on these risk factors when their offspring were also fed a HF diet.

Methods: Pregnant C57 mice on HF diet (45% kcal fat) were given the 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor pravastatin in their drinking water (5 mg/kg of body weight per day) from the time they were weaned until weaning of their offspring. Weaned offspring were then fed the HF diet until adulthood generating dam/offspring dietary groups HF/HF and HF + S/HF. These groups were compared with offspring from dams fed standard chow (C) which were fed chow diet post-weaning to adulthood (C/C). All data were expressed as mean ± SEM. One way ANOVA followed by post-hoc test was used. Significance was assumed if the P value was <0.05. Animal procedures were in accordance with the UK Animals (Scientific Procedures) Act 1986.

Results: HF+S dams showed significantly reduced total cholesterol concentrations and systolic blood pressure vs. HF dams ($P < 0.001$). The HF+S/HF offspring were significantly lighter, with lower systolic blood pressure and serum cholesterol concentrations vs. HF/HF ($P < 0.001$). HF/HF offspring also had elevated C-reactive protein (CRP) levels and these were reduced in the HF+S/HF animals to levels found in the C/C group ($P < 0.001$).

Conclusions: Long-term pravastatin administration to dams not only protects them from the deleterious effects of a HF diet but also protects their offspring from CV and metabolic risk factors in later life, even if these offspring consume a HF diet. Support: BUPA, Wessex Medical Research/ HOPE charity & BHF (UK).

1. M.M. Elahi *et al.*, *Br J Nutr.*, 10:1–6, 2009.
2. M.M. Elahi *et al.*, *Hypertension*, 51:939–944, 2008.

P-9A-388

Effect of vitamin D deficiency on cardiac function and susceptibility to ischemia/reperfusion injury in the adult rat heart

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We have recently demonstrated that maternal vitamin D deficiency in rats leads to increases in left ventricular wall volume, cardiomyocyte number and size, and in the proportion of immature mononucleated cardiomyocytes in the hearts of 4 week old rat offspring. Thus, exposure to vitamin D deficiency *in utero* and early life appears to lead to delayed maturation and subsequent enhanced growth (proliferation and hypertrophy) of cardiomyocytes in the left ventricle. The implications of these changes on cardiac function later in life are unknown.

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Objective: The aim of the present study was to investigate the effect of vitamin D deficiency in adult rats on cardiac function and the susceptibility to ischemia/reperfusion injury.
Methods: Four week old Sprague-Dawley female rats were fed either a vitamin D deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. At weaning the offspring remained on their respective diets until adulthood. Hearts of 16 week old male and female offspring (n = 8/group) were mounted on a Langendorff apparatus. Basal heart rate (HR), coronary flow, rate of contraction (+dp/dt) and relaxation (−dp/dt) and response to isoprenaline were recorded. The hearts were then subjected to 20 minutes of ischemia and $1\frac{1}{2}$ hours of reperfusion. At the end of the reperfusion period the left ventricle was sliced and incubated in 1% 2, 3, 5 triphenly tetrazolium chloride solution (TTZ), to determine infarct area using computerized planimetry.
Results: Left ventricle (LV) weight of vitamin D deficient females was increased (p = 0.02), but unaltered in the males. Basal cardiac function (HR, +dp/dt, −dp/dt) was not different in control and vitamin D deficient male and female offspring. However, basal coronary flow tended to be lower in hearts of vitamin D deficient rats and this was significant in males (p = 0.01). Isoprenaline increased HR, +dp/dt and −dp/dt in all animals. The isoprenaline-induced, increase in HR tended to be greater in vitamin D deficient males (p = 0.06), but there was no differences in contractile function between groups. After 55 minutes of reperfusion, HR had declined by 30% of that before ischemia, in both males and females, with HR being higher in vitamin D deficient males compared with control males; there were no differences between the female groups. After ischemia basal coronary flow was halved and now not different between control and vitamin D deficient groups. Basal cardiac function +dp/dt declined significantly, but was not different between groups, although the rate of relaxation (−dp/dt) was significantly slower in the vitamin D deficient males (p = 0.04), but not females. Strikingly, infarct area was 2-fold greater in vitamin D deficient hearts of both males and females (p = 0.006 and p = 0.03, respectively) compared with their control counterparts.
Conclusion: Basal and stimulated heart function does not appear to be affected by vitamin D deficiency although coronary flow is reduced in males. Importantly the hearts of vitamin D deficient rats are particularly susceptible to ischemia/reperfusion injury. Dysregulation of coronary flow and the extent of vascularisation may be factors which contribute to the vulnerability of ischemia/reperfusion injury.

P-9A-389

Effects of infant growth on endothelial function by ultrasound in very low birth weight infants as young adults

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Objectives: Young adults born prematurely with a very low birth weight (VLBW, <1500 g) have, in comparison to their term born peers, a higher blood pressure and higher insulin resistance. In contrast to individuals born at term their infant growth data are accessible during the time corresponding to the last trimester and time windows of vulnerability may be investigated. In a previous report, a lower gain of weight during two first weeks of life of preterm infants was beneficial for endothelial function. We aimed at estimating flow mediated dilatation (FMD) in adulthood and its associations with early infant growth in subjects born with VLBW and in a term born comparison group.
Methods: After excluding those with medication affecting endothelial function and those who were pregnant we report FMD in 92 VLBW and 66 age- and sex-matched term-born subjects. Birth weight means (and SDs) were 1140 (210) and 3662 (470) grams; and gestational ages were 29.5 (2.4) and 40.2 (1.1). Because rapid early growth during the first months of age may reflect either catch-up growth after difficulties causing intrauterine growth retardation, or relatively healthy extrauterine environmental conditions directly promoting growth, we assessed growth separately for the 56 VLBW subjects appropriate for gestational age (AGA, SD > −2.0 SD) and those 36 VLBW subjects who were born small for gestational age (SGA, < −2.0 SD). Appropriate institutional ethics committee clearance and participants' informed consent were obtained. At the age of 18 to 27 years the subjects underwent FMD measurement with ultrasound. It is a method measuring the endothelial function as a maximal percent dilatation of the lumen diameter in the right brachial artery during upper-extremity reperfusion after a 5-minute antebrachial cuff occlusion. Low FMD is associated with early atherosclerosis. We analyzed the effects of growth separately in the groups with multiple regression models predicting FMD, with adjustment for confounders (Table).
Results: Among the VLBW young adults, baseline diameter of the brachial artery was 3.28 mm (SD, 0.56) in contrast to 3.49 mm (0.64) among the term born (P = 0.003). FMD was 6.9% (3.9) in VLBW and 5.8% (3.3) in term subjects (P = 0.06). Each 10 g/d of lower weight gain velocity during the 2 first weeks of life was associated with 1.5 percent units lower FMD (0.3 to 2.8) among the VLBW subjects, but among the term born with no change in FMD in adulthood (P for interaction 0.03). In contrast, lower height growth soon after term was associated with higher FMD in adulthood (Table).
Conclusions: Although VLBW birth is associated with cardiovascular disease risk factors, it was not associated with a lower FMD. A lower FMD in adulthood was associated

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with a slower weight growth during two weeks after birth and, in within a VLBW SGA subset, with faster length growth after term. The results support the idea of vulnerable time periods during the third trimester.

Table. Association of change in FMD in percent units and growth from 44 to 48 post menstrual weeks.*

	p†	Term	VLBW	VLBWSGA	VLBWAGA
Length cm/month	.68	.7 (−.4 to 1.9)	−.7 (−1.7 to .2)	−2.4 (−4.2 to −.5)	−.2 (−1.4 to .9)
Weight 10 g/d	.10	.0 (−.2 to .2)	−.1 (−.2 to .1)	−.1 (−.3 to .2)	−.1 (−.2 to .0)
Head cm/month	.36	−1.7 (−3.5 to .1)	−.6 (−1.9 to .7)	−.07 (−2.21 to 2.34)	−1.58 (−3.31 to .15)

*Reported associations tell, e.g., that 1 cm/month faster length growth associates with 0.7 percent units higher FMD. Results are adjusted for age and sex. Similar results are obtained with additional adjustment for current height and BMI, smoking, parents' cardiovascular disease.
†P-values are for difference of growth effect between term and VLBW subjects.

P-9A-390

Local cardiac angiotensin system in the developmental origins of cardiovascular disorders: a promising prophylactic use of angiotensin II receptor blocker

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Objective: Increasing evidence demonstrated that undernutrition *in utero* is a risk factor of adult cardiovascular disorders (CVD). We developed a mice model of fetal undernutrition, showing augmentation of cardiac remodelling in adult offspring, enlargement and fibrosis, risk factors of CVD[1]. We investigated whether low dose treatment of candesartan, a angiotensin II receptor blocker (ARB), protects the development of cardiac remodelling of undernourished mice offspring, in comparison with hydralazine, a non-specific vasodilator.

Methods: Candesartan (50 mg/kg/day), hydralazine (10 mg/kg/day) or vehicle were continuously infused by subcutaneous mini-osmotic pump to undernourished (UN) offspring (30% maternal caloric restriction in later half of pregnancy) and normally nourished (NN) offspring form 9 wks until 17 wks, followed by systolic blood pressure (SBP) measurement and collection of heart tissues. Then, immunohistochemistry of angiotensin II, angiotensinogen mRNA expression, cardiac enlargement (CE; cardiac weight/body weight; mg/g), coronary perivascular fibrosis (CPVF; digital image analysis after Sirius Red Staining), were assessed in the heart tissues. Plasma angiotensin II levels were measured in UN and NN offspring at 8 wks.

Results: The immunostaining of angiotensin II and angiotensinogen mRNA expression were most intensive and significantly highest, respectively, in the cardiac tissue from vehicle-UN offspring, suggesting the up-regulation of local cardiac angiotensin system. By contrast the plasma angiotensin II levels in UN offspring was similar to those in NN offspring (43.3 vs. 43.4 pg/ml, $n = 14$), suggesting rather low level changes in systemic angiotensin system. Significant augmentation was observed in CE (5.08 vs. 4.30 mg/g, $n = 13$, $P < 0.01$), CPVF (27.5 vs. 25.1, $n = 13$, $P < 0.01$), and SBP (100.6 vs. 87.0 mmHg, $n = 13$, $P < 0.01$) in the cardiac tissues from vehicle-UN offspring, as compared with vehicle-NN offspring. Candesaltan treatment significantly ameliorated all of CE (4.13 vs. 5.08 mg/g, $n = 12$, $P < 0.01$), CPVF (24.6 vs. 27.5%, $n = 12$, $P < 0.01$), and SBP (91.7 vs. 100.6 mmHg, $n = 13$, $P < 0.01$) in the cardiac tissues from candesaltan-UN offspring, as compared with vehicle-UN offspring. By contrast, hydralazine did not improved CE (4.76 vs. 5.08 mg/g) in the cardiac tissues from candesaltan-UN offspring, as compared with vehicle-UN offspring, although it significantly ameliorated CPVF (24.5 vs. 27.5%, $n = 12$, $P < 0.01$) and SBP (77.6 vs. 100.6 mmHg, $n = 13$, $P < 0.01$).

Conclusions: Local cardiac angiotensin system is associated with developmental origins of cardiac remodelling, especially cardiac enlargement, suggesting a promising prophylactic use of ARBs against developmental origins of cardiovascular disorders.

1. Kawamura *et al.*, *Endocrinology* 148:1218, 2007.

P-9A-391

Effect of Boldo (*Peumus boldus* MOL.) infusion on the antioxidant capacity, hypercholesterolemia and genetic damage level in patients of a Family Health Center of Chillán

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Peumus boldus, Molina (Boldo) a native shrub to Chile has long been part of the folk medicine tradition of the chilean people. It is used as a carminative, cholagogue, choleretic, antidiarrheic, diuretic, eupeptic, digestive and hepatic. The chemical characterization of boldo leaves infusion has revealed the existence of the alkaloid boldine and the flavonoid catechin as the principal plant metabolites with a significant antioxidant capacity (AC)[1]. This is interesting to be analyzed because of the rol of free radicals in the formation of atherosclerotic lesions that are the cause of the cardiovascular disease[2]. Hypertension and dyslipidemia determine its seriousness[3]. On the other hand the preventive effect of flavonoid on cancer are attributed to a variety of mechanisms[4].

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Objective: Evaluate the effect of the total Boldo leaves infusion on the antioxidant capacity, hypercholesterolemia and the level of genetic damage in patients of a Family Health Center located in Chillán.
Methods: Experimental cross section study on a sample of 81 hypertensive patients showing dyslipidemia divides into 3 groups: i) negative control (n = 30), ii) treated with placebo (n = 27), iii) treated with total Boldo leaves infusion at 1,5% once a day during 4 weeks (n = 24). The antioxidant capacity (AC) was evaluated in plasma by the malondialdehide or MDA technique, at 532 nm and the genetic damage by the comet assay (Tail moment, TM) on cell obtained from a blood sample. The lipid profile was evaluated at the beginning and the end of the intervention. The data were expressed as average and standard deviation at $p < 0.05$ and statistically analyzed by means of Kruskall-Wallis, *t*-Student and the test of Scheffé for multiple comparisons. According to the Universidad del Bío-Bío ethics regulations a signed informed consent was obtained from each participant before the study started.
Results: The AC values from infusion treated group showed a significant statistical increase compared to control group in the age rank from 50 to 59 years. The values for TM (comet assay) showed statistical differences for the age rank from 50 to 59 years. The individuals from infusion treated group showed a significant decrease in the triglycerides levels for the age rank from 40 to 49 years ($p < 0.05$). These results are discussed in terms of the intervening variables of the study. Support: grant No.055109 3/R from the University of Bío-Bío.

	LIPOPEROXIDATION nmol MDA/ml plasma					TAIL MOMENT (μM)			
Years	Control (-) (n = 29)	Placebo (n = 27)	Infusion (n = 22)	*Chi*^{2}	*P*	Control (-) (n = 30)	Placebo (n = 27)	Infusion (n = 24)	*P*
40–49	0.039 ± 0.02	0.012 ± 0.003	0.019 ± 0.01	5.347	0.069	0,27 ± 0,02	0,66 ± 0,76	0,67 ± 0,59	*<0,001***
50–59	0.059 ± 0.05	0.023 ± 0.04	0.025 ± 0.01	7.202	0.027*	0,26 ± 0,21†	0,32 ± 0,25	1,48 ± 1,59†	*0,039**
60–69	0.042 ± 0.03	0.026 ± 0.02	0.029 ± 0.03	4.835	0.089	0,37 ± 0,39	0,37 ± 0,39	0,31 ± 0,19	0,982

*Kruskal-Wallis Test **Kruskal-Wallis † T-Student: *p = 0, 0230.*

	Control (-) (n = 30)			Placebo (n = 27)			Infusion (n = 24)		
YEARS	Before	Leter	*P*	Before	Leter	*P*	Before	Leter	*P*
40–49	306 ± 330	176 ± 101	0.593	200 ± 126	183 ± 53	0.715	183 ± 66	137 ± 62	*0.016**
50–59	189 ± 74	167 ± 65	0.448	425 ± 647	226 ± 202	0.063	169 ± 54	150 ± 43	0.163
60–69	222 ± 124	210 ± 96	0.856	173 ± 57	143 ± 49	0.148	208 ± 149	181 ± 117	0.282

*Kruskal-Wallis Test.

1. Lagos P., Zamorano-Ponce E., *et al. Phytother. Res.* 23: 1024–1027, 2009.
2. Zamora J., *Rev Chil Nutr* 34:17–26, 2007.
3. Velayos R., Suárez C. *Hipertensión* 18:418–428, 2001.
4. Muñoz A, Ramos F. *Revista Horizonte Médico* 7:23–31, 2007.

P-9A-392

Maternal obesity does not lead to exaggerated development of atherosclerosis in the offspring of apolipoprotein E deficient mice

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Objective: We recently reported that maternal obesity in C57BL/6J mice leads to the development of a metabolic syndrome-like phenotype in the offspring[1]. As metabolic syndrome is associated with increased risk of atherosclerosis we hypothesised that offspring of obese dams may demonstrate acceleration of atherosclerosis. To address this hypothesis we assessed the influence of maternal diet-induced obesity on development of atherosclerosis in atherosclerosis prone apolipoprotein E deficient (apoE$^{-/-}$) mice.
Methods: Female apoE$^{-/-}$ mice (C57BL/6J background) were fed either standard chow (3% fat) or a highly palatable, hypercalorific diet (14% fat, 37% sugar) for eleven weeks and throughout pregnancy and lactation. Male offspring were weaned onto standard chow and fed a Western diet (~0.2% cholesterol, 21% fat, 34% sugar) from six weeks of age. Atherosclerosis was assessed at six months of age. Quantification of atherosclerosis was performed by measurement of plaque area in sections of the brachiocephalic artery and aortic root stained for elastin (Verhoeff's Van Gieson) and by measurement of percentage of plaque: total area of the descending aorta (stained with Oil Red O). Quantification of atherosclerosis in the brachiocephalic artery was also performed by magnetic resonance imaging (MRI) using a gadolinium-based elastin-binding contrast agent. Assessment of plaque stability was carried out in sections of aortic root and brachiocephalic artery stained for collagen (picrosirius red), macrophages (Mac-3) and smooth muscle (alpha smooth muscle actin).
Results: Maternal serum total cholesterol concentration was similar between control and obese dams. Male and female offspring of obese dams (OffOb apoE$^{-/-}$) were heavier than offspring of control dams (OffCon apoE$^{-/-}$) over the time period studied ($p < 0.001$; RM ANOVA) with male offspring demonstrating hyperphagia ($p < 0.001$; RM ANOVA). At six months of age, serum leptin was significantly increased in OffOb apoE$^{-/-}$ *Versus* OffCon apoE$^{-/-}$, but no significant differences were observed in any other serum lipid analyte measured. No significant differences in plaque size in the aortic root or brachiocephalic artery or in the percentage of plaque area in the descending aorta of male offspring of apoE$^{-/-}$ mice were observed. By MRI, no significant differences in contrast-to-

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noise ratio (CNR) of plaque in the brachiocephalic artery were found between OffCon apoE$^{-/-}$ and OffOb apoE$^{-/-}$ (CNR [mean ± SEM] OffCon apoE$^{-/-}$: 41.38 ± 2.83, n = 6 vs. OffOb apoE$^{-/-}$: 37.91 ± 2.92, n = 6, p = NS; unpaired t-test). There was also no impact of maternal obesity on measures of plaque stability.

Conclusions: Exposure to maternal obesity during pregnancy and suckling does not influence the development of advanced atherosclerosis in the offspring of apoE$^{-/-}$ mice. It is possible there may be an effect on earlier lesions, however, maternal hypercholesterolemia remained unchanged against the already very high background apoE$^{-/-}$ control levels, and no effect on offspring inflammatory mediators or indeed lipid profiles were detected. Support: The British Heart Foundation (FS/05/045) and EARNEST, EU.

1. Samuelsson *et al.*, *Hypertension*, 51:383–392, 2008.

P-9A-393

Gender specific expression of markers of inflammation and atherosclerosis in blood vessels of aging maternal food restricted offspring

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Objective: We have previously demonstrated that maternal food restriction induces marked vascular remodeling in the newborn offspring which predisposes them to develop hypertension in adult life. The goal of this study was to characterize the impact of aging on expression of vascular markers of inflammation, oxidative stress and atherosclerosis in maternal food restricted offspring. We therefore determined the expression of C-reactive protein (CRP, marker of inflammation), nitrotyrosine (NT, marker of reactive nitrogen species and indicator of in vivo peroxynitrite levels), and Lectin-like oxidized low-density lipoprotein receptor (LOX-1, early marker of atherosclerosis) in aortas and mesenteric arterioles of 12 month old maternal food restricted (MFR) offspring by immunohistochemical method.

Methods: Pregnant Sprague-Dawley rats were fed a standard diet (control) or were 50% food restrictied (MFR) from day 10 of gestation to term. After birth, all offspring were nursed by control dams and weaned to ad libitum diet. Male and female offspring were sacrificed at 12 months of age. Aortas and mesentery from 5–6 animals from each dietary group derived from different litters were dissected, fixed in 4% paraformaldehyde and subjected to immunhistochemical analysis of CRP, NT and LOX-1. The integrated optical density (IOD), a measure of staining intensity was determined by Image Pro Plus software in a blinded fashion and used for comparisons by t-test.

Results: There was cell specific distribution of CRP, NT and LOX-1. In aorta, CRP was predominantly expressed in the adventitia and periaortic adipose tissue with less staining in the endothelium. Conversely, NT showed predominant expression in endothelial cell layer with less staining in the adventitia and periaortic adipose tissue, and LOX-1 was almost exclusively expressed in the endothelial cell layer. In mesenteric arterioles both CRP and NT were predominantly expressed in the endothelial cell layer. Overall, there was a highly significant increase ($P < .001$) in the expression of aorta CRP in MFR males (2.6 ± 0.2 vs. 0.9 ± 0.1) and MFR females (1.8 ± 0.1 vs. 0.8 ± 0.1) as compared to controls. Similarly, CRP in MFR mesenteric arterioles was significantly increased in MFR males 2.7 ± 0.3 vs. 1.0 ± 0.1) and females (2.1 ± 0.2 vs. 1.1 ± 0.2) as compared to respective controls. NT expression was also markedly ($P < .001$) up-regulated in aortas of MFR offspring (males: 5.3 ± 0.4 vs. 1.9 ± 0.2; females: 8.7 ± 0.4 vs. 4.6 ± 0.5). There were no statistically significant differences in NT expression in mesenteric arterioles in either gender between MFR and controls. Aortic LOX-1 expression was significantly ($P < .001$) increased in the MFR females (10.0 ± 0.7 vs. 6.3 ± 0.5) but not in males (5.8 ± 0.6 vs. 4.5 ± 0.5).

Conclusions: MFR induced offspring vascular inflammation and oxidative stress in both genders although the magnitude of increases in CRP and NT were more pronounced in the male offspring. This is consistent with marked elevated blood pressure and obesity seen in MFR males.

P-9A-394

Maternal nutrient restriction down regulates cardiac mitochondrial proliferation in male non-human primates at 0.5 gestation (G)

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Objective: Nutrition and other environmental stimuli influence developmental pathways, inducing permanent changes in metabolism and chronic disease susceptibility. How early environmental stress induces persistent molecular changes is not well understood. Cell specific ablation of mitochondrially-relevant genes in experimental models has been linked to both cardiac dysfunction and diabetes. More importantly, abnormal mitochondrial function is associated with obesity, diabetes and hypertension in man.

Method: We performed whole transcriptome analysis (TA) of RNA from male non-human primate (NHP) fetuses of *ad libitum* fed controls and dams 70% of *ad libitum* (MNR;

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adjusted to body weight) from 0.16G to tissue collection at 0.5G. Samples were taken from the free wall of the cardiac left ventricle.

Results: TA revealed 1786 significantly differentially expressed genes, with 968 up-regulated and 818 down-regulated by MNR. Of these, 41 genes play roles in mitochondrial respiratory chain/metabolism (10 up, 31 down-regulated), including cytochrome b5 type B (CYB5B; 126% down) and cardiolipin synthase 1 (CRLS1; 18% down), and 30 genes involved in mitochondrial genetics and protein transport (1 up, 29 down-regulated), including mitochondrial transcription factor A (TFAM; down 28%), mitochondrial translation initiation factor 2 (MTIF2; down 22%) and 16 genes encoding ribosomal proteins (down 11–30%).

Conclusions: A large number of mitochondrial transcripts are down regulated by MNR at 0.5 G in male cardiac left ventricle, including many components of complex I and complex V, mitochondrial ribosomes and mitochondrial protein import. Importantly, the expression of CRLS1, which acts as a co-factor for many mitochondrial enzymes including the adenine nucleotide translocator of complex IV (cytochrome oxidase), is also decreased. Finally, expression of the mitochondrial proliferation regulator, TFAM, is also decreased. These findings suggest that MNR from 0.16 to 0.5 G suppresses mitochondrial proliferation leading to decreased mitochondrial mass in cardiac left ventricle of the male NHP fetus.

P-9A-395

Maternal betamethasone has significant adverse outcomes for heart and coronary function in growth restricted offspring in a sheep model

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Objectives: Betamethasone (BM) is routinely administered to women at risk of pre-term delivery to increase fetal lung maturation and maximize survival of the newborn. Glucocorticoids have effects on a range of functions, including on the cardiovascular system. Pre-term neonates may be growth restricted and cardiovascular function may already be compromised. Our aim was to investigate the effects of maternal BM exposure on function in the isolated heart and coronary artery of the fetus/neonate in an ovine growth restriction model.

Methods: Ewes, 16, each carrying twins, were anesthetized with isoflurane. For one twin, 1 of the 2 umbilical arteries was ligated (SUAL). The umbilical artery of the other twin was handled but not ligated. For each twin, catheters were placed in the femoral artery and the amniotic cavity. On days 5 and 6 after surgery, 11.4 mg BM was administered *im* to the ewe. On day 7 the ewe was anesthetized and the fetal hearts removed into ice-cold Ca^{2+}-free saline. The hearts were quickly mounted on a Langendorff apparatus and perfused with HEPES-buffered, Ca^{2+}-containing solution at 37°C and 25 mmHg pressure. A saline-filled balloon was placed in each ventricles of the heart. Fluid flow was stopped for 20 min and then resumed for 1hr. Slices of left ventricle were treated to visualize infracted tissue. An additional cohort of offspring was allowed to be born and small coronary artery reactivity was tested in a wire myograph. Endothelial production of nitric oxide (NO) was blocked using L-NAME and prostanoid production was blocked with indomethacin.

Results: Fetal blood pressures were not affected by SUAL but were increased by 10 mmHg following BM treatment. Coronary flow rate, and rates of ventricular contraction and relaxation were increased in hearts from SUAL + BM fetuses. Infarct area was increased in SUAL hearts, with no effect of BM treatment (Table). Response of the hearts to challenge by β-adrenoceptor activation were also enhanced by SUAL.

	Control	SUAL	Control + BM	SUAL + BM
Coronary flow ml/min/g	1.08 ± 0.07	1.10 ± 0.04	1.11 ± 0.05	1.85 ± 0.07*
Contraction rate mmHg/s	235 ± 42	293 ± 24	277 ± 47	423 ± 56*
Relaxation rate mmHg/s	−244 ± 67	−297 ± 32	−266 ± 53	−484 ± 44*
Infarct area % total	2.2 ± 0.9	8.7 ± 0.4*	3.5 ± 0.9	8.3 ± 0.6*

In coronary arteries from SUAL neonates, responses to the vasoconstrictor U46619 were doubled ($p = 0.002$), total endothelium-dependent vasorelaxation was reduced (EC_{50} shifted right by an order of magnitude ($p = 0.004$), NO-induced relaxation was halved, endothelium-derived hyperpolarizing factor (EDHF) was reduced by 40% ($p = 0.006$), a dilator prostanoid, absent in control arteries, was present following SUAL, and there was significant arterial stiffening ($p = 0.003$).

Conclusions: A striking observation was that, independent of BM, intra-uterine growth restriction leads to exaggerated susceptibility of the heart to adrenoceptor stress and to temporary cessation of blood flow. The coronary vascular endothelium also released a vasodilator prostanoid, not present in control vessels. It is tempting to suggest that this may be compensatory for the marked reduction in the bioavailability of NO and EDHF following SUAL. Maternal BM treatment leads to a marked increase in coronary flow. Whether this facilitated the enhanced ventricular performance observed, or whether increased contractility stimulated the increase in perfusion awaits determination.

P-9A-396

Late gestation uteroplacental insufficiency and the lactational environment influence vascular function and arterial stiffness in adult growth restricted male offspring

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Objective: Intrauterine growth restriction occurs in 10% of pregnancies and a common cause is a poorly functioning placenta. This has been associated with increased risk of cardiovascular disease in the offspring in adulthood. The extent of morbidity may be further accentuated by poor nutrition in early life. Previously we have found that uteroplacental insufficiency is associated with impaired maternal mammary development, which further influences postnatal growth and leads to raised blood pressure in male rat offspring. The aim of this study was to investigate the roles of prenatal and postnatal nutritional environments on endothelial and smooth muscle reactivity and passive wall stiffness of resistance arteries from adult male offspring.

Methods: Fetal growth restriction arising from uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted, R) or sham surgery (Control, C) on day 18 of pregnancy in WKY rats. On postnatal day 1 offspring were cross-fostered onto Control or Restricted mothers. Control and Restricted male offspring were studied at 6 months of age. Rings of small mesenteric and femoral arteries were mounted on a wire myograph for testing of smooth muscle and endothelial function. Arteries were bathed in warmed, oxygenated physiological saline (PSS). Other segments of artery were mounted on a pressure myograph and bathed in PSS containing 0mM Ca^{2+} and 1mM EGTA for the assessment of passive mechanical wall properties.

Results: Mesenteric arteries from Restricted rats suckled on Restricted mothers (R-on-R) had a suppressed response to the α_1-adrenoceptor agonist phenylephrine ($P < 0.05$), and relaxation evoked by the nitric oxide donor, sodium nitroprusside (SNP), and endothelium-derived hyperpolarizing factor (EDHF) were impaired. Mesenteric arteries of R-on-R rats had increased wall stiffness ($P < 0.05$). In femoral arteries of R-on-R rats relaxation to SNP was impaired, while arterial wall stiffness and endothelium-dependent relaxation were unaltered. Deficits in vascular reactivity and wall stiffness in mesenteric and femoral arteries were alleviated in Restricted offspring that were cross-fostered onto control mothers (R-on-C). In contrast, Control offspring cross-fostered onto Restricted mothers (C-on-R) had markedly increased stiffening of the vascular wall in both mesenteric and femoral arteries.

Conclusions: This study demonstrates that late gestation uteroplacental insufficiency is associated with regional differences in endothelial and smooth muscle dysfunction and arterial wall stiffness in adult male offspring. The lactational environment further impacts on vascular function in both normally grown and growth restricted offspring. Improvement of the lactational environment alleviates vascular dysfunction in growth restricted offspring, while a poor lactational environment can lead to vascular dysfunction in normally grown offspring. Thus, the prenatal and early postnatal and lactational environments independently influence vascular function and wall stiffness and are determinants of cardiovascular function in adulthood.

P-9A-397

The relationship between cortisol concentrations in late pregnancy and systemic vascular resistance in childhood

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Objective: Prenatal exposure to glucocorticoids may "programme" a range of tissue-specific effects in the foetus, independently if the exposure is to exogenous glucocorticoids, to active steroids of maternal origin or to the foetus adrenal products. The consequences for animals and humans are consistent with a predomination of cardiometabolic and central nervous system effects. To assess the relationship between cortisol concentrations in the last trimester of pregnancy and systemic vascular resistance - SVR in childhood.

Table 1. Characteristics of the mothers.

Variables	N	%	Mean (SD)
Age (years)			31.2 (6.3)
<19	22	16.9	
19–25	54	41.5	
26–35	46	35.4	
⩾35	8	6.2	
Education (years)			6.1 (1.9)
⩽4	29	20.8	
5–8	54	41.5	
⩾8	47	37.7	
Smoking habit			5.5 (5.4)
Yes	13	10.0	
No	117	90.0	
BMI (weight/height^2)			27.9 (4.0)
20–25	42	32.3	
25–30	57	43.9	
30–35	26	20.0	
⩾35	5	3.8	
Cortisol in saliva (nmol/L)			14.2 (8.4)
⩽9.10	33	25.4	
9.11–12.42	33	25.4	
12.43–17.11	33	25.4	
⩾17.12	31	23.8	

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Table 2. Characteristics of the children.

Variables	N	%	Mean (SD)
Gender			
Male	61	46.9	
Female	69	53.1	
Age (months)			72.8 (4.6)
64–69	33	25.4	
70–72	40	30.8	
73–75	31	23.8	
76–90	26	20.0	
Birth weight (g)			3239.9 (480)
<2500	5	3.85	
2500–3000	36	27.69	
3000–3500	53	40.77	
⩾3500	36	27.69	
BMI (kg/m^2)*			15.7 (1.9)
Low weight	12	9.23	
Normal weight	96	73.85	
Risk overweight	14	10.77	
Overweight	8	6.15	
SVR (dyne/sec/cm^{-5})			2238.7 (684.4)
911–1715	33	25.4	
1716–2103	32	24.6	
2104–2642	33	25.4	
2643–4535	32	24.6	

*http://www.cdc.gov/growthcharts (low weight = <5th percentile; normal weight = 5–85th percentile; risk of overweight = 85–95th percentile; overweight = ⩾95th percentile).

Methods: This study is part of a cohort involving 130 Brazilian pregnant women and their children, ages 5 to 8 years. Maternal cortisol was determined in saliva by an enzyme immunoassay utilizing the mean concentration of 9 samples of saliva (3 in each different day), collected at the same time, early in the morning. SVR was assessed by the HDI/PulseWave CR-2000 Cardiovascular Profiling System®. The association between maternal cortisol and SVR in childhood was calculated by multivariate linear regression analysis.

Results: The characteristics of the pregnant women and respective children are presented in Tables 1 and 2. There was a statistically significant association between maternal cortisol concentrations in saliva and SVR in childhood ($p = 0.016$; $r^2 = 0.04$). The association persisted after controlling for body mass index, birth weight, age and gender of the children ($p = 0.048$; $r^2 = 0.10$).

Conclusion: Despite the low r^2 of the association between maternal cortisol and the SVR in childhood, in biological terms it is probably important, considering the number of factors that could intervene in the association throughout the prenatal life to childhood. As far as we know this is the first study in the literature assessing the association between cortisol concentrations in pregnancy and SVR in childhood. Overall, the data suggest that exposure to excess glucocorticoid in the prenatal period is associated to vascular complications in childhood, predisposing to cardiovascular diseases in later life.

P-9A-398

β_1 blocker treatment is ineffective in males and detrimental to cardiac function in females, following prenatal exposure to a maternal low protein diet

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Objective: Prenatal protein restriction *in utero* predisposes the adult offspring to hypertension. β adrenergic receptor blockade is one of the standard therapies for the treatment of hypertension. This study compares the effectiveness of β blocker therapy in adult rat offspring from control and protein restricted dams in attenuating the dose-response to the β adrenergic agonist isoproterenol, using the Langendorff-perfused heart preparation.

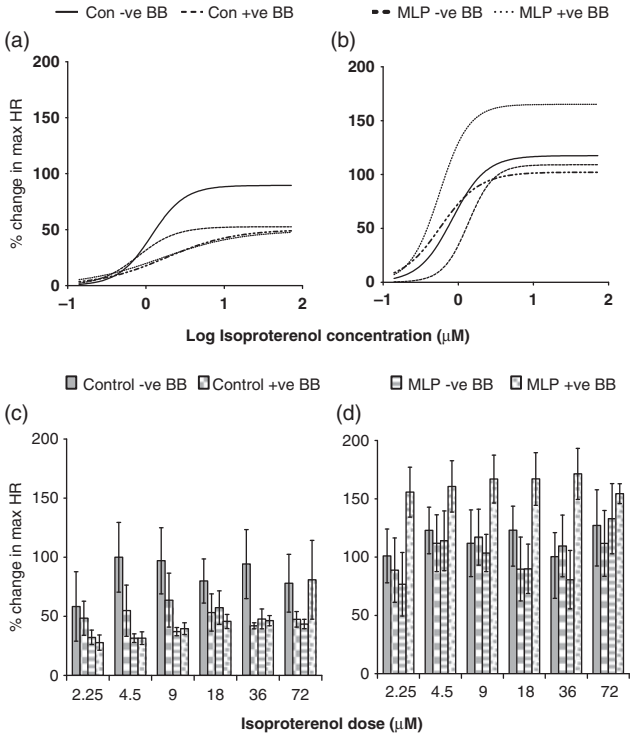

Figure 1. (a, b) Dose response curves (0–72 μM) of the effect of isoproterenol on the % change in heart rate relative to baseline and (c, d) comparison of treatment effects of specific doses between 2.25–72 μM in (a, c) male and (b, d) female Langendorff perfused hearts. Significant effects of β-blocker ($p < 0.001$; males and $p < 0.05$; females), Maternal diet, Dose, β-blocker × Maternal diet ($p < 0.001$; both sexes) and Maternal diet × Dose ($p < 0.01$; males) were determined from 3-way ANOVA.

Methods: Pregnant Wistar rats were fed either a control (CON, 180 g casein/kg) or a low protein diet (MLP, 90 g/kg)

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throughout gestation. At birth, mothers were transferred to a standard laboratory chow and the offspring weaned onto the same chow at 4 weeks of age. At 6 months, rats from each dietary group were treated with the β_1 blocker metoprolol (+ve BB). Metoprolol was administered via the drinking water at 100 mg/Kg a day for a 2 week period. Controls received water only (−ve BB). At cull, hearts were rapidly excised and cannulated via the aorta to a Langendorff perfusion system. Hearts were perfused with Krebs-Henseleit buffer bubbled with O_2/CO_2 in a retrograde manner and maintained at constant pressure (60 mmHg) and temperature (37–38°C). Left ventricular function was monitored by insertion of a latex balloon attached to pre-calibrated pressure transducers which enabled heart rate and ventricular pressure measurements to be assessed. Following 30 mins equilibrium, each heart received 100 μl of increasing doses of isoproterenol (0.14–72 μM) where contractile function parameters recovered to baseline before receiving the next dose.

Results: Metoprolol reduced the maximal heart rate response to the β agonist isoproterenol in CON males (Figure 1a and c) but had no effect in CON females (Figure 1b and d). Analyses of data from MLP males and females indicated significant interactions between β blocker treatment and diet ($p < 0.001$). In contrast with the control diet group, comparisons of mean values indicated that β blocker treatment had no effect on the response of the MLP male heart to isoproterenol (Figure 1a and c) and surprisingly metoprolol enhanced the stimulatory response of the MLP female heart (Figure 1b and d).

Conclusions: In conclusion β blocker treatment has sex specific effects. Its putative role in reducing the heart's response to isoproterenol was only observed in male offspring exposed to a control diet *in utero*. In contrast β blocker therapy in prenatally protein restricted offspring, was ineffective in males and detrimental to females. These findings cast doubt over the efficacy of β blocker agents to treat cardiac conditions in females. We suggest that beta blocker therapy for hypertensive individuals that were growth restricted at birth may either be ineffective or detrimental to males and females respectively. Further work is needed to determine the mechanisms underpinning these effects.

P-9A-399

Antioxidants prevent the deleterious effects of neonatal dexamethasone treatment on cardiovascular function in adult rats

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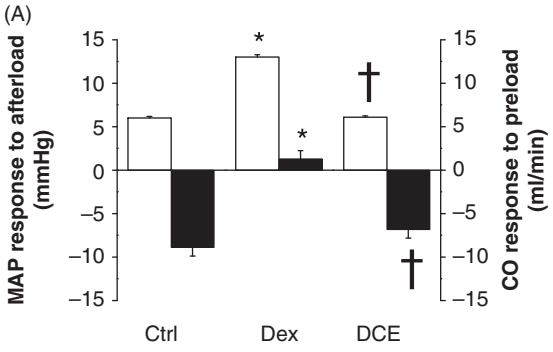
Objective: Although dexamethasone (Dex) is widely used to prevent and treat chronic lung diseases in premature infants, its short-term side effects on cardiovascular function have raised concerns about the clinical application. The mechanisms underlying these unwanted effects of glucocorticoids are not fully understood, but oxidative stress may play an important role [1,2]. In this study, adult rats were used to investigate: 1) the effects of neonatal Dex treatment on isolated cardiac and peripheral vascular function, and 2) whether postnatal co-administration with the antioxidant vitamins C and E ameliorates any detrimental effects of Dex.

Methods: Male Wistar pups received i.p. injections of a clinical regimen of dexamethasone (Dex; n = 8; 0.5, 0.3, 0.1 $\mu g.g^{-1}$) or Dex with vitamins C and E (DCE; n = 8; 200 $mg.kg^{-1}$ and 100 $mg.kg^{-1}$, respectively) on postnatal days 1–3 (P1-3); vitamins were continued from P4-6. Controls received equal volumes (10 $\mu l.g^{-1}$) of saline (Ctrl; n = 8) from P1-6. A fourth group received vitamins alone (CCE; n = 8). Rats were euthanized at P100 and the heart and a 3rd order femoral artery were dissected. Hearts were perfused under working mode and cardiac mechanical function was evaluated. Arterial rings were mounted on a wire myograph and concentration-response doses to vasoconstrictors and vasodilators were generated.

Results: Dex-treated animals had a lower heart weight relative to body weight (0.00274 ± 1.08E-4 vs 0.00333 ± 1.64E-4, $P < 0.05$) but cardiac output and hydraulic work were both maintained. However, the mean aortic pressure (MAP) response to increased afterload in Dex-treated animals was two-fold higher than in controls. In addition, in Dex-treated offspring, cardiac output (CO) responses to a decrease in preload were significantly reduced compared to controls. Interestingly, concomitant treatment of vitamins C and E restored the cardiac responsiveness to both changes in after- and pre-load towards control values (Fig 1A). Vitamins C and E alone did not show any cardiac effects relative to controls (data not shown). Relative to controls, Dex treatment markedly decreased the dilator response to MetCh (both sensitivity and max response, Fig 1B), and to SNP (sensitivity, pD_2 6.99 ± 0.05 *vs* 7.14 ± 0.09, $P < 0.05$). Dex also decreased the maximal response to K^+ (3.16 ± 0.23 *vs* 4.85 ± 0.23 N/m, $P < 0.05$). Concomitant treatment with vitamins C and E prevented all the above vascular effects of Dex.

Conclusions: Postnatal treatment with Dex in rats using a human clinical dosing regimen impaired the intrinsic control of cardiac function and depressed endothelial-dependent relaxation in the peripheral vasculature in adulthood. Postnatal co-administration of vitamins C and E prevented the programming effects of cardiovascular dysfunction of glucocorticoids. Support: The British Heart Foundation, The BBSRC and The Royal Society.



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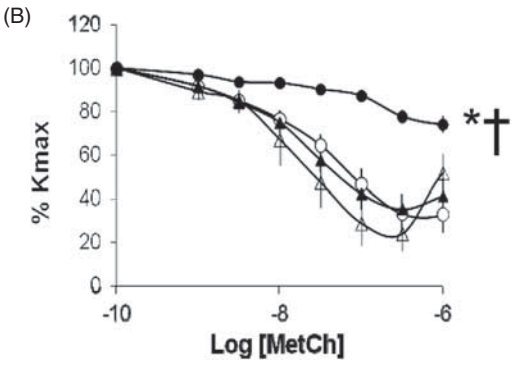

Fig 1. Mean ± SEM. **A**. Responses of MAP to increases in afterload (open bars), and of CO to decreases in preload (close bars). Significant differences ($P < 0.05$), * *vs* control, † *vs* Dex. **B**, Endothelium-dependent vasodilatation (MetCh) in femoral arteries. ○ Ctrl ● Dex, ▲Dex + VitCE, △Ctrl + VitCE. Significant differences ($P < 0.05$), *Max response, † sensitivity *vs* Ctrl.

1. S. Rajashree, R. Puvanakrishnan. *Mol Cell Biochem*, 181:77–85, 1998.
2. T. Iuchi *et al. Circ Res,* 92:81–87, 2003.

P-9A-400

Maternal protein restriction during mouse preimplantation development induces offspring vascular dysfunction and alters renin-angiotensin-system homeostasis

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Objectives: Studies in rodents and domestic animals have demonstrated that perturbations experienced during early development can influence future growth, metabolism, physiology and cardiovascular health. We have demonstrated previously that maternal low protein diet during discrete windows of gestation significantly elevated systolic blood pressure in adult offspring mice[1].
Methods: Following mating, female MF-1 mice were assigned to either normal protein diet (NPD; 18% casein) or isocaloric low protein diet for the entirety of gestation (LPD; 9% casein); or LPD exclusively during the preimplantation period (3.5 days), before returning to NPD for the remainder of gestation (Emb-LPD). All offspring were fed standard chow. All experiments were conducted using protocols approved by UK Home Office and local ethics committee.
Results: Mesenteric arteries from LPD and Emb-LPD males displayed significantly impaired vasodilatation to isoprenaline ($P = 0.04$ and 0.025 respectively), when compared to NPD arteries. Stereological investigation of mean glomerular number in female left kidneys at 28 weeks, and real-time analysis of type 1a Angiotensin II receptor, Na^+/K^+ ATPase transporter subunits and glucocorticoid receptor expression in offspring left kidneys, revealed no significant differences between treatment groups. LPD females had elevated serum angiotensin converting enzyme (ACE) activity ($P = 0.044$) whilst Emb-LPD males had elevated lung ACE activity ($P = 0.001$) compared to NPD offspring.
Conclusions: These data demonstrate that elevated systolic blood pressure induced by maternal dietary protein restriction during the preimplantation period or throughout gestation is associated with arterial vasodilator dysfunction and elevated ACE activity, but has minimal effects on kidney phenotype.
Support: The National Institutes of Health, [grant number U01 HD04435], BBSRC [BBF007450] and the Gerald Kerkut Charitable Trust.

1. A.J. Watkins *et al.*, *Biol Reprod.,* 78:299–306, 2008.

P-9A-401

Maternal smoking during pregnancy, fetal arterial resistance adaptations and cardiovascular function in childhood. The Generation R Study

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Objective: To unravel the mechanisms underlying the previously demonstrated associations between low birth weight and cardiovascular disease in adulthood, we examined whether maternal smoking during pregnancy leads to fetal arterial resistance adaptations and subsequently to fetal growth retardation and changes in postnatal blood pressure and cardiac development.
Methods: We performed a prospective cohort study from early fetal life onwards. Maternal smoking during pregnancy (non-smoking, first trimester smoking, continued smoking (<5 and ⩾5 cigarettes/day)) was assessed by questionnaires. Third trimester placental and fetal arterial resistance indices and fetal growth were assessed by ultrasound and Doppler. Postnatal blood pressure and cardiac structures were measured at two years of age. Analyses were based on 1,120

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children. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: First trimester smoking was not associated with third trimester placental and fetal blood flow adaptations. Continued smoking of ⩾5 cigarettes/day was associated with increased resistance in the uterine, umbilical, and middle cerebral arteries and a decreased flow and diameter of the ascending aorta. Among mothers who continued smoking, estimated fetal weight at 30 weeks and birth weight were most affected in children with the highest umbilical artery resistance. Fetal arterial resistance indices were also associated with aortic root diameter and left atrial diameter.
Conclusions: Our findings suggest that fetal arterial resistance adaptations may be involved in the pathways leading from maternal smoking during pregnancy to both low birth weight and cardiovascular developmental changes in childhood in the offspring. Future studies are needed to examine whether these adaptations have cardiovascular consequences in later life.

P-9A-402

Lifecourse predictors of adult fibrinogen concentrations: The Newcastle Thousand Families Study

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Fibrinogen is an independent predictor of cardiovascular disease. Previous research investigating early life effects on fibrinogen levels in adulthood has produced conflicting results.
Objective: The aim of this study was to examine the association between fetal, infancy and adult risk factors and fibrinogen levels at age 49–51 years, and to quantify the direct and indirect effects of these factors on fibrinogen using data from the Newcastle Thousand Families Study (NTFS).
Methods: The NTFS is a prospective study initiated in 1947 when all 1142 children born to mothers resident in the city of Newcastle upon Tyne in northern England were recruited. Detailed information on many aspects of their lives was collected prospectively during childhood, including early growth, illnesses and socio-economic conditions. At age 49–51, 574 study members returned detailed self-completion questionnaires and 412 attended for clinical examination. Plasma fibrinogen concentrations were measured in 173 men and 221 women. This was analysed, using linear regression, in relation to a range of variables at different stages of life, including family history of cardiovascular disease, birth weight (standardised for gestational age and sex), duration breast fed, housing conditions at birth, smoking and childhood illness histories, alcohol consumption, percent body fat, physical activity, and socio-economic status both at birth and in adulthood. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Poorer housing conditions at birth ($p = 0.001$), longer duration breast fed ($p = 0.025$) and higher adult alcohol consumption ($p = 0.002$) were significant independent predictors of lower fibrinogen concentration. In contrast, increasing body fat percentage ($p < 0.001$) and being a current smoker ($p < 0.001$) were independently predictive of significantly higher fibrinogen concentrations. No association was observed between fibrinogen concentration and standardised birth weight. Three significant interactions on adult fibrinogen levels were observed. 1) The effect of being a current smoker, relative to never smokers, was highest among those from the poorest quality houses at birth. 2) The effect of percent body fat was lower among never smokers. 3) The effect of percent body fat was greater among those with the highest alcohol consumption at age 49–51. A path diagram, exploring the relative contributions, including an exploration of indirect pathways, will be presented.
Conclusion: Concentration of fibrinogen in adulthood is influenced by a range of factors from different stages of life. Although birth weight was not a predictor, there were significant associations with housing conditions in early life and duration breast fed. The total variation explained by early life factors was less than half that explained by adult risk factors. Therefore, modification of adult exposures, particularly body fat and smoking, would be the most likely way to reduce the concentration of plasma fibrinogen in adulthood, which may also reduce cardiovascular disease risks.

P-9A-403

High or low-salt intake during pregnancy is associated with cardiac structural abnormalities in adult male offspring

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Introduction: epidemiological studies have shown that alterations in perinatal environment are associated with abnormalities in adulthood. Several studies have established a link between low birth weight and cardiovascular disease in adulthood.
Objective: to evaluate the effects of low or high salt intake during pregnancy on cardiac structure of adult male offspring.
Methods: Low- (LSD, 0.15%), normal- (NSD, 1.3%) or high-salt (HSD, 8% NaCl) diet was given to Wistar rats during pregnancy. During lactation all dams received NSD as well as the offspring after weaning until the 21st week of age (LS, NS and HS groups). Starting on the 21st week of age, 50% of each group was fed high-salt (hs, 4% NaCl) diet until 36 weeks of age (LShs, NShs, HShs). The other 50% was

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maintained on a NSD (LSns, NSns and HSns) for the same period. Echocardiographic features were obtained at 20 and 30 weeks of age.

Results: (mean ± SEM, n = 5–8/group): no differences were observed in tail cuff blood pressure and left ventricular (LV) to body mass ratio among offspring groups. At 20 weeks of age: inter-ventricular septum (IV-mm/kg) and posterior wall (PW-mm/kg) thickness was lower ($p < 0.05$) in HS (0.272 ± 0.008 and 0.275 ± 0.009) compared to NS (0.320 ± 0.013 and 0.323 ± 0.014) and LS (0.309 ± 0.008 and 0.314 ± 0.008). At 30 weeks of age: no differences were observed in IV and PW tickness between LShs, NShs and HShs. LV end diastolic diameter (LVd-cm/kg) was lower ($p < 0.05$) in HShs (1.215 ± 0.050) compared to NShs (1.445 ± 0.056) and LShs (1.369 ± 0.042) suggesting a concentric remodeling. Interestingly, IV ($p = 0.052$) and PW ($p < 0.05$) tickness was higher in LSns (0.316 ± 0.013 and 0.321 ± 0.012) compared to NSns (0.267 ± 0.020 and 0.267 ± 0.020) and HSns (0.259 ± 0.016 and 0.259 ± 0.016). IV and PW tickness was lower ($p < 0.05$) in LShs (0.264 ± 0.012 and 0.267 ± 0.013) compared to LSns.

Conclusions: according to these data, alteration in salt consumption during pregnancy is associated with cardiac abnormalities in adult male offspring. Offspring from HSD and LSD dams exhibit different patterns of cardiac response to a chronic salt overload during adulthood. Support: FAPESP and CNPq.

P-9A-404

Increased systolic blood pressure in rat offspring following a maternal low protein diet is normalized by maternal dietary choline supplementation

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Objective: An adverse prenatal environment may induce long-term metabolic consequences, in particular hypertension and cardiovascular disease. Although the mechanisms are unclear, this "programming" has generally been considered an irreversible change in developmental trajectory. A maternal low protein (LP) diet is well known to result in increased blood pressure in offspring. Choline has been shown to have direct blood pressure reducing effects in humans and animals. It has been suggested that endogenous choline synthesis via phosphatidylcholine is constrained during maternal LP exposure. The present study therefore investigated the effect of choline supplementation to mothers fed a low protein diet during pregnancy on systolic blood pressure (SBP) in offspring.

Methods: Wistar rats (day 120) were assigned to 1 of 3 diets to be fed ad-libitum throughout pregnancy: (1) control diet (AIN93G, 20% protein); (2) a LP diet (9% protein); (3) LP supplemented with choline (LPC). Dams were returned to the standard control diet at birth. Litter size was adjusted to 8 pups per litter to ensure standardised nutrition until weaning. Offspring were fed the standard control diet (AIN93G) from weaning for the remainder of the trial. Systolic blood pressure was recorded at postnatal day 150 using tail-cuff plethysmography.

Results: SBP was significantly increased in LP offspring compared to CONT animals and was normalized in LPC offspring (Figure 1). Effects of LPC reduction in SBP were similar in both males and females. These effects were independent of adult body weight and alterations in kidney size. Heart size relative to body weight was increased in LP females compared to both controls and LPC offspring but was not different in males.

Conclusions: The present trial shows for the first time that maternal supplementation with dietary choline during periods of LP exposure can normalize increased SBP observed in offspring in later life. Support: Foundation for Research, Science and Technology (FRST) and the National Research Centre for Growth and Development.

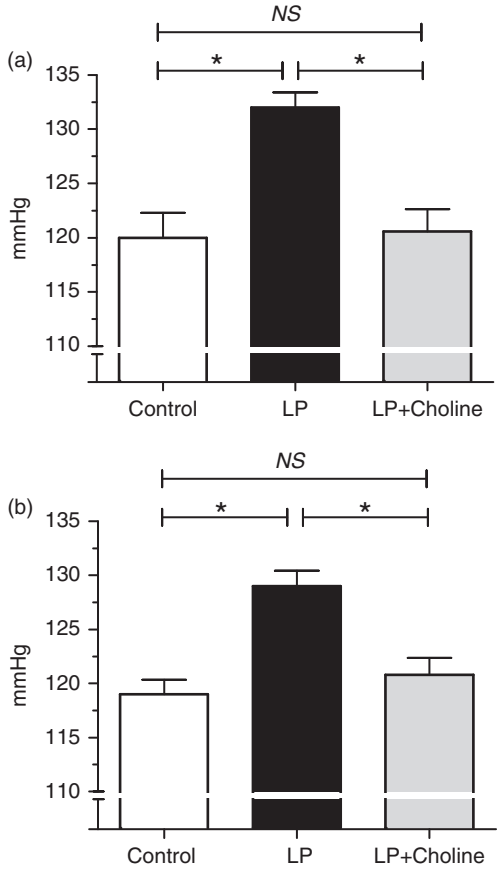

Figure 1. Systolic blood pressure in (a) female and (b) male offspring at postnatal day 150 following either a maternal control, low protein or low protein + choline diet during pregnancy. Data are means ± SEM, N = 8 per group, * = $p < 0.05$, NS = not significant.

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P-9A-405

Narrowing of the aorta after moderate preterm birth

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Objective: Preterm birth (is the major cause of infant death and morbidity, affecting 10–12% of all pregnancies. 82% of all preterm births are classified as moderately preterm (birth between 32 and 37 weeks of gestation). It has recently been demonstrated[1] that preterm birth is an independent risk factor for aortic narrowing in adolescents born preterm; there was ~16% narrowing in the thoracic aorta and ~19% narrowing in the abdominal aorta. However, it was not clear if this was due to preterm birth or with iatrogenic factors associated with treatment of preterm infants. We hypothesised that moderate preterm birth alone would lead to narrowing of the aorta. Our objective was to examine aortic structure in postnatal lambs following moderate preterm birth.

Methods: Preterm lambs were born at ~133 days of gestation; term is ~147 days. Arterial pressure was measured at 8 weeks and lambs were euthanized at 9 weeks after term equivalent age. Aortas were maximally dilated with papaverine hydrochloride prior to fixation in 4% formaldehyde. At least 4 sections from the first 4 cm of the thoracic aorta were embedded in paraffin and sectioned at 5 μm. Sections were then stained with H&E. Whole slides were scanned using the dotslide system (Olympus, Japan); lumen area and wall thickness were quantified using image analysis (Image Pro-Plus, Media Cybernetics, USA). Aortae from 8 term and 7 preterm lambs were compared.

Results: Preterm lambs were lighter than term controls at birth (term = 3.37 ± 0.24 kg, preterm = 4.39 ± 0.17 kg; $p < 0.05$), however at post mortem there was no difference in weight (term = 17.10 ± 0.59 kg, preterm = 17.14 ± 0.91 kg). There were no differences between groups in mean arterial pressure (term = 73.2 ± 2.6 mmHg, preterm = 77.1 ± 1.8 mmHg) or heart rate (term = 89.6 ± 7.1 bpm, preterm = 98.0 ± 6.9 bpm). There was also no difference in heart weight (corrected for body weight) between term and preterm lambs (term = 4.64 ± 0.25 g/kg, preterm = 5.15 ± 0.35 g/kg). Preterm lambs had significantly narrower aortae (calculated as aortic lumen area) compared with controls (preterm = 20.02 ± 1.22 mm^2, term = 24.12 ± 1.05 mm^2; n = 3 per group, p = 0.05). There was a trend for preterm lambs to have thicker aortic walls (preterm = 1062.85 ± 28.49 μm, term = 1081.75 ± 53.00 μm; n = 3 per group, p = 0.08).

Conclusion: Moderate preterm birth alone leads to aortic narrowing early in post-natal life in the absence of increased arterial pressure or heart weight. In this ongoing study, we are investigating the cause (e.g. collagen deposition, smooth muscle cell hyperplasia) of aortic narrowing.

1. Edstedt Bonamy AK *et al. Acta Paediatr* 97:1080–1085, 2008.

P-9A-406

Cafeteria diet aggravates structural cardiac impairments in adult offspring from maternal protein restricted rats

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Objective: This study aimed at evaluating the effects of post-weaning "cafeteria diet" upon cardiac morphological parameters in Wistar rats subjected to maternal protein restriction.

Methods: Wistar female rats were divided into two groups: normal protein (NP-19% protein) and low protein (LP-5% protein). Both diets followed AIN93-G dietary allowances. At 21 days, pups were divided into 8 groups, according to the post-weaning diet (SC – standard chow or HF – high fat diet): a) male NP-SC; b) female NP-SC; c) male NP-HF; d) female NP-HF; e) male LP-SC; f) female LP-SC; g) male LP-HF and h) female LP-HF. Cafeteria diet (30% lipids) was manipulated in our laboratory[1]. Body mass and blood pressure (BP) were verified weekly until 6 mo-old, when euthanasia occurred. Heart was removed after perfusion and had its volume measured by Scherle's method[2]. Stereological tools were used to analyze cardiac alterations[3]. For statistical analysis, ANOVA and post-hoc test of Tukey were used ($p < 0.05$). Appropriate institutional ethics committee clearance was obtained.

Results: HF diet caused overweight in NP-HF groups (both genders) at day 77, continuing until the end of the experiment (+34%, $p < 0.001$). At day 112, females LP-HF became heavier than LP-SC and this difference persisted until 6 months old (+42%, $p < 0.001$), showing that environmental factors can maximize the drawbacks of maternal protein restriction. This effect was also perceived when blood pressure (BP) was taken into account, where LP-HF animals from both genders showed the most expressive BP values at the end of the experiment (+50% in relation to SC group, $p < 0.001$). As for cardiac alterations, maternal protein restriction and post-weaning HF diet yielded greater cross-sectional cardiomyocyte area in males (+58%, $p < 0.0001$). Likewise, the left ventricle from LP-SC and NP-HF animals (males and females) were thicker than NP-SC (+30%, $p < 0.001$) and HF diet provoked a maximization of this finding in LP-HF groups (+35% when compared to LP-SC, $p < 0.001$). NP-HF animals from both genders showed higher amount of interstitial fibrosis than NP-SC (+75%, $p < 0.0001$). The same was observed when LP-HF was compared to LP-SC (+44%, $p < 0.001$). Myocardial vascularization was smaller in LP-SC and LP-HF males than in NP-SC (−50%, $p < 0.0001$), whereas NP-HF females showed a similar behavior (−43%, $p < 0.005$).

Conclusions: Both stimuli, maternal protein restriction and post-weaning high fat diet enhanced cardiac structural

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alterations. Therefore, our results ensure the paramount importance of intrauterine conditions as well as quality of post-weaning diet for the development of chronic diseases. Support: CNPq, Faperj, Capes.

1. V. Souza-Mello *et al.*, *Br J Nutr*, 98:1159–1169, 2007.
2. W. Scherle. *Mikroskopie*, 26:57–60, 1970.
3. B. Gregório *et al.*, *Am J Obstet Gynecol.* 199:82.e1–7, 2008.

P-9A-407

Inflammatory and fibrotic changes in the heart in a low protein rat model of intrauterine growth restriction

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Objective: Cardiovascular disease is a frequent complication of metabolic disorders associated with former intrauterine growth restriction (IUGR). Early structural and functional changes in the cardiovascular system after IUGR might contribute to the pathogenesis of cardiovascular disease. We tested the hypothesis that IUGR leads to structural changes in the rat heart before the onset of increased blood pressure.
Methods: We used a rat model of maternal protein restriction to 8% during gestation. Media-to-lumen ratios were measured in the heart vessels of newborn rats and rats at day 70 of life. Expression levels of markers of inflammation and fibrosis were assessed in the hearts of both time points by real-time RT-PCR. Immunohistochemistry for macrophage infiltration and collagen I deposition was performed. Mean arterial blood pressure was assessed intraarterially.
Results: Low protein diet of the mothers led to a reduction in body weight of the offspring (5.3 ± 0.2 vs. 6.5 ± 0.2 g in control pups; $p < 0.001$). In the hearts of newborn rats with IUGR the expression of inflammatory markers like MCP-1 and osteopontin, of profibrotic cytokines, like TGFβ and CTGF and of matrix molecules, like collagens I and IV, fibronectin, fibrillin-1 and elastin, as well as of the regulators of matrix deposition TIMP-1 and TIMP-2 were unaltered compared to controls. In contrast, all these parameters were significantly increased in 70 days old former IUGR rats. Media-to-lumen ratios of cardiac vessels after IUGR were inconspicuous in newborn rats, but increased at day 70 of life when compared to controls. More collagen protein deposition was also detected and macrophage infiltration into heart tissue tended to increase in IUGR at day 70 of life. At this time point, blood pressure was not different between IUGR and controls (101.3 ± 2.7 vs. 105.3 ± 1.6 mm Hg; ns).
Conclusion: In the low-protein model, IUGR leads to an increased inflammatory response and to fibrotic changes in the hearts of adolescent rats, which might lead to cardiovascular disease later in life. These changes are not a consequence of an increased blood pressure in IUGR.

P-9A-408

Comparison of two models of intrauterine growth restriction: bilateral ligation of the uterine arteries and maternal low protein diet induce different cardiovascular phenotypes

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Objective: Intrauterine growth restriction (IUGR) is often associated with an increased risk to develop cardiovascular diseases, like hypertension or atherosclerosis. Using a rat model of maternal protein restriction, we detected increased expression of matrix molecules and connective tissue growth factor in the vasculature of the offspring after IUGR. In this study, we compared cardiovascular changes in two different rat models of IUGR.
Methods: IUGR was induced either by low protein diet of the pregnant dam (LP), or by ligation of the arteria uterina (Lig). Vascular and heart tissue of the offspring was investigated for expression patterns of profibrotic markers and matrix molecules and for histological changes.
Results: Birth weight was reduced to a similar degree in LP (by 18.5%) and Lig (by 15.6%) offspring. In the vasculature of LP offspring, the expression of connective tissue growth factor and the matrix components collagen I, fibronectin and fibrillin-1 was increased. In Lig rats, however, the expression of these molecules was not increased. In the hearts of LP offspring, increased expression of the inflammatory cytokine MCP-1, of transforming growth factor-beta and connective tissue growth factor, as well as of collagens I and IV, fibronectin and fibrillin-1 was detected. This was not observed in Lig rats. Media-to-lumen ratio of the aorta was increased in LP, but not in Lig rats.
Conclusions: In the two models of IUGR, cardiovascular changes are not uniformly observed, but differ depending on the model used. We speculate that specific causes of IUGR induce different mechanisms of perinatal cardiovascular programming.

P-9A-409

Intrauterine growth restriction coupled with hyperglycemia leads to impaired cardiac function in adult rats: an echocardiography study

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Objective: Diabetes leads to pathological remodelling of the myocardium resulting in loss of cardiomyocytes and subsequent deposition of fibrillar collagen (fibrosis). It has previously been shown that the number of cardiomyocytes in

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the intrauterine growth restricted (IUGR) heart of rats, that have been exposed to maternal protein restriction, is significantly reduced at birth. Since cardiomyocytes cease proliferating soon after birth, we proposed that a reduced complement of cardiomyocytes at birth will render the IUGR heart particularly vulnerable to a secondary insult of hyperglycemia. Hence, the aim of the present study was to investigate the combined effect of IUGR, due to maternal protein restriction, coupled with hyperglycemia on cardiac function in rat offspring at 32 weeks of age.

Method: Female WKY rats were fed either a normal protein diet (NPD, 20% casein) or low protein diet (LPD, 8.7% casein) 2 weeks prior to mating, during pregnancy and lactation. At 23 weeks of age, streptozotocin (STZ; 50 mg/kg) was administered to the offspring to induce hyperglycemia. From 5 days after STZ injection, blood glucose levels were measured daily; long acting insulin (protophane) was injected (1-2U) daily to stratify blood glucose levels (mild, 7–10 mmol/L and moderate, 10–15 mmol/L; n = 8males per group). Morphological and functional analyses of the heart were performed using non-invasive transthoracic echocardiography and 2D B-mode imaging. Data were analysed by a two-way ANOVA with maternal diet (P_D) and induction of hyperglycemia (P_G) as factors.

Results: LPD offspring were born small and remained smaller than NPD offspring at 32 weeks of age ($P_D < 0.001$). In addition the induction of hyperglycemia led to a marked decrease in body weight at 32 weeks of age ($P_G = 0.001$). Both maternal diet and induction of hyperglycemia directly influenced heart size and cardiac function, however, the response to hyperglycemia was not different between the NPD and LPD offspring. LPD offspring exhibited a significantly increased relative LV mass and relative wall thickness ($P_D = 0.01$, $P_G = 0.007$), as well as a significantly decreased fractional shortening (%) of the left ventricle ($P_D = 0.04$, $P_G = 0.007$) at 32 weeks of age. Left ventricular diastolic volume was significantly decreased in LPD offspring ($P_D = 0.03$, $P_G = 0.05$), whereas, there was no difference in systolic volume. Stroke volume was also significantly reduced in LPD offspring ($P_D = 0.04$, $P_G = 0.003$). The ejection fraction (%) showed no difference between groups.

Conclusions: Maternal protein restriction in rats appears to lead to cardiac hypertrophy and impaired diastolic function in adult offspring and this is exacerbated in the presence of hyperglycemia. Reduced fractional shortening and stroke volume in LPD offspring indicates that there is impaired cardiac contractility such that the heart muscle is required to work harder to maintain a normal ejection fraction.

P-9A-410

Association between risk behavior and high blood pressure in adolescents

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Objective: To estimate the prevalence of high blood pressure in adolescents of high school students in southern Brazil and explore the association with risk behaviors.

Methods: Cross-sectional study with a representative sample of students in high school (14–18 years) the city of Maringá/PR/Brazil, including 991 (54.5% girls) from eight public and four private high school's selected through sampling in multiple stages. The outcomes considered of: blood pressure (BP). The BP was measured twice, with a minimum interval of two minutes, with teenagers sitting through an automatic, electronic and digital oscillometric (Omron MX3 Plus). The categorization of blood pressure was performed in three classes[1]: PA normal, borderline PA and high PA, adjusted for gender, age and percentile of height[2]. The independent variables investigated were: gender and intake of tabaco[3]. The consumption of tobacco (including consumption of tobacco in cigarettes, cigars and cigarillos), outcome was considered the adolescents said that consume a day at least one of the above products. Ethical aspects of the intervention study were in agreement with current guidelines in Brazil, and the study protocol was approved by the Ethical Committee of the Medical School of the University of São Paulo.

Results: Table 1 shows the prevalence of blood pressure according to the independent variables, the prevalence of high BP was 3.2%, significantly higher in boys. The prevalence of smoking was 5.7% overall (boys = 50.9%; girls = 49.9%). Observed that tobacco consumption is higher in adolescents with high blood pressure.

Table 1: Prevalence and confidence interval 95% ($CI_{95\%}$) of blood pressure categorization according to the independent variables.

Variable	Normal BP Prevalence % (CI 95%)	Borderline BP Prevalence % (CI 95%)	High BP Prevalence % (CI 95%)	*p*
Gender Girls (n = 540)	86.3 (83.1–89.1)	12.4 (9.7–15.5)	1.3 (0.5–2.7)	0.0001
Boys (n = 451)	83.6 (79.8–86.9)	10.9 (8.1–14.1)	5.5 (3.6–8.1)	
Tobacco consumption				0.50
No (n = 934)	84.9 (82.4–87.0)	11.9 (9.9 – 14.1)	3.2 (2.2 – 4.5)	
Yes (n = 57)	87.7 (85.5–89.7)	8.8 (7.1–10.7)	3.5 (2.5–4.9)	

The probability of the boys have high blood pressure is significantly higher than in girls (PR = 1.74 $CI_{95\%}$ = 1.43–2.13). Not observe significant associations between the habit of smoking and blood pressure levels.

Conclusion: We found alarming prevalence of high blood pressure, particularly in boys and the prevalence of tobacco

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consumption is alarming either, it is necessary to develop strategies that promote healthy lifestyles in this population.

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2. Kuczmarski RJ, *et al.*, *Vital Health Stat*, 11, 2002.
3. Malcon MC, *et al.*,. *J Adol Health.* 43:226–230, 2008.

P-9A-411

Analysis of biochemical changes in response to hypertension in the myocardium of adult rat offspring with intrauterine growth retardation using FTIR imaging micro-spectroscopy

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Objective: Epidemiological evidence suggests that intrauterine growth retardation (IUGR) is associated with an increased risk of cardiovascular disease later in life. We have previously shown that female IUGR rat offspring exposed to maternal protein restriction during pregnancy and lactation exhibit a decline in cardiac function as young adults. In order to gain an understanding of the etiology of the impaired cardiac function, the aim of this study was to compare the biochemical composition of the hearts from IUGR and non growth-restricted offspring and to examine the biochemical response to induction of hypertension.
Methods: Wistar Kyoto (WKY) rats were administered a low protein diet (LPD; 8.7% casein) during pregnancy and lactation until 2 weeks postnatally; controls were administered a normal protein diet (NPD; 20% casein). At 4 weeks of age offspring were weaned and placed on standard rat chow until the experimental endpoint. At 14 weeks of age hypertension was induced through a four week continuous infusion of a potent vasoconstrictor Angiotensin II (ANGII 200 ng/kg/min), while controls received saline. During this time systolic blood pressure was measured using a tail cuff method. At 18 weeks of age the offspring were perfusion fixed and organs collected. The hearts were weighed and the heart volumes were stereologically determined according to the Cavalieri method. Heart slices were embedded in paraffin and in 4 μm sections the biochemical changes in the left ventricle of LPD and NPD female normotensive (NPD $n = 9$; LPD $n = 7$) and hypertensive (NPD $n = 7$; LPD $n = 7$) offspring was examined using Fourier transform infrared (FTIR) imaging micro-spectroscopy. FTIR imaging provides a methodology to explore the underlying macromolecular chemistry and biochemical markers associated with the etiology of hypertension and heart disease.
Results: Birth weights of the LPD offspring were significantly lower compared to NPD offspring (6.5 ± 0.3 g and 7.6 ± 0.3 g, respectively), however in adulthood (18 weeks of age) this was no longer significant. Infusion of ANGII led to a decrease in body weight in NPD and LPD offspring ($P < 0.0002$). Absolute heart and left ventricular (LV) volumes were both significantly lower in LPD offspring at 18 weeks of age. Relative heart and LV volumes were significantly increased due to ANGII administration. Initial analysis of the FTIR spectra has shown that variations in tissue macromolecular composition can be observed within the tissue of individual heart sections and between the four groups that have been examined. FTIR maps of the LV wall showed significant variation between the experimental groups. FTIR images of the LV show the spatial location of protein, collagen and carbohydrate density. Further work is required to compare the FTIR images with histological stained micrographs of the same tissue regions.
Conclusions: FTIR imaging spectroscopy is showing promise as an independent modality for examining changes in the macromolecular chemistry of the adult IUGR heart under hypertensive conditions.

P-9A-412

A maternal methyl-rich dietary pattern characterized by high intakes of fish and nuts reduces the risk of congenital heart defects in the offspring

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Objective: There is now proof of principle that the inadequacies in the diet can have profound impact on epigenetic mechanisms regulating gene expression patterns throughout the life-course. Reproductive processes are known to be deranged by maternal malnutrition as well, of which particular folate has been associated with congenital malformations. Therefore, our objective was to identify maternal dietary patterns affecting the availability of methyl-groups used for DNA and histone methylation, and investigate whether these dietary patterns are associated with the risk of congenital heart defects (CHD) in the offspring.
Methods: From 179 case mothers of a child with CHD and 231 control mothers food intake was assessed from food frequency questionnaires (FFQ) collected 14 months after the index-pregnancy as a proxy of the periconceptional maternal

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dietary intake. In maternal blood concentrations of methylation biomarkers were determined, i.e., S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) and folate. Food groups were summarized from individual FFQ items which were entered as predicting variables in the reduced rank regression (RRR) method while the methylation biomarkers served as response measures. The RRR calculated combinations of food groups, e.g., dietary patterns, which optimally predict the methylation biomarkers. Linear and logistic regression model were used to asses associations between dietary patterns, log-transformed biomarkers and risk of CHD. Confounders were maternal age, BMI, and educational level at the study moment, and periconceptional B-vitamin supplementation, smoking, alcohol and medication use.

Results: Two dietary patterns were identified with RRR. The first showed high intakes of snacks, sugar products, nuts and beverages and was associated with increased SAH concentrations (β 0.92, $p < 0.001$) and therefore labelled as the methyl-poor dietary pattern. The second dietary pattern, the methyl-rich dietary pattern, was characterized by high intakes of nuts, fish and other seafood and was positively associated with SAM (β 0.44, $p < 0.001$), folate in red blood cells (β 0.01 $p < 0.01$) and in serum (β 0.01 $p = 0.03$) while being inversely associated with SAH (β −0.08, $p < 0.001$). The methyl-poor dietary pattern did not affect CHD risk. In contrast, a strong maternal use of the methyl-rich dietary pattern was associated with a nearly 70% reduced risk for having a child with CHD (OR 0.32, 95%CI 0.18–0.59).

Conclusion: This study shows that high intakes of snacks and refined sugars reduce the availability of methyl-groups in blood. On the other hand high intakes of fish and nuts increase their availability. Most interesting is the finding that the use of a methyl-rich dietary pattern by moms-to-be seems to contribute to the prevention of CHD. This could be a link between the periconceptional maternal nutrition and epigenetic regulation of embryonal development and intrauterine growth.

P-9A-413

Anthropometrical measurements in the life course and blood pressure in young Chilean adults

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Several factors concur in the regulation of blood pressure, among which nutritional status is of prime importance.

Objective: To determine the association between anthropometrical variables at birth, 12 months and adult and blood pressure in young adults.

Methods: This was a non-concurrent longitudinal study comprising 1000 subjects, aged 22–28 years, born in Limache, Valparaíso, Chile. Weight, length, gestational age at birth and weight at 12 months were obtained through clinical records. These measurements were standardized in z scores at birth (WAZ 0) and at 12 months (WAZ 12) and increment between both ages was calculated. Weight, height and blood pressure were obtained during a physical exam as adults.

Results: Mean systolic and diastolic blood pressure were 114.6 and 72.5 mm Hg respectively. There was an inverse association between WAZ 0 and systolic pressure (Beta = −1.21 mm Hg, 95% Confidence Interval (CI): −2.32 to −0.67), but no association between WAZ 12 and systolic pressure. Also, direct associations between the increment of weight during the first year (Beta = 1.19 mm Hg, CI: 0.48 to 1.90) and between body-mass index and systolic pressure (Beta = 0.50, CI: 0.32 to 0.69) were registered, controlling by age, schoolarity and gestational age. Similar associations were obtained for diastolic blood pressure.

Conclusions: The association between the anthropometrical measurements and blood pressure differs throughout life: lower weight at birth, high weight increment during the first year and weight excess in adult life are risk factors. These data adds evidence for a careful management of low birth weight avoiding excessive gain weight in order to reduce hypertension risk at older ages.

P-9A-414

Association between risk behavior and high blood pressure in adolescents

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Objective: To estimate the prevalence of high blood pressure in adolescents of high school students in southern Brazil and explore the association with risk behaviors.

Methods: Cross-sectional study with a representative sample of students in high school (14–18 years) the city of Maringá/PR/Brazil, including 991 (54.5% girls) from eight public and four private high school's selected through sampling in multiple stages. The outcomes considered of: blood pressure (BP). The BP was measured twice, with a minimum interval of two minutes, with teenagers sitting through an automatic, electronic and digital oscillometric (Omron MX3 Plus). The categorization of blood pressure was performed in three classes[1]: PA normal, borderline PA and high PA, adjusted for gender, age and percentile of

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height[2]. The independent variables investigated were: gender and intake of tabaco[3]. The consumption of tobacco (including consumption of tobacco in cigarettes, cigars and cigarillos), outcome was considered the adolescents said that consume a day at least one of the above products. Ethical aspects of the intervention study were in agreement with current guidelines in Brazil, and the study protocol was approved by the Ethical Committee of the Medical School of the University of São Paulo.

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The probability of the boys have high blood pressure is significantly higher than in girls (PR = 1.74 $CI_{95\%}$ = 1.43–2.13). Not observe significant associations between the habit of smoking and blood pressure levels.

Conclusion: We found alarming prevalence of high blood pressure, particularly in boys and the prevalence of tobacco consumption is alarming either, it is necessary to develop strategies that promote healthy lifestyles in this population.

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2. Kuczmarski *et al. Vital Health Stat*. 11:2002.
3. Malcon *et al. J Adol Health.* 43:226–30, 2008.

P-9A-414B

Aortic growth retardation after preterm birth

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Objective: Adolescents born preterm have a smaller aorta than controls born at term.[1,2] We hypothesize that preterm termination of placental circulation and the concomitant decrease in aortic blood flow impair aortic growth after preterm birth. To test this hypothesis, aortic growth was studied in preterm infants during the first 6 months of life and compared to the normal feto-neonatal growth of the aorta in subjects born at term.

Methods: We performed a longitudinal study of aortic growth in 22 very preterm infants (birth weight 1277 ± 189 g, gestational age 26–30 weeks), and in 16 healthy control subjects born at term (birth weight 3350 ± 400 g). Aortic dimensions were determined at three consecutive time points: 3 months before term (after birth in preterm infants and intrauterine assessments of control subjects at gestational week 28), at term and at 3 months of corrected postnatal age. We measured abdominal aortic diameters at the diaphragmatic level by angle-corrected M-Mode ultrasonography. Appropriate institutional ethics committee clearance and parents' informed consent were obtained. Results are presented as mean ± SEM.

Results: At the first measurement - corresponding to 28 weeks of gestation - end-diastolic aortic diameter did not differ between infants born very preterm (3.7 ± 0.13) and controls, i.e., healthy fetuses examined in utero (3.9 ± 0.14 mm, $p = 0.25$). At an age corresponding to term, the end-diastolic diameter of the aorta was significantly lower in infants born very preterm (5.0 ± 0.20 mm) as compared to infants born at term (6.1 ± 0.21 mm, $p < 0.001$). This could not be explained by differences in body weights (mean 3670 and 3350 g, respectively, $p = 0.24$). At 3 months of corrected postnatal age (preliminary data, recruitment ongoing), infants born preterm (so far 4 infants included) continued to have a smaller aorta (end-diastolic diameter 5.9 ± 0.32 mm) as compared to controls (6.8 ± 0.36 mm, $p = 0.13$, $n = 3$).

Conclusion: Aortic growth is restrained after preterm birth. Previous studies show that such growth deceleration may lead to long-lasting narrowing of the aorta.[1,2] The significance of these findings for future cardiovascular health in people born preterm is currently unknown and needs to be explored.

1. A.K. Edstedt Bonamy *et al.*, *Pediatr Res.*, 58:845–849, 2005.
2. A.K. Edstedt Bonamy *et al.*, *Acta Paediatr.,* 97: 1080–1085, 2008.

P-9A-414C

Maternal undernutrition alters offspring expression of vascular microRNAs and mRNAs

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Objective: We have previously reported that maternal undernutrition is associated with vascular remodeling of the extracellular matrix (ECM), and decreased expression of VEGF protein and angiogenesis in the newborn offspring. One potential mechanism by which gene expression is regulated is through micro RNAs (miRNAS). The role of

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miRNAS in fetal programming of adult disease has not been explored to date. We hypothesized that maternal undernutrition will influence the expression of offspring vascular miRNAs, and through this mechanism alter the mRNA expression of genes involved in ECM remodeling such as collagen, elastin, and angiogenesis such as VEGF.

Methods: Aortas from male offspring of maternal food restricted (MFR, 50% food restriction from day 10 of gestation to term) or control dams were collected at postnatal ages day 1 (p1) and 12 months. MiRNA profiling was performed by Ocean Ridge Biosciences (Jupiter, FL) using custom-developed microarrays containing 237 NCode 2 rat miRNAs oligonucleotide probes of 35–44-mer manufactured by Invitrogen and spotted in duplicate. The intensity of each oligo probe based on averaging of triplicate spots was determined, normalized, and analyzed using GeneSpring 7.0 Software (Silicon Genetics, Redwood City, CA). The same specimens were also subjected to large scale gene expression profiling by hybridization of 20 μg of cRNA to rat Ref-8 v3 Expression bead chip (Illumina), consisting of 17,133 oligonucleotide probe sets. Confirmation studies for select genes were performed by Western blotting, immunohistochemistry and real time RT-PCR. Results were analyzed by ANOVA and Tukey test.

Results: Out of the 237 rat miRNAs profiled, at p1, 14 miRNAs were up-regulated by at least 1.5 fold and 17 miRNAs were down-regulated in MFR aortas. At 12 months of age, 6 miRNAs were up-regulated by greater than 1.5 fold whereas 52 miRNA were down-regulated in the MFR aortas. There was a marked difference in the expression profile of the miRNAs between the p1 and 12 month control aortas, with significantly higher expression of miRNAS in the p1 aortas as compared with 12 month aortas. The predicted target genes of miRNAs commonly altered in the two age groups in MFR aortas included genes regulating angiogenesis (VEGF, FGF, TGF, IGF-2, ephrins, neuropilin, NOTCH), ECM components and remodeling (collagen, elastin, ADAMTS, matrix metolloproteinases), cell proliferation and apoptosis (cyclin, annexin, caspase) and signaling molecules (MAP kinases). Many of these predicted target genes of miRNAs that were altered in expression were also identified by the mRNA arrays.

Conclusions: Our data for the first time demonstrates that the maternal uterine environment alters the expression of offspring vascular miRNAs, and this may represent a novel epigenetic mechanism for regulation of multitude of genes involved in control of a wide range of biological processes such as development, differentiation, metabolism, apoptosis and angiogenesis. Supported by NIH grant R03 HD054920-01 (O.K.).

P-9A-414D

Differential reactivity to insulin in human umbilical and chorionic vessels from normal and gestational diabetic pregnancies

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Gestational diabetes (GD) is associated with increased synthesis of the potent vasodilator nitric oxide (NO) by the human placenta macro and microvascular endothelium[1], and increased umbilical vein plasma levels of insulin[2]. Insulin also induces NO-dependent vasodilatation in human adult systemic forearm arteries[3] and up regulates endothelial NO synthase (eNOS) expression and activity in human umbilical vein endothelium[4].

Objective: We evaluated whether insulin modulates vascular reactivity in the macrocirculation (umbilical vessels) and microcirculation (chorionic vessels) of the human placenta from normal and GD pregnancies.

Methods: Placentae from normal or GD pregnancies were obtained. Appropriate institutional ethics committee clearance and participants' informed consent were obtained. Vessel rings isolated from umbilical (UA) and chorionic (CA) arteries and veins (UV, CV), were mounted on a wire–myograph. Vascular reactivity (isometric force) to KCl-preconstricted vessels (37.5 mM) was assayed in response to insulin (0.001–10 nM) in the absence or presence of the NOS inhibitor N^G-nitro-L-arginine methylester (L-NAME, 100 μM, 30 min). Responses were expressed as a percentage of relaxation relative to maximal effects of KCl (% Kmax) and adjusted to concentration response curves.

Results: Insulin dilated UA from normal (NP-UA) and GD (GD-UA) pregnancies ($21 \pm 0.4\%$ and $20 \pm 0.5\%$, respectively), an effect inhibited by L-NAME only in NP-UA ($68 \pm 5\%$). Insulin relaxed NP-UV and GD-UV ($17 \pm 1\%$ and $12 \pm 1\%$ respectively), an effect blocked by L-NAME in NP but partially inhibited ($18 \pm 2\%$) in GD. In NP-CA and -CV no effect of insulin was observed. In GD-CA and -CV insulin relaxed $31 \pm 5\%$ and $6 \pm 2\%$, respectively. Both effects were blocked by L-NAME. The insulin-induced reactivity in umbilical vessels (arteries and veins) was directly dependent on foetal weight in term GD ($r^2 = 0.9$), leading to vasoconstriction in small for gestational age newborn (2400–2490 g) and dilatation in normal and large for gestational age newborn (2830–3900 g). L-NAME only blocked the effect of insulin in LGA newborns.

Conclusions: Thus, insulin shows a prominent vasoactive effect mainly in umbilical vessels with a lower effect in chorionic vessels. The vasoactive effect of insulin in umbilical vessels from normal pregnancies requires NOS activity, but in gestational diabetes this effect is NOS-independent. It is suggested that vascular reactivity to insulin is different in macro compared with microvessels of the human placenta, an effect that also depends on foetal weight in newborns from gestational diabetic pregnancies. Support: FONDECYT 1070865/1080534. BK holds CONICYT-PhD fellowship.

1. F. Westermeier *et al.*, *Curr Vasc Pharmacol.*, 2009 (In press).
2. E.A. Reece *et al.*, *Obstet Gynecol Surv.*, 49:64–71, 1994.
3. T.S. Herman *et al., Endothelium*, 11:231–239, 2004.
4. M. González, L. Sobrevia. *Curr Vasc Pharmacol.*, 2010 (In press).

P-9B-415

Birth weight and body composition in a remote Aboriginal Australian community

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Objectives: To describe the relationship between birth weight (BW) and body composition in one remote Australian Aboriginal community with high rates of chronic disease related morbidity and mortality.
Methods: 1,078 adults participated in a chronic disease health screen between 2004 and 2006. 309 males and 214 females had a recorded BW. Ages were 20-49 years. Height, weight, fat free mass (FFM) BMI, waist circumference (WC), and percent fat (%Fat) were assessed. Data were analysed in gender specific BW quartiles. Appropriate institutional ethics committee clearance and patients consent were obtained.
Results: Mean (SD) BW was 2.80 kg (0.54) for males and 2.72 kg (0.50) for females. The tables give mean values of body composition variables by BW quartiles.

Table 1: Mean (SD) of body composition variables by BW quartiles in males.

BW	Height (cm)	SD	Weight (kg)	SD	FFM (kg)	SD	BMI (kg/m^2)	SD	WC (cm)	SD	%Fat	SD
1.15–2.49	171.9	5.7	63.0	14.2	51.6	5.3	21.3	4.6	82.4	13.2	16.1	9.2
2.50–2.79	170.9	5.2	62.8	11.8	51.8	5.3	21.4	3.7	81.6	11.0	16.2	7.8
2.80–3.16	171.9	4.9	63.5	11.8	52.0	5.6	21.5	4.0	82.2	10.6	15.9	7.8
3.17–4.69	172.7	7.2	71.5	17.9	56.4	5.4	23.8	5.0	86.4	13.9	19.2	8.9
p	0.4380		0.0001		0.0001		0.0001		0.0031		0.0014	

Table 2: Mean (SD) of body composition variables by BW quartiles in females.

BW	Height (cm)	SD	Weight (kg)	SD	FFM (kg)	SD	BMI (kg/m^2)	SD	WC (cm)	SD	%Fat	SD
1.07–2.40	159.2	5.3	62.9	15.5	41.6	5.0	24.8	6.0	91.2	15.3	34.1	9.0
2.41–2.72	159.9	5.3	62.1	14.3	40.8	3.9	24.3	5.5	88.5	16.0	33.0	9.4
2.74–3.03	161.1	5.0	65.8	19.3	41.7	5.1	25.5	7.7	93.1	19.2	35.6	10.2
3.04–4.31	161.5	6.0	67.4	19.2	43.6	5.7	25.8	6.7	92.3	15.4	36.1	9.0
P	0.1455		0.2081		0.0817		0.4378		0.2920		0.1876	

Conclusion: Males of the highest birth weight quartile had higher weight, FFM, BMI, WC, and %Fat relative to those with lower birth weights. None of the parameters were high relative to representative Australian standards (AusDiab)[1]. There were similar trends, although not significant in weight and FFM in females. Notably, however, females over the entire birth weight spectrum had average WC measurements in the obese range by nonAboriginal standards (≥88 cm)[1], without a trend by birth weight. The promoters of the preferential central deposition of fat in females, with its especial conservation in those of lower birth weights, is worthy of much further study.

1. D.W. Dunstan *et al.*, *Diabetes Res Clin Pract.*, 57: 119–129, 2002.

P-9B-416

A study on neonatal body parameters, insulin and leptin levels

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Objective: The "Thrifty Phenotype" hypothesis predicts that more heart disease and glucose intolerance will be seen in a population that is undergoing transition from sparse to better nutrition. This concept is of greatest relevance to developing countries, where fetal growth restriction still affects large number of people, where economic progress is leading to the emergence of childhood and adult obesity and where CVD and type 2 diabetes are rising rapidly. Therefore, the present study was designed to develop a baseline data on body parameters, insulin and leptin levels of neonates, as there is a dearth of data available for specific regions around the country.
Methods: Venous blood from umbilical cord was collected at the time of delivery. Serum was separated and stored at −20°C of temperature. Leptin and insulin were assayed through ELISA technique. Weight, Length and head circumference were measured using standard methods.
Results: The findings of present study reflect higher growth restriction rate in late pregnancy. Mean birth weight of the neonates was 2.82 kg and there was negligible difference in the means of male and female babies. Similar trends in relation to gender were seen for length, head circumference and ponderal index (means 49.37 cm, 33.26 cm and 2.35 respectively). Percent incidence of malnutrition was highest by weight for height (showing wasting/asymmetrical growth), followed by ponderal index, weight for age and head circumference (Z-score). Height for age (Z-score) which stands for stunting showed lowest incidence of malnutrition. Mean insulin levels of total subjects were 7.81 μIU/ml (7.33 μIU/ml in females and 8.31 μIU/ml in males). Mean leptin levels of total subjects were 11.18ng/ml (14.77 ng/ml in females and 7.58ng/ml in males). Leptin concentration in females was twice as those in males. Both leptin and insulin

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levels were lower in LBW than in normal birth weight babies. Leptin was found to be key component influencing the interrelationship of growth, body parameters and hormonal levels in neonates.
Conclusion: Leptin seems to be the key component influencing the interrelationship of growth, body parameters and hormonal levels of neonates.

P-9B-417

Body composition of neonates – an urban hospital based study

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Objective: The present investigation aimed at studying the body parameters and body composition of neonates born to mothers from middle income group (MIG) and low income group (LIG) families and to correlate the same with the maternal nutritional status in the last trimester of pregnancy.
Methods: The final study sample consisted of 300 mothers (200 from MIG and 100 from LIG) in the last trimester of pregnancy. Cases of gestational diabetes, prematurity and twins were excluded. Maternal anthropometry (height and weight) was recorded and the hemoglobin levels were estimated using cyanmethemoglobin method. Neonatal anthropometry namely birth weight, length, head circumference (HC), chest circumference (CC), mid upper arm circumference (MUAC) and abdominal circumference (AC) were measured within 48 hours of birth. The skin fold thickness at triceps and suprailiac was taken using Harpenden calipers. All the anthropometric measurements were taken using standard procedure. The body composition was studied in terms of percent body fat, Total Body Fat (TBF), Fat Free Mass (FFM), Central Fat/Total Fat ratio, Arm Muscle Area (AMA), Mid Upper Arm Fat Area (UFA), Arm Fat index (AFI). The study was cleared by the departmental ethical committee and written consent was obtained from the subjects before hand.
Results: About 71% of the women weighed less than 60 kg at the 9th month of pregnancy. Almost 54% of the women were taller than 155 cm and 64% of them had hemoglobin level below 11 g/dl. The overall incidence of low birth was 31% in the present study. The mean birth weight and length of the neonates were 2.61 kg and 48.48 cm respectively. Mean HC, CC, AC and MUAC were 31.30 cm, 29.90 cm, 27.29 cm and 9.02 cm respectively. The mean triceps SFT was 3.10 mm and supra iliac SFT was 2.09 mm.All the neonatal body parameters were significantly higher in the NBW neonates as compared to the LBW neonates. Percent incidence of malnutrition by head circumference for age Z-score was the highest (60%) in the moderate and severe form indicating low brain preserving tendency in the neonates. Neonates in the present study showed low muscle and subcutaneous fat depot. The mean percent body fat, TBF and FFM was higher in male neonates, the difference being significant for percent body fat and TBF. The NBW neonates had significantly higher mean MUAC indices as compared to LBW neonates. Mean total body fat and fat free mass were significantly higher in NBW neonates as compared to LBW neonates. Birth weight, anthropometric indices and ponderal index were positively correlated with the measures of body composition. Significantly higher mean birth weight, length, HC, CC, AC, MUAC, TSF and SSF was found in neonates born to mothers weighing $\geqslant$60 kg and/or with normal haemoglobin levels.
Conclusion: Concerted efforts are required to improve maternal nutritional status for better birth outcome.

P-9B-418

The influence of maternal ethnicity on birth anthropometry of newborns in a multiethnic Caribbean population

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Objective: Intrauterine growth retardation could partially account for the high prevalence of coronary artery disease and type 2 diabetes mellitus among Trinidadians of South Asian ancestry. As such we assessed the whether there were ethnic differences in birth anthropometry and explored possible determinants of the various indices measured.
Methods: A retrospective study was conducted using medical records from three consecutive years at the Mt. Hope Women's Hospital from which a 1 in 3 random sample of live full term births was obtained. Those excluded were twin births, pre-term neonates and cases in which ethnicity was not recorded. Ethnicity of the newborn was determined from maternal and paternal ethnicity. Analysis of covariance was used to determine whether the ethnic differences in birth anthropometry remained significant after controlling for confounding variables. Stepwise regression was performed to identify the independent predicators of birth anthropometry. The study was approved by the hospital administration and by the institutional ethics committee of the Faculty of Medical Sciences, University of the West Indies. All patient identifiers were removed from the data set.
Results: The sample comprised 4106 live births (51% male) of whom 2035 (48%) were African; 1665 were South Asian (40%) and 496 (12%) were of Mixed ancestry. At booking the prevalence of anaemia (Hb $<$ 11.0 g/dL) among African

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mothers was significantly higher than that of South Asian mothers (29 vs 26%). Compared with South Asians, African and Mixed mothers were significantly ($p < 0.001$) taller (167.6 ± 6.7; 166.2 ± 6.6; 161.0 ± 6.4 cm), heavier (72.3 ± 14.7; 69.2 ± 14.2; 62.3 ± 13.4 kg), had higher BMI (25.7 ± 5.1; 24.9 ± 4.4; 24.0 ± 4.7 kg/m^2), systolic (118.3 ± 15.1; 117.2 ± 15.7; 114.6 ± 14.3 mmHg) and diastolic (75.2 ± 10.3; 75.3 ± 10.4; 73.9 ± 10.5 mmHg) blood pressure. When classified according to the ethnicity of the mother, the newborns had gestational ages that were not different. Compared to newborns of African and Mixed mothers those born to South Asian mothers had significantly lower ($p < 0.001$) birth weight (3.17 ± 0.58; 3.17 ± 0.57; 2.98 ± 0.55 kg), length (50.0 ± 3.1; 50.1 ± 3.3; 49.5 ± 3.0 cm), ponderal index (26.1 ± 4.4; 26.0 ± 5.0; 25.2 ± 4.3 kg/m^3) and head circumference (33.9 ± 1.9; 34.0 ± 2.0; 33.5 ± 1.7 cm). The difference in birth weight remained significant after controlling for maternal age, gestational age and haemoglobin at booking, parity, height, BMI, blood pressure and gestational age and gender of the newborn. Stepwise regression analyses indicated that mother's age and ethnicity, and infant's gender and gestational age at birth were independent predicators of birth weight, length and head circumference.

Conclusions: There are ethnic differences in birth anthropometry among Trinidadian newborns which cannot be fully explained by maternal characteristics. It is possible that the intrauterine environment might account for some of these differences. Support: Campus Research and Publication Fund, University of the West Indies, Trinidad & Tobago.

P-9C-419

Maternal flaxseed diet during lactation programmed thyroid function in young and adult offspring

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Objective: The Flaxseed (*Linum Usitatissimum*) presents several substances with beneficial effects as carotenoid, vitamin A and D, glycoside, linamarin and mucilage which is supposed to reduce glycemia, cholesterol, obesity, consequently protecting the cardiovascular system. As some of those effects may be mediated by thyroid hormones, in this study we evaluated the thyroid function in young and adult male rats whose mothers were fed a linseed diet during lactation.

Methods: Virgin rats (200–220g) were mated and after birth were divided into two experimental groups: control (C), with free access to a casein diet containing 17% protein, 52% carbohydrate, 7% lipid and 5% fiber; and flaxseed (F), with free access to a diet containing 17% protein from casein (12%) and flaxseed (5%), 54% carbohydrate, 8% lipid and 5% fiber exclusive from flaxseed. Diet treatment started at birth, which was defined as day 0 (d0) of lactation, and was ended at weaning (d21). After weaning, all pups received a standard laboratory diet and body weight (BW) and food intake (FI) were monitored until adulthood. Only male offspring were studied and the sacrifice occurred when they were 21 or 180 days-old and it was collected blood to determine the serum concentration of TSH, free T_4 (FT_4), total T_3 (TT_3) and leptin by radioimmunoassay. The data are reported as means ± S.E.M. Unpaired Student's *t*-test was used and the significance level was set at $p < 0.05$.

Results: The animals from F mothers (F group) at 21 and 180 days-old had no changes in BW and FI. At 21 days-old the F group presented lower TT_3 (−30%, $p < 0.05$), higher TSH (+84.6%, $p < 0.05$), normal FT_4 and higher leptin (69.4%, $p < 0.05$) serum concentrations. At 180 days-old, they had lower FT_4 (−28.3%, $p < 0.05$) with normal serum concentrations of TT_3, TSH and leptin.

Conclusions: Maternal flaxseed treatment during lactation imprints a thyroid dysfunction at weaning characterized by a primary hypothyroidism. Since T4 did not alter, it is possible that T4 deiodination or clearance is lower. The adult offspring had an inappropriate lower TSH secretion considered the lower T4 levels with normal T3, which suggest that pituitary and peripheral deiodination could be increased[2]. In conclusion, maternal flaxseed diet during lactation may have consequences upon thyroid function in the offspring. Support: The National Council for Scientific and Technological Development (CNPq), the State of Rio de Janeiro Carlos Chagas Filho Research Foundation (FAPERJ), Federal University Fluminense (UFF) and State University of Rio de Janeiro (UERJ).

1. K. Prasad. *Atherosclerosis*, 197:34–42, 2008.
2. S.C. Dutra *et al.*, *Horm Metab Res.*, 35:268–70, 2003.

P-9C-420

Impact of human activities on aluminum contamination in the drainage canals in the Nile Delta, Egypt

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Sabal drainage canal is one of the largest canals in the Nile Delta. It extends for 48 km through Menufiya Province and it pours into Rosseta Branch of the River Nile. Aluminum is released into Sabal drainage canal through the emissions of the recycled aluminum industries. Numerous unauthorized aluminum industries are scattered in the villages and suburban areas around such drain. The industry based on the melting of the used aluminum materials, reformation, polishing by sand and

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washing by flow water. Another source of Aluminum comes from the discharge of stations of water purification that contain huge amount of aluminum sulfate (alum) which are used as a coagulant for suspended solid particles. In addition, the extensive use of the clay in the industry of packed bricks along Sabal drainage canal is considered as anther sources of Aluminum. The field and laboratory studies of water samples and different fish tissues (muscles, liver and ovary) collected over one year from Sabal drainage revealed the following items: 1. Water samples collected from Sabal drainage canal have higher levels of Aluminum if compared to those collected from other localities. 2. The levels Aluminum in the muscle, liver and the ovary of Tilapia zillii collected from Sabal drain are higher in comparison to those detected in fish from other localities. Moreover, the level of Al exceeds the international permissible limits. From a public health standpoint, the increased concentrations of Aluminum in water samples and the muscles, liver and the ovary of T. zillii from Sabal drain is a matter of concern as this metal represent a health risk for fishermen and human consumers. Therefore, the following suggestions are important: 1. Constant elimination of illegal aluminum industries in the area of Sabal drainage canal is necessary. 2. It is very important to replace the smaller and old stations of sewage treatment by another modern type capable of collecting and treating huge amount of sewage, with high efficiency of treatment and purification.

P-9C-421

Multi-organ effects of prenatal alcohol exposure: long-term implications

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Objective: During human pregnancy, episodic exposure of the fetus to ethanol (EtOH) is not uncommon. However, the effects of episodic alcohol exposure on organ development and functional outcomes are not well understood. As fetal EtOH exposure may be a potent but little recognised cause of developmental programming, we have used a sheep model to explore the effects of prenatal EtOH exposure on a range of tissues both before and after birth. Our objective was to determine the effects of repeated EtOH exposure during the last trimester equivalent on the development and postnatal function of major organs.

Methods: Pregnant ewes with implanted jugular vein catheters were infused with either saline or EtOH (0.75 g/kg) for one hour daily from 95 days of gestational age (DGA) until 133 DGA (term ~147 DGA). At 125 DGA, the animals underwent surgery for the insertion of a fetal arterial catheter for blood sampling and arterial pressure/heart rate recording, and an amniotic catheter. One group of animals (alcohol exposed and controls) underwent necropsy at 134 DGA, while another group were born and studied at 8 weeks after birth.

Results: Maternal and fetal plasma EtOH concentrations reached maximal values of ~0.11 g/dL at 1 hour after infusion onset, but returned to baseline within 8hours. EtOH exposure resulted in transient mild hypoglycemia in the ewe and fetus as well as maternal acidemia and delayed fetal hypoxemia. EtOH exposure did not affect fetal body or organ weights, and no gross anomalies were seen. In fetal lungs, EtOH exposure led to increased collagen mRNA expression and collagen deposition, and reductions in mRNA expression of surfactant proteins A and B to ~33% of control levels. mRNA expression of pro-inflammatory cytokines IL-1β and IL-8 was reduced by ~90% compared with controls[1]. In the fetal kidneys, there was an 11% reduction in nephron endowment following EtOH exposure[2]. In fetal arteries from several vascular beds, EtOH led to altered smooth muscle reactivity and endothelial function, and a profound increase in arterial stiffness. In the fetal brain, we found that EtOH led to thinning of the corpus callosum and a decreased number of microglia in white matter.

Conclusions: Daily exposure of the fetus to EtOH during the later stages of gestation can impact upon the development of multiple organs. Mechanisms may include fetal hypoxemia, hypoglycemia and oxidative stress. If the alterations in organ structure persist after birth, they could impair health in later life. On-going studies are examining the postnatal effects of prenatal EtOH exposure.

1. Sozo F *et al. Am J Physiol*, 296:L510–L518, 2009.
2. Gray SP *et al. Am J Physiol,* 295:R568–R574, 2008.

P-9C-422

Is pre- or post-natal secondhand smoke exposure associated with childhood overweight? Hong Kong's "Children of 1997" birth cohort

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Objective: Paternal smoking,[1,2] and perhaps pre-natal secondhand smoke (SHS) exposure,[3] may be modifiable causes of childhood overweight. However, social patterns of smoking and childhood overweight make such observations open to residual confounding. Studies from non-Western socio-historical contexts such as Hong Kong, where most women do not smoke (<4%), are valuable in clarifying whether the association between SHS exposure and childhood overweight observed in

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western societies is biologically-mediated or socio-economically confounded, and whether there are any critical windows for SHS exposure (i.e. prenatal vs. postnatal).

Methods: We used multivariable linear regression to examine the adjusted associations of SHS exposure with body mass index (BMI) and height z-scores at 7 years relative to the 2007 World Health Organization growth reference, among 6,713 children with non-smoking mothers (74% follow-up) from a population-representative, Hong Kong Chinese birth cohort, ''Children of 1997'', born in April and May 1997. We classified SHS into mutually exclusive categories representing increasing doses from different sources (paternal smoking or any household smoking). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Compared to infants with no pre-natal SHS exposure from non-smoking households, infants from non-smoking households exposed to pre-natal SHS had higher BMI z-scores, but not height, at 7 years (mean difference 0.08, 95% confidence interval (CI) 0.003 to 0.15) as did infants from households where fathers smoked daily (0.12, 95% CI 0.04 to 0.21), adjusted for sex, birth order, highest parental education and mother's place of birth.

Conclusions: Our findings, although preliminary, suggest that an association of pre-natal SHS exposure and paternal smoking with child overweight could possibly be biologically mediated, with the key exposure perhaps before birth. Given the known harms of smoking, reducing SHS exposure from conception as a precautionary action for childhood overweight might be warranted. *Acknowledgements*: We thank colleagues at the Student Health Service and Family Health Service of the Department of Health for their assistance and collaboration and Connie Hui for assisting the record linkage.

1. S.D. Leary *et al.*, *Obesity (Silver Spring)*, 14:2284–2293, 2006.
2. R. von Kries *et al.*, *Am. J. Epidemiol.*, 156:954–961, 2002.
3. A.M. Toschke *et al.*, *Eur J Pediatr.*, 161:445–448, 2002.

P-9C-423

Organochlorine compounds and rapid early growth: findings from the INMA Environment and Childhood Project

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Objectives: Rapid growth in the first months of life is a strong risk factor for long-term obesity, although relatively little is known about factors that promote this growth pattern. It has been hypothesized that chemicals with endocrine disrupting properties may influence obesity risk, but empirical data is limited. This paper explores associations between several organochlorine compounds (OCs) and rapid infant growth as a marker of long-term obesity risk.

Methods: Data come from the Spanish INMA-INfancia y Medio-Ambiente (Environment and Childhood) Project in Sabadell, Barcelona, a birth cohort which recruited 657 women during the 1st trimester of pregnancy with subsequent follow-up to assess infant health. Average daily weight gain during the first 6 months was estimated, and rapid growth defined as >0.67 SDs of weight gain/day. Multivariable logistic regression was used to evaluate odds of rapid growth associated with lipid-adjusted levels of several OCs measured in maternal first trimester serum (DDE, DDT, PCBs, bHCH and HCB), adjusting for covariates including birthweight, gestational age, child sex, breastfeeding duration, parity, and maternal age, education, smoking, and weight gain in pregnancy. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Overall, maternal OC levels were not significantly higher among rapid growers. Among mothers of normal pre-pregnancy weight (body mass index $<25\ kg/m^2$), however, the prevalence of rapid growth was significantly higher in the top (39.8%) vs. bottom (19.2%) quartile of maternal DDE ($P < 0.05$). While there was no association among infants of mothers overweight or obese prior to pregnancy, there was a significant positive association between maternal DDE levels and rapid growth among those born to mothers of normal weight (interaction $P < 0.05$ for overweight $\times$ DDE). Other contaminants were not meaningfully associated with rapid early growth.

Conclusions: This analysis suggests elevated maternal DDE levels may be a risk factor for rapid growth in infancy. This finding is consistent with two recent studies (Verhulst *et al*, 2009; Karmaus *et al*, 2009) which found intrauterine exposure to DDE, but not several other chemicals, to be associated with obesity later in life. Future studies are needed to better understand the potential role of environmental obesogens in the obesity epidemic, including their possible role in rapid early weight gain. Support: Spanish Ministry of Health, Instituto de Salud Carlos III, the Generalitat de Catalunya-CIRIT, and the European Union projects EARNEST FOOD-CT-2005-007036 and NewGeneris FOOD-CT-2005-01632.

1. Karmaus W *et al. Occup Environ Med*. 66:143–149, 2009.
2. Verhulst SL *et al. Environ Health Perspect*. 117:122–126, 2009.

P-9C-424

Long-term effects of maternal nicotine exposure during lactation on body adiposity, lipid profile and hypothalamic leptin signaling pathway

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Objective: Some studies have shown a strong relationship between stressful events (nutritional, hormonal or environmental) in early life and the development of adult chronic diseases. Epidemiological and experimental data show an association between maternal smoking and later obesity. Here, we studied whether maternal nicotine exposure during lactation influences the adiposity, leptin signaling pathway and others metabolic parameters in adult rat offspring.
Methods: On the 2nd day of birth, lactating rats were subcutaneously implanted with osmotic minipumps and divided into: 1) NIC - releasing nicotine (6 mg/Kg/day) during 14 days of lactation; 2) C, releasing saline for the same period. After weaning, offspring's body weight gain and food intake were evaluated until they were 180 days-old, when they were killed to evaluate serum hormones (leptin, insulin and corticosterone) by radioimunoassay as well as adiposity by computed tomography and histological analyses. We also determined glycemia, triglycerides, total cholesterol and its fractions (VLDL, LDL and HDL) and proteins of leptin signalling pathway in hypothalamus by Western blotting.
Results: 180 days-old NIC offspring presented higher body weight (+11%, $p < 0.05$), central and total adiposity (+32% and +36%, $p < 0.05$, respectively) as well as higher subcutaneous and epididymal adipocyte area, hyperleptinaemia (+38%; $p < 0.05$) and lower hypothalamic JAK-2 and pSTAT-3 expression (−41% and −56%, $p < 0.05$, respectively). These rats did not show any change on serum insulin, corticosterone, lipid profile and glucose levels.
Conclusions: We evidenced that early maternal nicotine exposure during lactation programes for obesity, hyperleptinaemia and probably for hypothalamic leptin resistance in adulthood. Thus, regarding neonatal exposure, we suggest that nicotine can be one of the tobacco compounds responsible for later central obesity (one of the components of the metabolic syndrome), but not for the other two components (insulin resistance and dyslipidemia). Support: CAPES, CNPq and FAPERJ.

P-9C-425

Maternal nicotine exposure during lactation programs adrenal function of rat offspring

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Objective: Maternal tobacco smoke during gestation programs for later obesity and hypertension. Previously, we showed that adult offspring whose mothers were nicotine exposed only during lactation developed obesity, hyperleptinaemia and hypothyroidism. As nicotine affect the medullary adrenal function as well as catecholamines and glucocorticoids have well-known effects on cardiovascular system, in the present study we evaluated the adrenal function of adult offspring whose dams were nicotine exposed during lactation.
Methods: On the 2nd day of birth, lactating rats were subcutaneously implanted with osmotic minipumps and divided into: 1) NIC - releasing nicotine (6 mg/Kg/day) during 14 first days of lactation; 2) C, releasing saline for the same period. Offspring were killed at 15 and 180 days-old. Total catecholamines (adrenaline and noradrenaline) content and "in vitro" caffeine-stimulated secretion were measured using the trihydroxyindole method. Adrenal tyrosine hydroxylase (TH) content was evaluated by Western blotting. Serum corticosterone was evaluated by radioimmunoassay.
Results: At 15 days-old, NIC pups presented higher catecholamine content (+41%, $p < 0.05$) and lower TH expression (−33%, $p < 0.05$). When adult, NIC offspring presented higher catecholamine content (+32%, $p < 0.05$) and TH expression (+38%, $p < 0.05$), but lower in vitro catecholamine release (−13%, $p < 0.05$). Serum corticosterone levels were not changed in these animals on both periods.
Conclusion: We evidenced that nicotine exposure during lactation induces short and long-term effects upon the adrenal medullary function. In suckling pups, maternal nicotine exposure seems to supress catecholamine production and release. In adult offspring, postnatal NIC exposure programs for higher catecholamine production. However, it seems that the caffeine-stimulated secretion of catecholamines by the adrenal are impaired. This programming effect could suggest a worse adaptation to a risk situation. Support: CAPES, CNPq and FAPERJ.

P-9C-426

Maternal nicotine exposure during lactation programs the hypothalamus-pituitary-thyroid axis of adult rat offspring

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Objective: Maternal tabacco smoke during gestation programs for later obesity[1,2]. Previously, we showed that adults offspring whose mothers were nicotine exposed only during

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lactation had obesity, hyperleptinaemia and secondary hypothyroidism. Leptin acts through JAK-2/STAT-3 signaling pathway[3] and stimulates the hypothalamic-pituitary-thyroid axes[4–;7]. Therefore, our aim was to evaluate the proteins of the leptin signaling pathway in the hypothalamic-pituitary-thyroid axis of adult rats whose dams were nicotine exposed during lactation.

Methods: After 48h from birth, lactating rats were implanted osmotic minipumps and divided into: NIC group (n = 8) - releasing nicotine (6 mg/Kg/day, s.c.) for 14 days; C group (n = 8) – releasing saline for the same period. Offspring were killed when they were 180 days-old. Proteins expressions of leptin pathway (Ob-R, JAK-2, STAT-3, phosphorilated STAT-3, SOCS-3) were analyzed by Western blotting.

Results: In hypothalamus, adult NIC offspring showed lower JAK-2 and pSTAT-3 contents (−42% and −56%, $p < 0.05$). In pituitary, there was no change on the leptin signaling pathway. In thyroid, NIC group presented higher Ob-R (+123%, $p < 0.05$), but lower pSTAT-3 (−33%, $p < 0.05$) and SOCS-3 expressions (−68%, $p < 0.05$).

Conclusions: The secondary hypothyroidism in NIC offspring can not be related to changes in leptin signaling pathway in pituitary gland. On the other hand, once leptin increases TRH, the lower proteins expression of leptin pathway in hypothalamus suggest a central leptin resistance that could explain the central origin of the hypothyroidism. In the thyroid, the lower pSTAT-3 and SOCS-3 suggest a lower leptin sensitivity. Thus, maternal tobacco smoke in lactation can affect the regulation of the thyroid axis by leptin through nicotine effects. Support Source: CNPq, FAPERJ e SBEM (Thyroid Department).

1. R. Von Kries *et al. Am J Epidemiol.,* 156:954–961, 2002.
2. M. Z. Goldani *et al. Braz J Med Biol Res.,* 40:1231–1236, 2007.
3. A. Sahu. *Endocrinol.*,145:2613–2620, 2004.
4. Ortiga-Carvalho *et al. J Endocrinol.,* 174:121–125, 2002.
5. L. Jin *et al. Endocrinol.*,141:333–339, 2000.
6. K. W. Nowak *et al. Int J Mol Med.,* 9:31–34, 2002.
7. O. Isosaki *et al. Endocrinol J.,* 51:415–423, 2004.

P-9C-427

Does folic acid supplementation in early pregnancy modify the associations of maternal smoking during pregnancy with fetal growth and birth weight? The Generation R Study

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Objective: Maternal smoking is associated with decreased fetal growth and subsequently lower birth weight. Folic acid supplement use shows opposite effects. The objective of this study was to examine the modifying effect of periconceptional folic acid supplement use on the associations of maternal smoking during pregnancy with fetal growth and birth weight.

Methods: This study was based on 6,265 mothers participating in a population-based prospective cohort study from early pregnancy onwards. Smoking habits were assessed by questionnaires in each trimester of pregnancy. Folic acid supplement use was assessed at enrolment in the study. Folic acid supplementation was categorized into optimal use (preconception start of supplementation), suboptimal use (start during first 10 weeks of pregnancy), and no use. Fetal head and abdominal circumference and femur length were repeatedly measured during pregnancy. Birth weight was obtained from midwife and hospital registries. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Of all mothers, 39.7% had optimal folic acid supplementation use and 31.1% suboptimal folic acid supplementation use. And 8.2% smoked in first trimester only and 16.7% continued smoking during pregnancy. Both continued smoking and suboptimal folic acid supplementation were associated with lower birth weight (both $p < 0.01$). We found a significant interaction between smoking and folic acid supplementation use ($p < 0.01$) suggesting that the adverse effects of smoking were reduced by folic acid supplementation. The largest protective effect of folic acid supplement use on estimated fetal weight was observed in third trimester among mothers who continued smoking. The beneficial effects on birth weight of optimal folic acid supplementation use was 60 (95% CI: 25, 94) grams among non smokers, 106 (95% CI: 24, 189) grams among first trimester only smokers and 75 (95% CI: 26, 123) grams among continued smokers. Adjustment for potential confounders did not materially affect the effect estimates.

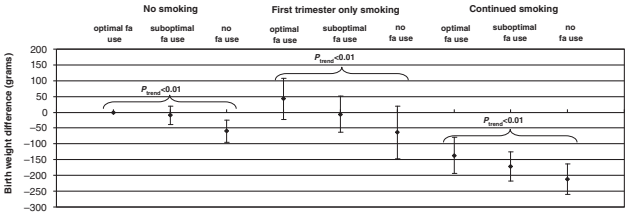

Figure 1: Differences in birth weight in different categories of smoking and folic acid (fa) supplementation use.

Conclusions: We can conclude from our results that the association between maternal smoking during pregnancy and birth weight is modified by folic acid supplementation use.

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In third trimester of pregnancy the same modification is found on estimated fetal weight. Further studies are needed to assess the underlying mechanisms.

P-9C-428

Interactions of prenatal and adulthood pesticide exposures, gender, and ageing: implications for the fetal basis of adult disease hypothesis in a murine model of Parkinson's disease

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Objective: Environmental insults during periods of neurodevelopment have been hypothesized to initiate or cause susceptibility to neurodegenerative processes, such as the degeneration of the nigrostriatal dopamine system seen in Parkinson's disease (PD). Using two pesticides that are associated with PD in epidemiological studies and in animal models, we've previously shown that prenatal exposure to the fungicide maneb (MB) increases susceptibility to the neurotoxic effects of adulthood paraquat (PQ) exposure. This multiple hit paradigm was hypothesized to be a useful model for investigating a relationship between PD and the developmental origins of adult disease hypothesis.
Methods: Pregnant C57Bl/6 mice were injected with saline or 2 mg/kg MB on gestation days 10–18. The large cohort of male and female offspring generated was divided into subgroups who were injected with saline or PQ (10 mg/kg) every-third-day from 7 to 13.5 weeks of age (total of 15 doses), and sacrificed at 3, 15, 30, or 45 weeks after the final injection (up to ~14 months of age); for one subset of animals, sacrificed at the latest time-point, open field activity assessments were made during the exposure period and coinciding with each of the other time-points.
Results: Late-appearing (not until 30-weeks post-PQ exposure) and persistent robust behavioral changes (e.g. ~30–40% reductions, relative to other groups, in ambulatory distance in a 1-hour session) were observed only in males with prenatal-MB+adulthood-PQ exposure, whereas females did not exhibit this phenotype. Analysis of striatal dopamine, the dopamine metabolites DOPAC and HVA, serotonin, and the serotonin metabolite HIAA did not reveal any significant effects of prenatal or adulthood exposure on levels of the parent neurotransmitters dopamine or serotonin; however, significant interactions of gender, prenatal exposure, adult exposure, and/or ageing were noted for DOPAC, HVA, dopamine turnover, HIAA, and serotonin turnover. The results suggest that whereas striatal neurochemical effects of adulthood PQ exposure in prenatal-saline exposed animals are transient (i.e. returning to normal within several weeks of cessation of exposure), males and females exposed to PQ in the context of prenatal-MB exposure show long-lasting disruptions (notable 45-weeks post-PQ). Activity of the 20S proteasome was also assessed in striatal tissue, and decreases were noted for prenatal-MB males at 3 weeks. At 45 weeks, however, prenatal-MB + adulthood-PQ males and females exhibited relative decreases in proteasome activity, with a greater effect seen in males, suggesting interactions of exposure history, gender, and ageing.
Conclusions: The results of this study, combined with other results in our lab, suggest that prenatal MB exposure increases the neurotoxic effects of later PQ exposure, that gender modulates the effect, and that ageing exacerbates these interactions. The emergence of a behavioral phenotype, disruptions in striatal dopamine and serotonin homeostasis, and striatal proteasome dysfunction are suggestive of a neurodegenerative process that may model PD or act as a risk factor for the disorder in the context of neurodevelopmental and adulthood environmental "multiple hits." Further work will determine the applicability of this model to linking the FeBAD hypothesis with PD. Support: NIH ES016277 and ES159792.

P-9C-429

Prenatal exposure to cadmium induces long term vascular plasticity in rats

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An adverse environment during intrauterine life may be predictive of several pathologies in adult life including cardiovascular (CV) disease[1]. We have recently demonstrated that prenatal exposure to cadmium (Cd^{2+}), a ubiquitously distributed environmental toxicant, and main component of tobacco smoke induces endothelial dysfunction and cardiac hypertrophy at adult age, constituting adaptive responses to maintain cardiac function (A.M. Ronco *et al*, *unpublished*). In this work we hypothesized that, in addition to those described effects, prenatal exposure to Cd^2 also induces cardiovascular plasticity at adulthood through mechanisms involving oxidative stress.
Objective: to study the effect of prenatal exposure to Cd^{2+} in cardiac response to induced experimental myocardial infarction performed at adult age, and whether this is associated to the expression of aortic heme-oxygenase (HO-1), a gene induced by agents that cause oxidative stress.
Methods: virgin female Wistar rats were mated and then treated with 30 ppm of Cd^{2+} (as $CdCl_2$ in drinking water) during the whole pregnancy period. Birth weight and size

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were first measured and, animals were left growing under normal conditions of distilled water and food *at libitum* until 60 days old. At this age, experimental myocardial infarction was produced by ligation of the left coronary artery as previously described[2]. After 72 h post ligation, the size of the infarction was measured with the triphenyltetrazolium staining technique and the infarct region was measured by planimetry. The magnitude of the infarction was expressed as percentage of the volume of the infarct region related to the total cardiac volume. The cardiac function post infarction was evaluated by echocardiography. Cardiac histology was analyzed in hearts fixed in 4% paraformaldehyde, embedded and cut into 4 μm sections, and HO-1 expression in aortic tissues was determined by real time PCR.

Results: birth weight and size was not affected by the Cd^{2+} treatment. At 60 days old, the Cd^{2+}- treated group showed a significantly lower size infarct region than the control group, with percentages of 7.1 ± 1.5% vs 19.6 ± 2.8% of damaged tissue respectively (n = 7). This result was corroborated by echocardiographic analysis showing no effect in the LV ejected volume (LVEV; 0.21 ± 0.06 vs 0.19 ± 0.05 ml/beat) and in the fractional shortening % (FS; 52 ± 7 vs 51 ± 7) for control and Cd^{2+}-treated postinfarction group respectively. Histological analysis revealed increased vascularization of cardiac tissue. Also, the Cd^{2+}-treated group showed increased expression of HO-1, indicating a protective vascular response against oxidative stress induced by Cd^{2+}.

Conclusions: prenatal exposure to Cd^{2+} induces oxidative stress leading to foetal adaptive responses to maintain cardiac function at least until 60 days old. These responses including increased expression of HO-1 and vascularization in vascular tissues may represent compensatory mechanisms against the oxidative stress insult during foetal development. Support: Fondo Nacional de Ciencia y Tecnología (Chile) Fondecyt N° 1071110.

1. C. Gicquel *et al., Best Pract Res Clin Endocrinol Metab*. 22:1–16, 2008.
2. H. Yin *et al., J Biol Chem* 280:8022–8030, 2005.

P-9C-430

Maternal arsenic exposure, impaired glucose tolerance during pregnancy, and early programming of child insulin resistance and obesity

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Objective: Accumulating evidence has shown an increased risk of Type 2 diabetes in general populations with arsenic exposure, but little is known about exposures during pregnancy and the association with gestational diabetes (GD). Infants born to mothers with GD are at increased risk of adverse birth outcomes, subsequent impaired glucose tolerance, and obesity. We studied 532 pregnant women and their newborn infants living proximate to the Tar Creek Superfund Site in Ottawa County, Oklahoma (USA) to investigate if arsenic exposure is associated with impaired glucose tolerance during pregnancy and adverse infant birth and growth outcomes.

Methods: Maternal blood and hair were collected at delivery and analysed for arsenic concentration using inductively coupled plasma mass spectrometry with dynamic reaction cell (DRC-ICP-MS). Maternal plasma glucose was measured between 24–28 weeks gestation after a 1-hour oral glucose tolerance test (GTT) as part of routine prenatal care. Infants born to these women are being followed from birth for growth and developmental outcomes. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Maternal arsenic concentrations ranged from 0.2 to 24.1 μg/L (ppb) (mean ± SD, 1.7 ± 1.5) and 1.1 to 724.4 ng/g (ppb) (mean ± SD, 27.4 ± 61.6) in blood and hair, respectively. One-hour plasma glucose levels ranged from 40 to 284 mg/dL (mean ± SD, 108.7 ± 29.5); impaired glucose tolerance was observed in 11.9% of women when using standard screening criterion (>140 mg/dL). Adjusting for age, Native-American race, pre-pregnancy body mass index, Medicaid use, and marital status, women in the highest quartile of blood arsenic exposure had 2.8 higher odds of impaired GTT than women in the lowest quartile of exposure (95% confidence interval, 1.1–6.9) (p-trend = 0.008). Neonatal plasma glucose levels ranged from 25 to 130 mg/dL (mean 69.6 ± 17.3); 6% of infants were born with hypoglycemia (<45 mg/dL) which is often a consequence of being born to a diabetic mother. At birth, almost 7% of infants were greater than 90th percentile for weight; this increased to 25% and 28%, respectively, at 12 and 24 months of age.

Conclusions: Among this population of pregnant women, arsenic exposure was associated with increased risk of impaired GTT at 24–28 weeks gestation and, therefore, may be associated with increased risk of GD (1). Prenatal exposures may also trigger early programming events related to the risk of insulin resistance and obesity in offspring. Better understanding of mechanisms responsible for development of obesity and identification of modifiable risk factors such as environmental exposures, diet, and activity patterns, may lead to efforts at primary prevention. Acknowledgements: Dr. Ettinger was supported by US National Institute of Environmental Health Sciences (NIEHS) grant number: K01-ES014907. This study was supported by NIEHS grant numbers: P42-ES05947; P30-ES00002; P01-ES012874; R01-ES014930; R01-ES013744; and US Environmental Protection Agency (EPA) STAR Research Assistance Agreement No. RD-83172501.

1. Ettinger AS, *et al. Environ Health Perspect*, 117: 1059–1064, 2009.

P-9C-431

Maternal smoking during pregnancy and blood pressure levels in early adulthood - intrauterine effect or familial confounding?

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Objective: To investigate the association between maternal smoking during pregnancy and systolic and diastolic blood pressure levels in offspring in early adulthood. Furthermore, we aimed to investigate if found associations could be explained by familial confounding.
Methods: The study population consisted of all men born between 1982 and1988 who were conscripted for military service in Sweden in 2000–2006. Information about maternal smoking during pregnancy, birth and parental characteristics was obtained from the Swedish Medical Birth Registry and merged with information about the offspring's blood pressure levels from the Conscript Registry. To address the question of familial confounding we identified brothers by using the Multigeneration Registry and compared blood pressure levels in brothers where only one had been exposed to smoking during gestation. We performed logistic regression analyses to estimate β -coefficients (with 95% confidence intervals). Appropriate institutional ethics committee clearance was obtained.
Results: Smoking during pregnancy was associated with an increase of diastolic blood pressure. The association was attenuated after adjustment for potential confounders, including body-mass index, but remained significant; $\beta = 0.24$ (95% CI; 0.03–0.44) for moderate smokers (1–9 cigarettes per day) and $\beta = 0.46$ (95% CI; 0.19–0.73) for heavy smokers (≥10 cigarettes per day). However, the fully adjusted within-sibling analyses showed no significant effect on diastolic blood pressure $\beta = 0.055$ (95% CI; −0.005–0.114). Neither did we see an effect on systolic blood pressure in sons to mothers who had been smoking during pregnancy.
Conclusions: Maternal smoking during pregnancy was associated with an increase of diastolic blood pressure in early adulthood. However, as there were no significant association within brothers in the sibling analyses, this increase in diastolic blood pressure could be due to familial confounding rather than an effect of maternal smoking *per se.* Support: The Swedish Research Council (grant nr K2007-70X-20510-01-4).

P-9C-432

Assessing Bisophenol A exposure in long term stored urine specimens

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Bisphenol A (BPA), an estrogenic contaminant has been linked to adverse developmental effects in laboratory animals. Evaluating developmental effects of this compound in humans is logistically difficult and expensive. Assessing BPA in urine samples already collected for fecundability or gestational studies carried on in the past might be a promising method to advance our understanding of the effects of BPA on human development.
Objective: To assess the long term stability of BPA. As the half-life of BPA is <6 hours, we also decided to evaluate within-person temporal variability in environmental exposure to BPA.
Methods: We evaluated BPA levels in samples from the North Carolina Pregnancy Study, which included daily urine specimens collected 22–24 years ago.
Results: Total BPA concentration was measured by mass spectrometry in first-morning urine samples from 60 women of reproductive age. We analyzed three urine samples collected approximately two and four weeks apart. To assess cycle effects, we evaluated specimens from both the follicular and luteal phases of the menstrual cycle. Temporal variability was assessed with mixed model regression and correlations. In our sample, the phase of the menstrual cycle was not associated with urinary levels of BPA. We observed a slight, yet not statistically significant, increase in BPA levels during the three-year collection period. The inter-quartile range was: 1.1 to 3.1 ng/mg (BPA/creatinine), slightly higher than the levels observed in NHANES specimens collected 3–11 years later. Samples collected 2 weeks apart were more closely correlated than those collected 4 weeks apart (Spearman rho = 0.5 and 0.3 respectively).
Conclusions: The similarity between the BPA levels measured in our sample and those reported by NHANES, plus the association between BPA levels and the length of the sampling intervals, suggest that BPA is relatively stable during long-term freezer storage. The correlations imply that over periods of weeks BPA exposures is relatively stable. Our results suggest that urine stored over prolonged time periods could be use to study developmental effects of BPA exposure.

P-10A-433

Influence of breastfeeding, gestational and perinatal variables on medicine use at the ages of 3, 12 and 24 months: the 2004 Pelotas (Brazil) Birth Cohort Study

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Objectives: To evaluate the effect of breastfeeding, gestational and perinatal variables, on medicine utilization among children aged 3, 12 and 24 months.
Methods: Standardized questionnaires were administered to mothers of children belonging to the 2004 Pelotas (Brazil) Birth Cohort Study at the ages of 3, 12 and 24 months. Medicine utilization in the 15 days prior to each interview was assessed. The medicines packaging were requested to collect detailed information on each medication reported. Independent variables included: delivery complications or health problems (yes/no), utilization of neonatal intensive care units (yes/no), low birth weight (<2500 g), birth order, type of delivery, maternal morbidity during pregnancy (yes/no), feeding patterns at 3, 12 and 24 months of age (never breastfed, breastfed in the past but not at the time of the interview, still breastfed). After unadjusted analyses, all associations were tested using Poisson regression, including adjustment for confounding variables based on a theoretical framework. Confounders included in the regression were: sex, skin color, maternal age and schooling, socioeconomic status and health insurance. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Sample sizes were 3,985 at 3 months, 3,907 at 12 months, and 3,868 at 24 months. The prevalence of medicine utilization within the 15 days prior to the interview was around 65% at ages 3 and 12 months, and around 55% at 24 months of age. Variables significantly associated with medicine use at 3 months of age only were: health problems taking place at delivery (prevalence ratio (PR) 1.10; 95%CI 1.02:1.18), low birthweight (PR 1.11; 95%CI 1.02:1.21) and no breastfeeding (PR 1.50; 95%CI 1.22:1.84). Firstborns were 10% more likely than others to use medicines at 3 and 12 months of age. Those admitted to neonatal intensive care units at birth were 16% more likely to use medicines at 24 months of age. Children born by C-section were 5% and 7% more likely than others to use medicines at 12 and 24 months of age, respectively. Maternal morbidity during pregnancy was associated with 16% and 12% higher use of medicines at 3 and 24 months, respectively.
Conclusions: Medicine use, an indicator of overall health status, is, at least, partially programmed by factors operating in early life. Future follow-up visits of the same cohort will enable us to test the long-term effect of these exposures on medicine use throughout childhood, adolescence and adulthood.

P-10A-434

Perinatal iron deficiency programs long-term metabolic dysfunction and blood pressure dysregulation

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Objective: The World Health Organization estimates that more than 66% of the global population has some degree of iron deficiency (ID). Importantly in the context of the developmental origins of health and disease, the populations most are risk are pregnant women and those of child-bearing ages. ID during gestation has been reported to cause hypertension in adult offspring, although the underlying mechanisms have not been elucidated. Given that results from the Framingham study suggest that 78% of essential hypertension in men have a component directly attributed to obesity, we investigated the long-term cardiovascular consequences of perinatal ID (PID), and whether these were associated with metabolic dysfunction. Specifically, we determined the effect of PID on (i) weight gain and food consumption, (ii) visceral adipose tissue (VAT) deposition (iii) locomotor activity, and (iv) systemic and renal hemodynamic properties.
Methods: Female Wistar rats were fed a purified low-iron diet (3 mg/kg Fe) prior to and throughout gestation, and a control diet (225 mg/kg Fe) thereafter; control rats were fed the control diet throughout the entire study. At 12 wk, offspring were instrumented with radiotelemetric transducers for continuous assessments of locomotor activity and hemodynamic parameters. At 36 wk, rats were killed and visceral fat pads were removed and weighed.
Results: Offspring of iron deficient mothers had significantly reduced hematocrits (PID: 0.227 ± 0.028 vs. Control: 0.383 ± 0.032; $P < 0.001$) and bodyweights (PID: 5.4 ± 0.2 g vs. Control: 6.1 ± 0.1 g; $P < 0.001$) compared to controls at birth. These differences in bodyweight persisted into adulthood. However, despite having lower body weights, PID offspring had 15% greater VAT deposition ($P < 0.001$), and were 25% less active in their home cages than their control counterparts ($P < 0.001$). In a parallel series of experiments, imposition of a high-fat diet (40% fat energy) exacerbated the differences in VAT deposition between adult control and PID offspring. Mean arterial pressures, as assessed by radiotelemetry from 10 weeks of age onward, were significantly elevated in the PID group compared to controls ($P < 0.05$), and exhibited a nearly 2-fold greater change in response to altered sodium intake ($P < 0.05$). Furthermore, in anaesthetized rats, we determined that the relationship between renal artery pressure and renal interstitial hydrostatic pressure (RIHP), which represents a fundamental control mechanism of sodium excretion, was blunted by 34% in the PID group compared to controls ($P < 0.001$).
Conclusion: PID results in increased visceral adiposity and altered energy metabolism, which is associated with enhanced blood pressure responsiveness to dietary salt. Whether these two phenotypes are mechanistically linked in this highly relevant model of developmental programming is currently being investigated. Support: The Canadian Institutes of

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P-10A-435

Trypsin effect in heme iron bioavailability in humans

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It has been established that meat present in the diet increases heme iron bioavailability[1]. There is a lack of information regarding the effect that some of the proteins found intraluminally may have. Recent studies using the Caco-2 cell model have shown that Trypsin increases heme iron absorption[2]; however, there is no evidence that this also occurs in humans.

Objective: To establish the effect of the Trypsin in the bioavailability of heme iron in humans.

Methods: Fourteen apparently healthy women between 35–45 years of age were selected to participate in one iron absorption study. Informed consent was obtained from all the volunteers prior to the absorption studies. The women received 5 mg of iron as heme intrinsically labeled with either 3 uCi 55Fe or 1 uCi 59Fe plus 1.7 g of mucin. On days 1, 2, 14 and 15 the subjects intake heme alone, heme + Trypsin, partially digested hemoglobin + Trypsin, partially digested hemoglobin + mucin + Trypsin, respectively. On days 14 and 28 blood samples were collected to assess iron status and to determine the amount of circulating radioactivity using the Eakins and Brown double isotope technique. Repeated measures ANOVA was used to compare the mean percent bioavailability of iron from the test meals.

Results: Geometric mean (range ± 1SD) of heme iron absorption was de 5.1% (3.1–8.3), 2.9% (1.6–5.1), 7.3% (4.1–13.1) y 6% (2.7–13) for heme alone, heme + Trypsin, partially digested hemoglobin + Trypsin, partially digested hemoglobin + mucin + Trypsin, respectively (ANOVA, $p < 0.05$).

Conclusion: Heme iron bioavailability in humans improves in presence of the globin degradation products by Trypsin. Support: Fondecyt Grant 1061060.

1. M. Layrisse, C. Martinez-Torres. *Am J Clin Nutr.* 25:401–441, 1972.
2. Uc *et al. Am J Physiol,* 287:G1150–G1157, 2004.

P-10A-436

Maternal supplementation with vitamin A or β-carotene and cardiometabolic risk among pre-adolescent children in rural Nepal

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Objectives: Vitamin A deficiency is common among women in developing countries. While vitamin A plays an important role in fetal renal and cardiovascular development, there has been little research on the effects on vitamin A or β-carotene during pregnancy on cardiovascular risk later in life.

Methods: Women of reproductive age were enrolled in a cluster-randomized, double blind, placebo-controlled trial of weekly supplementation with 7000 μg retinol equivalents of preformed vitamin A or β-carotene from 1994–1997 in rural Nepal. Women received their assigned supplements before, during and after pregnancy. Over a study period of three years, 17,361 infants were born to women enrolled in the trial. In 2006–2008, we revisited and assessed 13,171 children aged 9–13 y to examine impact of maternal supplementation on early biomarkers of chronic disease. Blood pressure was measured 3 times using an automated oscillometric device on all children. Among a subsample of 1390 children, venous blood was collected for plasma glucose (fasted), Hb1Ac and lipids and a morning urine specimen was collected to measure the ratio of microalbumin/creatinine. Detailed anthropometry was also conducted. Appropriate institutional ethics committee clearance and participants' informed consent were obtained. Results: The mean ± SD age of children at follow-up was 10.9 ± 0.8 y. Their mean ± SD systolic and diastolic blood pressure was 97.2 ± 8.2 and 64.6 ± 8.5 mm Hg, respectively. About 5.0% had high blood pressure (≥120/80 mm Hg), 4.7% in males and 5.4% in females. Blood pressure rose with age and was higher among girls than boys (Figure 1). Children had a mean ± SD BMI of 14.5 ± 1.3 kg/m^2 and waist circumference of 55.4 ± 3.7 cm. The 90th percentile cutoff for BMI and waist circumference in this population was 16.08 kg/m^2 and 60.1 cm, respectively. The mean HbA1c was 5.1 ± 0.30% and fasting glucose was 72 mg/dl (IQR: 65–78). The prevalence of microalbuminuria (≥30 mg/g creatinine) was 4.8%; 3.5% in males and 6.2% in females. High triglycerides (≥150 mg/dl) and low HDL (<40 mg/dl) were 9 and 85%, respectively. There were no treatment group differences in mean blood pressure, fasting glucose, HbA1c, triglycerides, and total, or HDL-cholesterol overall and after adjustment for age and gender. Nor were there differences between groups in the risk of hypertension, microalbuminuria or dyslipidemia in unadjusted and adjusted models. There was evidence of a significant interaction between treatment group and fatness: prenatal vitamin A or β-carotene supplementation protected against the risk of hypertension among children who had a high waist circumference, although not with high BMI. There were no apparent interactions between treatment group and gender on either of these outcomes.

Conclusions: Maternal supplementation with vitamin A or β-carotene had no overall impact on cardiovascular risk

in this population of pre-adolescent children in rural Nepal, but may be protective against hypertension if the children become overweight. *Acknoedgements:* The authors acknowledge funding from the Bill and Melinda Gates Foundation, Seattle, WA.

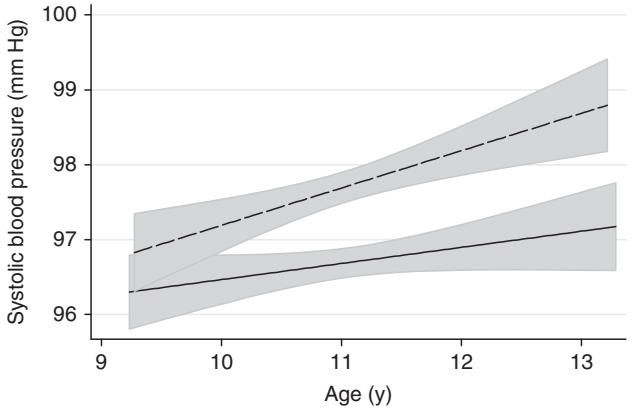

Figure 1: Systolic blood pressure differs by age and sex among children in rural Nepal.

P-10A-437

Blood pressure and kidney function at 4.5 years of age in the offspring of the MINIMat trial: effect of maternal food and multiple micronutrient supplementation

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Objective: To investigate the impact of improved prenatal nutrition on blood pressure in rural Bangladesh by following offspring born during the Maternal and Infant Nutrition Interventions in Matlab (MINIMat) trial.
Methods: Mothers had been randomised in early pregnancy (week 10 gestation) and were either encouraged to access Government food provision as soon as possible or they were given no such promotion and were expected to access these services later in pregnancy, which is the usual care in this community. The food packets provided an additional 600kcal per day. In addition, mothers had been randomised at week 14 of gestation to receive daily supplements containing either 30 mg Fe + 400 μg folate (Fe30F); 60 mg Fe + 400 μg folate (Fe60F); or a multiple micronutrient supplement (MNS) containing 15 vitamins and minerals at around the recommended daily allowance. The offspring were recruited into the follow-up study when they were 4.5 years old. Blood pressure was assessed in triplicate using an automated device (Omron 705IT). Kidney function was assessed in a sub-sample of children by ultrasound measurement of size and volume (n = 1148) and via glomerular filtration rate calculated from laboratory analysis of plasma cystatin c (n = 1334). Appropriate institutional ethics committee clearance and participants' informed consent were obtained prior to fieldwork.
Results: Blood pressure was assessed for 2312 children who had been born at term, representing 71% of the original live singleton births during the trial. After adjustment for age, sex, wealth index, gestational age and season of birth, no difference was observed for systolic blood pressure in relation to food (mean difference: 0.42 mmHg; CI: −0.21, 1.05; p: 0.19) or micronutrient (mean difference MNS vs both Fe groups: 0.004 mmHg; −0.66, 0.67; p: 0.99) intervention. Diastolic blood pressure was higher in individuals whose mothers had followed the usual care and accessed food provision later in pregnancy (mean difference: 0.57 mmHg; CI: 0.03, 1.11; p: 0.04). There was no difference in diastolic blood pressure in relation to micronutrient supplementation (mean difference: 0.42 mmHg; CI: −0.15, 0.99; p: 0.15). Neither kidney volume nor glomerular filtration rate were found to be affected by either of the maternal interventions.
Conclusions: In contrast to an earlier study in Nepal[1], we found no evidence that maternal micronutrient supplementation was related to offspring systolic blood pressure. However, mothers who had been promoted to access additional food packages early in pregnancy had offspring with slightly lower diastolic blood pressure at 4.5 years of age. The importance of such a marginal difference is debatable but may suggest childhood health improvements that could be promoted by simple community interventions traditionally designed to impact on maternal and newborn health.

1. A. Vaidya *et al. Lancet*, 371:492–9, 2008.

P-10A-438

Vitamin B_{12} and folic acid supplementation and plasma total homocysteine concentrations in pregnant Indian women with low B_{12} and high folate status

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Objective: Maternal vitamin B_{12} deficiency and hyperhomocysteinemia predict poor pregnancy outcome, foetal adiposity and insulin resistance. There is little appreciation of the widespread maternal vitamin B_{12} deficiency amongst practicing clinicians and policy makers in India. We investigated

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details of vitamin supplements, and circulating concentrations of vitamin B_{12}, folate, and total homocysteine in pregnant Indian women.

Methods: We enrolled 184 (94 rural, 90 urban) pregnant women, 163 (86 rural, 77 urban) in this observational, non-intervention study, were measured 3 times (18, 28, 34 weeks gestation). History of dietary intake and supplements was obtained. From the information provided at each visit total supplemented dose of B_{12} and folic acid received up to the last study visit (34th week) was calculated. Three groups were formed according to supplementation; those receiving no supplementation, those receiving only folic acid, and those receiving B_{12} with folic acid.

Results: At enrolment 80% rural and 65% urban women had low vitamin B_{12} concentration; two rural women had low folate concentration. During pregnancy 95% of urban and 85% rural women received folic acid; 84% urban and 12% rural women also received vitamin B_{12} in combination with folic acid (both $p < 0.001$ compared to urban). In women receiving no supplementation ($n = 17$) plasma vitamin B_{12} and folate did not change from 17 to 34 weeks of pregnancy and homocysteine increased ($p < 0.05$). Homocysteine concentrations at 34 weeks gestation in women receiving only folic acid ($n = 71$, mean 8.4 (95% CI 7.8, 9.1) μmol/L) were comparable to the unsupplemented group ($n = 17$, 9.7 (7.3, 12.7), $p = 0.15$), but women who received vitamin B_{12} supplement dose of >1000 μg ($n = 42$, all with folic acid) had lower concentrations (6.7 (6.0, 7.4), $p < 0.001$, compared to the unsupplemented group. Increasing dose of vitamin B_{12} ($r_s = -0.31$, $p = 0.006$) but not of folic acid ($r_s = -0.19$, $p = 0.11$) was associated with lower plasma total homocysteine concentration.

Conclusions: In vitamin B_{12} insufficient, folate replete pregnant women, vitamin B_{12} supplementation is associated with a reduction of plasma total homocysteine concentration in late pregnancy. Our results suggest that vitamin B12 supplementation may be used to balance 1-C metabolism in Indian mothers.

Support: International Atomic Energy Agency (IAEA).

P-10A-439

Associations of long chain polyunsaturated fatty acid concentrations with birth outcome and gender in term Indian mothers

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Objective: Our earlier studies have shown that mothers delivering preterm babies have lower plasma and erythrocyte docosahexaenoic acid levels as compared to term babies. There is a need to examine these long chain polyunsaturated fatty acids (LCPUFA) levels in low birth weight babies born at term. The purpose of the study was to examine the maternal and cord LCPUFA concentrations and their associations with birth outcome in term deliveries.

Methods: Pregnant women ($n = 253$) delivering at term were recruited at Bharati hospital Pune, India. They were divided into two groups based on their babies' birth weights 1) normal birth weight (NBW) i.e. ⩾2.5 kg ($n = 188$) and 2) low birth weight (LBW) i.e.<2.5 kg ($n = 65$). Each group was further divided into two groups according to the baby's sex i.e. male NBW (M-NBW), female NBW (F-NBW), male LBW (M-LBW) and female LBW (F-LBW) groups. The LCPUFA were estimated using the gas chromatograph. The omega 3 fatty acids included alpha linolenic acid, eicosapentaenoic acid and docosahexaenoic acid while omega 6 fatty acids included linoleic acid, gamma linolenic acid, di-homo-gammalinolenic acid, docosapentaenoic acid and arachidonic acid. The study was approved by institutional ethics committee and participants' informed consent was obtained.

Results: Maternal plasma docosahexaenoic acid concentration was lower ($p<0.05$) in LBW (1.2 ± 0.4 g/100 g fatty acids) as compared to NBW (1.3 ± 0.5 g/100 g fatty acids) group. Similarly maternal erythrocyte arachidonic acid levels were lower ($p < 0.05$) (13.3 ± 2.6 g/100 g fatty acids) in LBW group as compared to NBW group (14.5 ± 2.8 g/100 g fatty acids). In contrast, cord plasma docosahexaenoic acid was higher ($p < 0.05$) (2.4 ± 0.9 g/100 g fatty acids) in LBW as compared to NBW (2.1 ± 0.8 g/100 g fatty acids) group. Similarly cord erythrocyte arachidonic acid (19.3 ± 3.5 g/100 g fatty acids) levels were also higher ($p < 0.01$) as compared to NBW groups (17.4 ± 2.6 g/100 g fatty acids). Lower ($p < 0.05$) plasma (1.29 ± 0.49 g/100 g fatty acids) docosahexaenoic acid concentrations were seen in mothers delivering male NBW babies as compared to mothers delivering female NBW babies (1.40 ± 0.50 g/100 g fatty acids). Cord plasma docosahexaenoic acid ($n = 160$, $r = -0.152$, $p = 0.05$) and erythrocyte arachidonic acid ($n = 153$, $r = -0.236$, $p = 0.003$) were negatively associated with birth weight.

Conclusions: Reduced maternal and increased cord LCPUFA levels may indicate an efficient preferential transport especially of arachidonic acid and docosahexaenoic acid to LBW fetuses. A differentially altered LCPUFA status in mother due to altered metabolism may significantly influence the birth outcome.

P-10A-440

Folic acid, vitamin B12 and omega 3 fatty acid metabolism and levels of homocysteine in pre-eclamptic women

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Objectives: Elevated plasma homocysteine has been implicated in vascular changes and oxidative stress contributing to endothelial dysfunction in pre-eclampsia. Our earlier studies have shown increased oxidative stress leading to reduced docosahexaenoic acid (DHA) levels in pre-eclamptic women. In animals we have shown that maternal folic acid supplementation alters brain essential polyunsaturated fatty acid levels especially omega 3 fatty acids in the offspring. Folate and vitamin B_{12} are the major determinants of one carbon metabolism in which S-adenosyl methionine (SAMe) is formed which helps to maintain methyl group supply for various macromolecules like DNA, neurotransmitters and membrane phospholipids. The present study for the first time examines the levels of key micronutrients (folic acid, vitamin B_{12}) and omega-3 fatty acids, vital components of one carbon metabolism and resultant homocysteine concentrations in pre-eclamptic women and compares it with normotensive women.

Methods: 49 pre-eclamptic and 57 normotensive women were recruited at Bharati hospital, Pune, India. Plasma folate and vitamin B_{12} levels were estimated by the fluorescence polarization immunoassay while homocysteine estimation was performed by the micro particle enzyme immunoassay method. The important long chain polyunsaturated fatty acids were estimated using the gas chromatograph. The omega 3 fatty acids included alpha linolenic acid, eicosapentaenoic acid and docosahexaenoic acid while omega 6 fatty acids included linolenic acid, gamma linolenic acid, di-homo-gammalinolenic acid, docosapentaenoic acid and arachidonic acid. The study was approved by institutional ethics committee and participants' informed consent were obtained.

Results: Mean levels of homocysteine were significantly elevated in pre-eclamptic women (14.28 ± 7.31 μmoles/L) as compared to normotensive women (11.11 ± 4.37 μmoles/L) ($p = 0.007$) despite similar levels of both folic acid and vitamin B_{12}. Erythrocyte DHA concentrations were lower in the pre-eclamptic women (3.87 ± 0.91 g/100 g fatty acids) as compared to normotensive women (3.45 ± 1.05 g/100 g fatty acids), ($p < 0.05$). After adjusting for age, BMI and gestation folic acid levels showed a positive association ($n = 38$, $r = 0.317$; $p = 0.046$) with maternal omega 6 levels while erythrocyte omega 3 levels ($n = 38$, $r = -0.453$; $p = 0.003$) were negatively associated with plasma homocysteine concentrations.

Conclusions: Increased folate concentrations during pregnancy may lead to increase in omega 6 fatty acids which are pro inflammatory and may promote vasoconstriction. Our study for the first time provides evidence for the associations of altered omega-3 fatty acids especially DHA, metabolism and resultant increased homocysteine concentrations in pre-eclampsia.

P-10A-441

Anaemia during pregnancy in the Arauco province, Chile

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Objective: Anaemia caused by iron deficiency is one of the main nutritional problems in the world. Recent studies in two of the most important cities in Chile, Santiago[1] and Concepcion[2], indicate that anaemia prevalence in pregnant women was 13% and 14.5%, respectively. The prevalence of maternal anaemia in other parts of the country is unknown. The Arauco province is located in the south of the country. It has the highest indicators of poverty and the largest proportion of indigenous population, the so-called mapuche people, in Chile. The aim of this study is to establish the prevalence of anaemia in pregnant women of this province and its association with maternal nutritional status.

Methods: Prospectively collected anonymous data from pregnant women who initiated their pregnancies and attended prenatal clinics of the Arauco province during 2006 was used. The CDC criterion[3] was applied to diagnose anaemia. This criterion allows for the effect of gestational age on anaemia diagnosis and permitted to analyse just first haemoglobin data available coming from different weeks of gestation. The Rosso-Mardones[4] curve was used to determine maternal nutritional status and statistical differences between means and proportions were calculated using the t-student and ANOVA. Appropriate institutional ethics committee clearance was obtained.

Results: The 1305 women included, presented the following mean variables: a) gestational age (weeks) at the first haemoglobin test: 13.9 ± 6.9; second test: 29.5 ± 10.1; third test: 36.5 ± 12.4. b) The haemoglobin (g) values at the first test were: 12.3 ± 1.1; at the second test: 11.6 ± 1.1; and at the third test: 12.2 ± 6.9. c) The proportion of cases without haemoglobin data at the first test were: 13.9%; at the second test: 25.5%; and at the third test: 75%. Anaemia during pregnancy was mostly diagnosed using haemoglobin data from the first blood test (86.1%); few cases provided haemoglobin data from the second and third blood tests. Anaemia prevalence diagnosed in the complete cohort of pregnant women of the province of Arauco reached 21%. When this prevalence was distributed by maternal nutritional status, there was not statistically significant differences between categories, although there was a tendency to be higher in low weight for height mothers ($p = 0.06$).

Conclusions: Anaemia prevalence at the beginning of pregnancy could be fairly diagnosed, adding a few cases with a higher gestational age to complete the information. The prevalence of anaemia in that period is larger in the Arauco province than two recently studied cities of Chile. This is in agreement with above mentioned socio-economic data and demands to improve

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prevention activities. Prospectively collected data from pregnant women during 2007 will be added to this information in the future and may improve the analysis presented.

1. F. Mardones *et al.*, *Rev Med Chile*, 131:520–25, 2003.
2. F. Mardones *et al.*, *Arch Latinoam Nutr*, 58:132–8, 2008.
3. Institute of Medicine, National Academy of Science. Nutrition During Pregnancy. Washington, DC: National Academy Press, 1990.
4. F. Mardones, P. Rosso. *Matern Child Nutr,* 1:77–90, 2005.

P-10A-442

Vitamin D deficiency in pregnancy and risk of cesarean section in rural India

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Objective: Vitamin D plays an important role in promoting skeletal growth and maintaining muscle strength. A recent study[1] in US has demonstrated that vitamin D deficiency is a risk factor for cesarean section in pregnancy. We investigated the association between maternal vitamin D status in pregnancy and risk of a cesarean delivery in the Pune Maternal Nutrition Study (PMNS).
Methods: The PMNS database has information on maternal pre-pregnancy size, socio-economic status (SES), education, food intake (24-h recall, food frequency questionnaire), physical activity and circulating levels of nutrients and metabolites (glucose, lipids) during pregnancy. We also measured maternal serum 25-hydroxy vitamin D [25(OH) D] (vitamin D) at 28 weeks gestation by ELISA method. Mothers with concentrations below 20 ng/mL were considered vitamin D deficient[2]. Delivery details (mode of delivery, complications if any) were recorded. Women were classified into 3 groups according to mode of delivery: normal vaginal, assisted vaginal and cesarean section. Neonatal size was measured within 72 h of birth. The data were compared between the three groups using analysis of variance. Risk of cesarean section was analysed using multiple logistic regression. Appropriate institutional ethics committee clearance and participants' consent were obtained.
Results: Of 780 deliveries, 727 (93.2%) were normal vaginal, 19 (2.4%) assisted vaginal and 34 (4.4%) cesarean section (22 primary). There was no difference in age and SES between the three groups, however mothers who delivered by cesarean section were more educated ($p = 0.01$), had higher BMI ($p = 0.03$) and higher hip circumference ($p = 0.01$) before conception. Plasma glucose concentrations were similar in the 3 groups. Twenty two percent of women had vitamin D deficiency. Cesarean women had lower vitamin D concentrations (median 21.1 ng/mL) compared with the other two groups (26.2 ng/mL, $p = 0.007$). Eight percent of vitamin D deficient women had cesarean section compared with 3.5% of those with normal concentrations ($p = 0.05$). Babies delivered by cesarean section had larger head circumference compared to the other two groups ($p = 0.001$), however gestational age at delivery and gender ratio was similar in the three groups. Multiple logistic regression revealed that maternal vitamin D deficiency [(OR 2.40, 95% CI: 1.04, 5.50, $p = 0.03$), and short stature (OR 2.68, 95% CI: 1.06, 6.71, $p = 0.03$) predicted cesarean section, independent of various confounding factors (maternal age, height, BMI, waist circumference, hip circumference, fat percent, gestational age, and neonatal head circumference, length and gender). Vitamin D deficiency predicted cesarean delivery even in those with primary cesarean section (OR 2.90, 95% CI: 1.06, 7.93, $p = 0.03$).
Conclusions: In rural India, low maternal vitamin D status in pregnancy is associated with an increased risk of cesarean section. Support: The Wellcome Trust, UK.

1. A. Merewood *et al.*, *J Clin Endocrinol Metab* 94:940–945, 2009.
2. Lips P. *Endocrine Rev*, 22:477–501, 2001.

P-10A-443

Dietary Assessment Methods (DAME) for micronutrient intake in pregnant women: a systematic review

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Objetive: The EURopean RECommendations Aligned (EURRECA) Network of Excellence needs clear guidelines for assessing the validity of reported micronutrient intakes amongst vulnerable population groups. In this review, the studies on dietary methods used to assess micronutrient intake during pregnancy are presented.
Methods: A systematic literature search was conducted for studies validating the methodology used for measuring the usual dietary intake during pregnancy. The quality of each validation study selected was assessed using a scoring system developed by EURRECA. Validation of FFQs were categorised according to whether the study used a reference method that reflected short-term intake (<7days) long-term intake (≥7 days) or used biomarkers. A correlation coefficient for each micronutrient was calculated from the mean of the correlation coefficients from each study weighted by the quality of the study.

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Results: Seventeen papers were selected for inclusion in this review which included the validation of 15 FFQs, 2 dietary records, one diet history and an iron intake check list. Estimates of 26 micronutrients by 6 FFQs were validated against 24 hr recalls indicating good correlation for 6 micronutrients. Estimates of 24 micronutrients by 2 FFQs were validated against EDRs and all had good or acceptable correlations. Estimates of 14 micronutrients by 3 FFQs were validated against weighed dietary records indicating good correlations for five. Six FFQs were validated against biomarkers, presenting good correlations only for folic acid.
Conclusions: FFQs appear to be most reliable for measuring short-term intakes of vitamin E and B_6 and long-term intakes of thiamin. Apart from folic acid, biomarkers do not add any more certainty as to the reliability of intake methods. When frequency methods are used for assessing micronutrient intake, the inclusion of dietary supplements improves their reliability for most micronutrients. Support: European Union.

P-10A-444

Antenatal supplementation with folic acid reduces kidney dysfunction and risk of metabolic syndrome while folic acid+iron+zinc improves growth among 6–8 year old children in rural Nepal

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Objectives: Micronutrient deficiencies are common among women during pregnancy in much of the developing world, and are associated with poor fetal growth and other adverse outcomes in infancy. Low birth weight has been linked to an increased risk of chronic disease, yet few have examined the long-term effects of micronutrient deficiency during gestation on risk factors for chronic disease in later life.
Methods: We conducted a community-based, cluster-randomized, controlled trial of micronutrient supplements provided to women beginning in early pregnancy in the rural terai of Nepal. Women were randomized to receive daily supplements of either a control; folic acid; folic acid+iron; folic acid+iron+zinc; or a multiple micronutrient. All supplements contained vitamin A. A total of 4130 infants were born during the trial from 1999–2001. Children were re-visited and enrolled in a follow-up study from 2006–2008. Blood pressure was measured using an automated oscillometric device. Height, weight, mid-upper arm circumference, waist circumference, triceps skinfold, and subscapular skinfold thicknesses were measured. Early morning blood and urine specimens were collected to assess glycated hemoglobin, plasma cholesterol (including HDL and LDL fractions), triglycerides, glucose, insulin, and urinary microalbumin/creatinine ratio. The homeostasis model assessment (HOMA) was used to estimate insulin resistance, using fasting glucose and insulin. Microalbuminuria was defined as urinary microalbumin/creatinine ≥30 mg/g and metabolic syndrome was defined using a modified National Cholesterol Education Program definition. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: A total of 3524 children were enrolled in the follow-up study (~93% of surviving children). Relative to the controls, maternal supplementation with folic acid + iron + zinc resulted in a modest improvement in height of 0.64 cm (95% CI: 0.04, 1.25) and a reduction in triceps skinfold thickness of 0.25 mm (−0.44, −0.06) and subscapular skinfold thickness of 0.20 mm (−0.33, −0.06). There were no other differences in anthropometry between treatment groups. Maternal supplementation with folic acid reduced the risk of microalbuminuria by 44% (OR: 0.56; 95% CI: 0.33, 0.92) and metabolic syndrome by 37% (OR: 63; 95% CI: 0.41, 0.97). There appeared to be a protective effect among all groups that received folic acid supplementation during gestation (Figure 1). There were no other between-group differences in blood pressure, cholesterol, triglycerides, or markers of insulin resistance.
Conclusions: In this poor, rural population with prevalent micronutrient deficiency, micronutrient supplementation of women during pregnancy can have long-lasting effects on the growth and risk of chronic disease in her offspring. *Acknowledgements:* The authors acknowledge funding from the Bill and Melinda Gates Foundation, Seattle, WA.

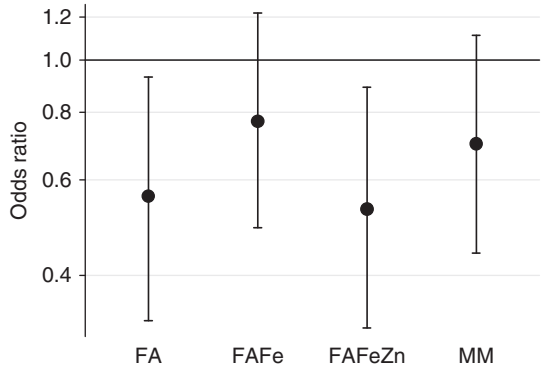

Figure 1: The risk of microalbuminuria (urinary microalbumin/creatinine ratio ≥30 mg/g) in children by maternal supplement group. FA, folic acid; FAFe, folic acid + iron; FAFeZn, folic acid + iron + zinc; MM, multiple micronutrients.

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Higher maternal plasma folate concentration but not vitamin-B12 concentration during pregnancy is associated with higher cognitive function scores in 9–10 year old children in South-India

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Objective: Data linking maternal micronutrient status during pregnancy with offspring cognitive function are scarce. Our main objective was to examine whether maternal plasma folate and vitamin-B12 concentrations during pregnancy are associated with cognitive performance in the children.
Methods: Between September 2007–May 2008, cognitive function was assessed in 536 (259 boys and 277 girls) healthy children, aged 9–10 years, from the Mysore Parthenon birth cohort, using 3 core tests from the Kaufman Assessment Battery for children and additional tests measuring long-term retrieval/storage, attention and concentration, visuo-spatial and verbal abilities. Maternal plasma folate and vitamin-B12 concentrations were measured from the stored blood samples collected at 30 ± 2 weeks of gestation. We also collected data on a variety of potential confounders like maternal age, parity, gestational age at birth, birth size, sex, children's current age, size, years at school and folate and vitamin-B12 concentrations, parents' area of residence, educational attainment and current socio-economic status. Associations of maternal plasma folate and vitamin-B12 concentrations with cognitive function were examined by multiple linear regression analysis using stata version 10. The study was approved by the Holdsworth Memorial Hospital, Mysore, research ethics committee and informed verbal consent was obtained from parents and children.
Results: During pregnancy 22 (4%) women had 'folate deficiency' (plasma folate concentration <7 nmol/l) while 228 (42.5%) had 'vitamin-B12 deficiency' (plasma vitamin-B12 concentration <150pmol/l). Maternal folate correlated with maternal vitamin-B12 ($r = 0.1$, $p = 0.015$) and with the children's folate at 9.5 years ($r = 0.2$, $p < 0.0001$).Maternal vitamin-B12 correlated with the children's vitamin-B12 at 9.5 years ($r = 0.2$, $p < 0.0001$). In multiple linear regression, there was a 0.2 SD increase in all the children's cognitive scores per SD increase in maternal folate concentration ($p < 0.05$ for all) and the associations with Atlantis (learning ability and long-term storage and retrieval), verbal-fluency (broad retrieval ability, speed and flexibility of verbal thought process), Kohs block design (visuo-spatial ability) and coding-WISC-III (visual-motor processing speed and coordination, short-term memory, attention and concentration) were independent of the confounding variables listed above. There were no associations between maternal vitamin-B12 concentrations and tests of cognitive function. There were no significant interactions between vitamin-B12 and folate in relation to cognitive scores.
Conclusions: In this Indian population higher maternal plasma folate, but not vitamin-B12, concentration during pregnancy independently predicts better childhood cognitive performance.

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P-10A-446

Reversal of developmental programming with vitamin B_{12} supplementation – a pilot study

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Objective: In the Pune Maternal Nutrition Study, maternal imbalance of folate and vitamin B_{12} (B_{12}) status during pregnancy showed unfavourable associations with body composition, and metabolic and cognitive functions in the offspring, which may reflect intra-uterine programming by maternal nutritional status. Our objective was to study the possibility of reversal of these effects with oral supplementation of near physiological doses of B_{12} and folic acid in these offspring during early adolescence.
Method: This was a randomized, placebo-controlled, double-blind, 2×3 factorial trial. Vitamin B_{12} was given as 2 or 10 μg capsules, with or without 200 μg folic acid, forming six groups (B_0F_0, B_2F_0, $B_{10}F_0$, B_0F_{200}, B_2F_{200}, $B_{10}F_{200}$), for 12 mo. The following parameters were measured at baseline and after 12 mo: anthropometry (height, weight, skin fold thickness, waist circumference), grip strength, body composition (DXA), cardiovascular (CV) risk parameters (glucose, insulin, lipids, blood pressure) and different tests of cognitive performance. KEM Hospital Research Centre's ethics committee clearance, parents' informed, written consent and children's assent were obtained.
Results: One hundred and six children of mean age 9 y were randomised and allocated supplements; 105 completed the trial. There was no interaction between effect of B_{12} and folic acid supplementation in relation to change in body composition, CV risk parameters and cognitive function ($P > 0.05$ for all), and hence their effects were analyzed separately: B_0 vs. B_2 vs. B_{10}; and F_0 vs. F_{200}. After 12 mo the plasma B_{12} concentrations increased in B_{10} by 140 (95% CI: 110, 168) pmol/L, in B_2 by 54 (95% CI: 25, 83) pmol/L and

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decreased in B_0 by 1 (95% CI: −29, 27) pmol/L. In F_{200} group folate concentrations increased by 13.1 (95% CI: 10.0, 16.0) pmol/L and in F_0 fell by 2.3 (95% CI: −5.3, 0.7) pmol/L. Plasma tHcy fell by a mean 2.9 (95% CI: −3.9, 11.8) μmol/L in B_{10} and by 0.48 (95% CI: −1.5, 0.6) μmol/L in B_2, compared to a rise of 2.2 (95% CI: 1.2, 3.2) μmol/L in B_0. In F_0, plasma tHcy increased by 0.18 (95% CI: −0.82, 1.19) μmol/L compared to a fall of 0.91 (95% CI: −1.91, 0.84) μmol/L in F_{200}. Supplementation had no effect on growth, body composition, HOMA-R, lipids, BP, grip strength and cognitive scores in any group ($P > 0.05$ for all) after 12 mo. ***Conclusion:*** Daily oral supplementation with physiological doses of B_{12} for 12 mo was effective in improving circulating vitamin B_{12} status, but did not influence body composition, CV risk parameters or cognitive performance. Our findings suggest a possible irreversibility of developmental programming effects in the offspring, at least through early adolescence, at the doses and duration of B_{12} supplements we used. TRIAL REGISTRATION: ISRCTN 59289820. Support: The Wellcome Trust, UK.

P-10A-447

Assessment of nutritional quality in healthy pregnant women of the Canary Islands

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Objetive: to describe the composition of the diet of healthy pregnant women of the Canary Islands and to estimate the nutritional quality using the *Healthy Eating Index* (HEI). ***Methods:*** cross-sectional study based on 103 women aged 18-40 years, who gave birth at the University Hospital Materno-Infantil of Gran Canaria. Food consumption and macro and micronutrient intake were estimated using a food frequency questionnaire used in the Canary Island Nutrition Survey (ENCA) and the HEI was calculated. This index includes 10 components and the maximum possible score of the index is 100 points. Appropriate institutional ethics committee clearance and participants' informed consent were obtained. ***Results:*** the score of the index was 54,9. This result remains below the optimum score of ⩾80, which is considered good the diet quality of pregnant women in our study population. The average score of the first 5 components of the index showed that cereal consumption was below the daily portions recommended for pregnant women, whereas vegetables, fruit, milk and meat consumption surpassed the recommendations. A significant number of pregnant women did not reach the 50% of the recommendations for iron, folate and vitamin D intake (36.9%, 26.2% and 38.8%, respectively). At least 30% of the population exceeded 200% of the recommendations for proteins, thiamin, niacin, riboflavin, vitamin C and vitamin A. ***Conclusions:*** dietary advice for improving the diet quality during pregnancy and the supplementation of mainly iron and folate are necessary. Support: Danone S.A.

P-10A-448

The association between maternal dietary intake and antioxidant status with maternal and fetal physical outcomes – study methodology

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Objective: A poor dietary intake and low body level of antioxidant nutrients may be particularly important due to the surge of maternal oxidative stress during pregnancy (Scholl & Stein, 2001). However, little attention has been paid to the potentially important issue of antioxidant nutrient status in pregnancy and related outcomes (Allen LH, 2005). Hence, this cross sectional study aims to focus on the maternal dietary intake and total serum antioxidant status as well as serum selenium status in relation to both maternal and fetal physical outcomes.

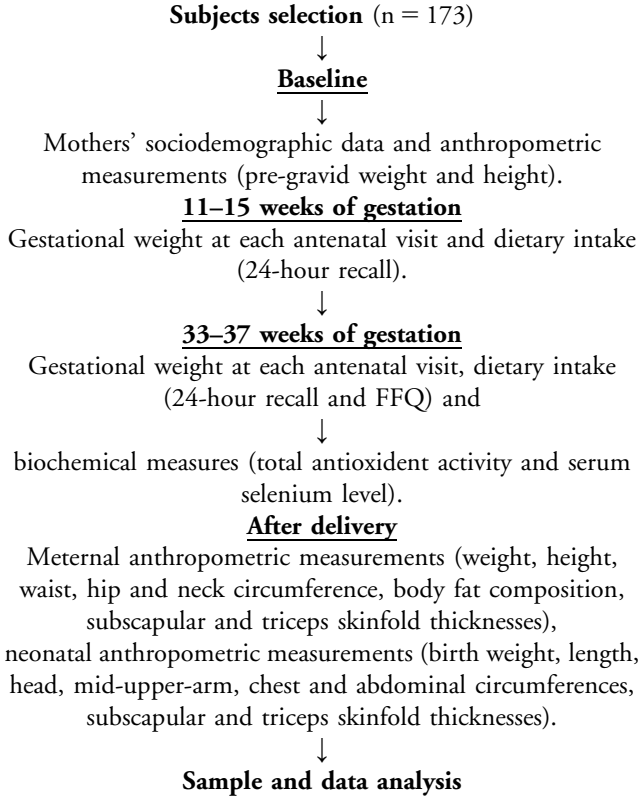

Figure 1. Flow Chart of Research Activities.

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Methods: A total of173 pregnant women and their newborns will be recruited from the Obstetric and Gynecology Clinic, Universiti Sains Malaysia Hospital for this cross sectional study. To increase accuracy, mothers with gestational age more than 6 weeks during first antennal visit will be excluded. Maternal dietary intakes (24-h recall and food frequency questionnaire), serum total antioxidant status (using ELISA method) and selenium status (using atomic absorption spectrophotometry method) will be measured at 11–15 weeks of gestation and 33–37 weeks of gestation. Maternal and neonatal anthropometric measurements will be taken after delivery. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Complete results shall be made available for publication by July 2010. Conclusions: Findings from the study will address the importance and beneficial role of antioxidant nutrient balance and selenium intake for pregnant mother in resulting optimal maternal and infant outcomes. Possible collaborative research works are welcomed.

1. T.O. Scholl, T.P. Stein, *J. Matern. Fetal. Med.,* 10: 182–185, 2001.
2. L.H. Allen, *Am J Clin Nutr.*, 81:1206S–12S, 2005.

P-10A-449

Effect of micronutrients and exercise during pregnancy on factors related with non-transmissible chronic diseases. A randomized controlled trial

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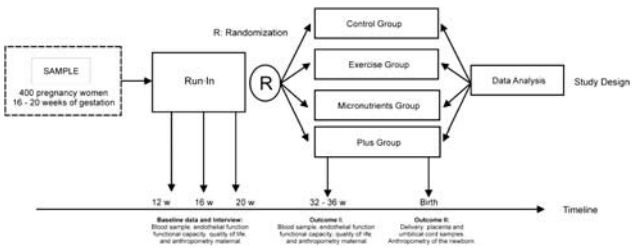
Manifold studies had indicated the relation between metabolic alterations and fetal growth with the development of Non-transmissible chronic diseases-NCTD in adult age. It has been proposed that maternal factors(endothelial function-EF, oxidative stress-OS and adipokynes levels-AL) and placental ones(mitochondrial dysfunction-MD) are the precursory mechanisms of fetal metabolic alterations and of the later development of NCTD. Also, it has been suggested that possible supplementation with micronutrients and the physical exercise during the gestation could regulate both maternal and placental factors. For these considerations, is important to clarify if the proposed factors are related with maternal and fetal metabolic alterations and if the supplementation with micronutrients and/or the physical exercise during the gestation can regulate them. This could be an early and original choice to strengthen the NTCD prevention in the population.
Objectives: 1. To evaluate the maternal and placental metabolic factors associated to NCTD in the newborn. 2. To evaluate the effect of the physical exercise and the micronutrients supplementation during the pregnancy on the maternal and neonatal EF, OS and AL and also in the placental MD and the newborn's anthropometry.
Method: Study Type: experimental. Study Design: Basic Science, Randomized, Blind(Investigator, Outcomes Assessor), Active Control, Blocks Assignment, Effectiveness Study. Appropriate institutional ethics committee clearance and participants' informed consent will be obtaining. Subjects: 400 first time pregnancy women, between 16-20 gestational weeks, are going to be randomized and assigned to the following groups(Diagram 1): *1. Control Group:* they take placebo(maltodextrina) and usual prenatal care. *2. Exercise Group:* they take placebo and practice regular aerobic physical exercise: Walking(10 minutes), aerobic exercise(30 minutes), stretching(10 minutes) and relaxation exercise(10 minutes). Exercise will be performed at three sessions per week. *3. Micronutrients Group:* micronutrients supplementation of Zinc 30 mg, Magnesium 400 mg, Beta-carotene 9 mg, Tocopherol 30 mg, vitamin C 200 mg and Niacin 100 mg and usual prenatal care. *4. Combinated interventions Plus-Group:* micronutrients supplementation plus regular aerobic physical exercise. Measures will be taking at the start and at the end of the intervention. Placental sample will be taking at the delivery moment. Brachial artery flow-mediated dilation, Dimetilarginine Asymmetric, Nitrates-Nitrites and cyclicGMP, F2-isoprostanes, Adiponectin, Leptin, Tfam, Citrate Sintase, Citocrome C Oxidase, Oxide Nitric sintase, Superoxide dismutase, Maternal Anthropometric indicators, Functional capacity (VO_{2max}), Neonatal Anthropometric indicators.
Expected Results: There will be changes in the outcome (OS, AL, mitochondrial and endothelial dysfunction).
Conclusions: measures between the interventions groups, indicating that micronutrients supplementation and physical exercise during pregnancy will play an important role in order to avoid fetal programming of chronic diseases. This will help to clarify whether the proposed items are related to metabolic abnormalities and if complementation with micronutrients during pregnancy and/or regular physical exercise can be an early and innovative alternative to strengthen the prevention chronic diseases in the population. Support: Instituto Colombiano de Ciencia y Tecnología ''Francisco José de Caldas'' (COLCIENCIAS-Project Code 110645921540). Trial registration: NCT00872365.



Graphic 1. Study design.

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P-10A-450

Health care management: current nutrition problem in kenyan society

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Objective: The current daily meals for all Kenyans are creating many medical nutrition problems. For people of 35 years old, mental fatigue, back ache, joint muscle pains, swollen joint (arthritis like) Neuropathy have become major clinical presentation. Current meals lack Zinc, Selenium, Manganese, Magnesium, Calcium, and Copper, Vitamins A and B and essential amino acids. This is due to over cooking and high use of polished grains. In modern society control was initiated presenting with above.
Methods: Nutrition health clinics were started in 2001. All patients over 35 years old were enrolled in programs. Their routine food eating and nutrition composition was recorded first. Locally available foods rich in Zinc, Selenium, Manganese, Copper, Vitamins A, B and other essential amino acids were incorporated in daily meals for the family. Breakfast, lunch or supper forms to monitor mainly clinical changes and BMI were used for one (1 year). Artificial micro-nutrients rich on above chemicals (Beta Vit) were used for serious cases but later changed to natural diet supplying the same.
Results: 75% over 50% people who were put on above chemicals received there indicated probably. It is also proved highly effective in reducing micronutrient deficiencies as well as improving vitality.
Conclusion: Our study showed Routine foods eaten by Kenyans lack antioxidant essential mineral: Nutrition value is destroyed by over cooking (vitamin A) there is high intake of non essential amino acids (proteins). Our study shows that the above current medical problems facing Kenyan society can easily be controlled by people feeding on food rich in Zinc, Selenium, Vitamin A, B, C and K and Manganese, Magnesium, Copper, Vitamins A and B and essential amino acids. Polished foods are creating many nutrition medical problem societies. Those who remained on convention treatment continued.

P-10B-451

"Similarities in the inequalities" and physical activity in young adults

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Objective: Low birth weight individuals are known to be more vulnerable to develop metabolic syndrome later in life[1]. In addition, it is known that these individuals exercise less in adulthood, a behavioral trait that may contribute to their increased disease risk[2]. Physical activity is a known health protective factor, benefiting both metabolic and psychological aspects. The objective of this study is to verify early and late determinants of physical activity in young adults.
Methods: 2063 individuals from a birth cohort study born from June 1978 to May 1979 in Ribeirão Preto, Brazil, were studied at age of 23/25 years old. Logistic regression was performed using three models: (a) Early model considering the variables birth weight, gestational age, maternal schooling and smoking data collected shortly after birth; (b) Late model considering individual's gender, schooling and smoking (c) Combined (early + late) model. Physical activity was evaluated using the International Physical Activity Questionnaire (IPAQ), stratifying the individuals in two categories: active and sedentary.
Results: In the early model, low birth weight (RR = 1.207, 95% CI 1.023–1.423) and low level of maternal schooling (RR = 1.213, 95% CI 1.028–1.430) were risk factors for sedentary activity. In the late model, poor schooling (RR = 1.138, 95% CI 1.021–1.269) and female gender (RR = 1.385, 95% CI 1.285–1.516) were associated with sedentary behavior. In the combined model, low birth weight, female gender and individual' schooling were significant. In addition, we found an interaction between birth weight and individual' schooling, in which sedentary behavior is statistically more prevalent in individuals born with low birth weight only if they have higher levels of education.
Conclusions: This study shows that the early environment interacts with later life course events in a complex manner, with low birth weight individuals practicing less physical activity, possibly related to behavioral programming effects[2], but this is seen only in groups with higher levels of education. Low birth weight individuals with lower educational backgrounds exercise more probably due to their increased labor related physical activity. Therefore, variables from early development such as birth weight and social insertion in later life course interact to determine an individual's physical activity. We demonstrated here an example of the "Similarities in inequalities" model[3], in which social inequalities are linked to similar health outcomes (in this case, physical activity).

1. C.N. Hales, D. J. Barker, *Br Med Bull.* 60:5–20, 2001.
2. E. Kajantie *et al.*, *PAS Annual Meeting*, 2009.
3. P.P. Silveira *et al.*, *Lancet*, 366:451–452, 2005.

P-10B-452

Early exercise training increases heart weight without hypertension later in life irrespective of birth weight

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Objective: Fetal growth restriction induced by uteroplacental insufficiency in rats has previously been shown to result in high blood pressure, particularly in males. Furthermore our lab has previously reported a nephron and cardiomyocyte deficit in offspring born small, which may contribute to the development of cardiovascular disease. We studied the effects of early and late exercise training on blood pressure as well as heart, kidney and body weights on growth restricted male rats.
Methods: Uteroplacental insufficiency was induced on day 18 of pregnancy and resulted in *Restricted* litters which were compared to sham-operated *Controls*. In order to control for the reduction in litter size in *Restricted* litters, sham-operated *Reduced litter* offspring were included, where litter size was reduced at birth, previously reported to alter postnatal lactation and growth. Offspring remained sedentary or underwent Early (5–9 weeks) or Late (20–24 weeks) exercise training. Exercise training involved treadmill running 5 days/week, 60 minutes/day. We examined blood pressure by tail cuff and body weight at 8, 12, 16, 20 and 24 weeks of age as well as heart and kidney weight at 9 or 24 weeks ($n = 10$).
Results: Restricted offspring were smaller than sham operated offspring 1 day after birth. There was no effect of early exercise on body weight at all ages, however, at 24 weeks rats that underwent late exercise were lighter than those who remained sedentary or underwent early exercise training ($P < 0.05$). At 24 weeks of age both early and late exercise training was associated with significantly larger absolute and relative heart weights ($P < 0.05$). Interestingly this increase in heart mass with exercise training was not apparent immediately after early exercise at 9 weeks. Late exercise training also cause relative kidney weights to be larger ($P < 0.05$). At 16 weeks of age the Control and Restricted offspring were not different from each other but were smaller than offspring exposed to a Reduced litter after birth ($P < 0.05$). Interestingly at 8 and 12 weeks Reduced litter offspring had higher blood pressure compared to Control and Restricted offspring regardless of exercise training ($P < 0.05$) and by 24 weeks Reduced litter offspring had significantly heavier absolute kidney and heart weights but not when normalized to body weight. Furthermore at 16, 20 and 24 weeks of age blood pressure was not different between any groups.
Conclusion: A major finding of this study is that a short period of exercise training early in life results in increased heart mass in adulthood. Furthermore the increased heart weight in adulthood with both early and late exercise was not associated with differences in blood pressure. Late exercise training was beneficial in reducing body weight and may prevent the onset of adult disease in high risk populations.

P-10B-453

Adults with very low birth weight have lower resting energy expenditure than their peers born at term – The Helsinki Study of Very Low Birth Weight Adults

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Objective: Adults born preterm have increased risk factors of cardiovascular diseases. Low resting energy expenditure (REE) may, for example by increasing the risk of obesity, also contribute to cardiovascular risk. We studied the effects of very low birth weight (VLBW; <1500 g) on REE.
Methods: In conjunction with the Helsinki Study of Very Low Birth Weight Adults[1] indirect calorimetry was used to measure REE and dual X-ray absorptiometry (DXA) to measure lean body mass in 118 VLBW individuals and 121 individuals who were born at term (mean age: 22.5 years [95% CI 22.2–22.8]). Appropriate institutional ethics committee clearance and informed consent were obtained.
Results: As compared with controls, VLBW adults had on average 1.4 kg/m^2 (95% CI 0.5 to 2.4) lower BMI, and 5.9 kg (95% CI 4.1 to 7.7) lower lean body mass, but similar body fat percentage ($p = 0.9$). Their REE was also lower (1589 kcal/24h [95% CI 1537 to 1642]) than in controls born at term (1695 kcal/24h [95% CI 1640 to 1749]). The table shows that the difference remained similar when adjusted for age, sex, parental education and daily smoking. However, it attenuated after additional adjustment for body mass index and became non-significant when adjusted for lean body mass. Among the VLBW group, those born small for gestational age (SGA) had 8.7% ($p = 0.02$) lower REE than those born appropriate for gestational age (AGA). Again, the difference was no longer statistically significant when adjusted for lean body mass.

Table. Linear regression models showing differences in resting expenditure (95% CIs) between VLBW and term born young adults, unadjusted and adjusted by covariates in different models.

Measurement	Model*	N of Subjects	Mean difference term-VLBW (95% CI)	P Value
Resting Energy Expenditure (kcal/24h)	Unadjusted	239	−6.3% (−10.4 to −2.1)	0.004¶
	1	239	−6.3% (−9.2 to −3.3)	<0.001¶
	2	238	−6.4% (−9.3 to −3.3)	<0.001¶
	3	238	−3.4% (−5.9 to −0.9)	0.009¶
	4	238	1.5% (−0.7 to 3.8)	0.2

*1 Adjusted for age and sex, 2 Adjusted for 1+ parental education (4 levels) and daily smoking, 3 Adjusted for 2+ BMI (kg/m^2), 4 Adjusted for 2+ lean body mass (kg).

Conclusions: Adults with VLBW have lower resting energy expenditure than their peers born at term. This is attributable

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to their lower lean body mass. We previously reported a lower degree of physical activity in VLBW adults[1]. These findings suggest that, while there is no evidence of increased rates of obesity in young adult age, people with VLBW may constitute a risk group for developing obesity and related metabolic traits at a later age.

1. P. Hovi *et al. NEJM,* 356:2053–2063, 2007.

P-10B-454

Predictors of physical activity and energy expenditure in Afro-Caribbean children

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Objectives: The worldwide incidence of obesity in children is increasing. The underlying mechanisms are poorly understood, but early life events may influence physical activity in later life. We hypothesized that maternal size during pregnancy and birth size are determinants of physical activity energy expenditure (PAEE) in children. Also, childhood PAEE would be inversely related to adiposity and cardiovascular risk factors.

Methods: The Vulnerable Windows Cohort Study is a longitudinal observational study of Jamaican mother/child pairs recruited during the antenatal period. Maternal anthropometry was measured in each trimester. Anthropometry of the children was measured at birth, at 6 weeks, 3 monthly to 2 years and then every 6 months. Physical activity was measured in 100 boys and 122 girls at mean age 13.4 years (range 11.5–14.9 years) using the Actical activity monitor (Mini-Mitter, Inc.). Resting energy expenditure was measured using Oxymax (Columbus Instruments). Cardiovascular risk factors (blood pressure, fasting glucose, insulin and lipids) were also measured. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: The girls had greater fat mass and percentage fat. Boys spent a greater proportion of the day being active ($P < 0.001$) and expended more energy than girls (12.3 ± 3.3 vs. 9.6 ± 2.8 kcal/kg/d; $P < 0.001$). Maternal weight was positively associated with child's mean Actical count ($r = 0.18$; $P = 0.007$) and energy expenditure ($r = 0.16$; $P = 0.02$). There were similar, though weaker, associations with mother's height (P-values <0.05). PAEE was not associated with birth weight (P-values >0.3). Maternal weight after adjusting for child's age and sex was also positively correlated with child's fat-free mass ($P < 0.001$), fat mass ($P < 0.001$) and percentage fat ($P = 0.01$). Total physical activity after controlling for age and sex was positively associated with body composition i.e. fat mass, fat-free mass, percentage fat and waist circumference (P-values <0.001). However, these associations became non-significant after adjusting for current weight. Age- and sex-adjusted PAEE was positively associated with triglycerides, insulin levels and systolic blood pressure (P-values <0.05), but not after adjusting for current weight and height. Conversely, PAEE was associated with fasting glucose even after controlling for age, sex, weight and height ($r = -0.16$; $P = 0.02$).

Conclusion: Maternal size, but not birth weight, is a determinant of childhood PAEE. Physical activity, unlike maternal size, is not strongly associated with childhood body composition. Physical activity is independently and inversely related to fasting glucose levels.

P-10B-455

Higher physical activity and aerobic fitness attenuates the influence of birth weight on fasting insulin in youth

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Objective: Low birth weight is associated with increased metabolic risk, including insulin resistance and type 2 diabetes in later life. We examined the influence of birth weight on fasting insulin. We also examined whether physical activity and aerobic fitness modify the associations between birth weight and later fasting insulin.

Methods: The European Youth Heart Study (EYHS) is a mixed longitudinal population based cohort study of 10 and 15 year old children in Denmark, Portugal, Estonia and Norway. Maternally reported birth weight data was available for 2,928 participants after exclusion of very low BW (<1.5 kg, n = ?). Body weight and height was measured with the volunteer wearing light clothing and sexual maturity was recorded according to Tanner categories. Overnight fasting blood samples were collected, separated and stored at -80°C within 30 minutes. Fasting insulin, used as a measure of insulin resistance and was assessed either using an enzyme immunoassay or by 2-site immunometric assay with 125I or alkaline phosphatise labels (r between methods = 0.94). Aerobic fitness (watt/kg) was assessed using a progressive, maximal cycle ergometer test. Physical activity (PA) was objectively measured using a hip worn accelerometer (MTI

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Actigraph). Overall PA was expressed as counts per minute (cpm), and time (min/day) spent in moderate and vigorous activity (MVPA) was defined as >2000 cpm per day. Appropriate institutional ethics committee clearance and participants informed consent were obtained.

Results: Lower birth weight was associated with higher fasting insulin, adjusted for sex, age group, country and sexual maturity (ß = −0.037, 95%CI −0.07, −0.003, p = 0.031), this association was also independent of maternal BMI and waist circumference (ß = −0.069, 95%CI −0.104, −0.035, p < 0.001). This association was partially attenuated with further adjustment for aerobic fitness (ß = −0.068, 95%CI −0.104, −0.033, p = 0.001). The models were repeated adjusting for total physical activity (cpm), which partially attenuated the association (ß = −0.047, 95%CI −0.090, −0.004, p = 0.030). Following adjusting for MPVA per day the association between birth weight and fasting insulin ceased to be significant. (ß = −0.034, 95%CI −0.083, 0.061, p = 0.180). There was no evidence for an interaction between birth weight and aerobic fitness or between birth weight and either total physical activity or MVPA.

Conclusions: Lower birth weight is associated with higher fasting insulin, which was independent of central adiposity, measured by waist circumference. This association was partially attenuated by adjustment for aerobic fitness. The association between birth weight and fasting insulin ceased to be significant following adjustment for time spend in moderate and vigorous physical activity. Suggesting increased physical activity levels may be useful to reduce the deleterious influence of lower birth weight on later fasting insulin.

P-10B-456

Early growth and leisure time physical activity in later life

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Objective: To assess the role of early growth on leisure time physical activity (LTPA) in later life among men and women.

Methods: We examined 2003 individuals born in Helsinki, Finland between 1934 and 1944. Of them, 1967 individuals with adequate information on their LTPA were included in this study. LTPA was assessed by the validated exercise questionnaire (KIHD-12 month leisure time physical activity history). Subjects' birth and serial growth measurements were obtained from birth, child welfare and school health records. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Participants with higher engagement to LTPA, showed a more favourable adult anthropometric and body composition profile than those who were less active. Weight and height at birth, and weight at 2 years were positively associated with intensity of total LTPA (p = 0.04, p = 0.01 and p = 0.03), respectively. Height at 2, 7 and 11 years were positively associated with intensity of conditioning LTPA (p = 0.01, p = 0.04 and p = 0.004). Weight and height at 2, 7 and 11 years were all positively associated with energy expenditure of total LTPA (p = 0.03, p = 0.02, p = 0.01, p = 0.02, p = 0.03 and p = 0.03), respectively. Furthermore, height at 2 and 11 years were positively associated energy expenditure of conditioning LTPA (p = 0.02 and p = 0.03).

Conclusions: Body size and growth from birth to 11 years may play an important role in programming of leisure time physical activity in later life.

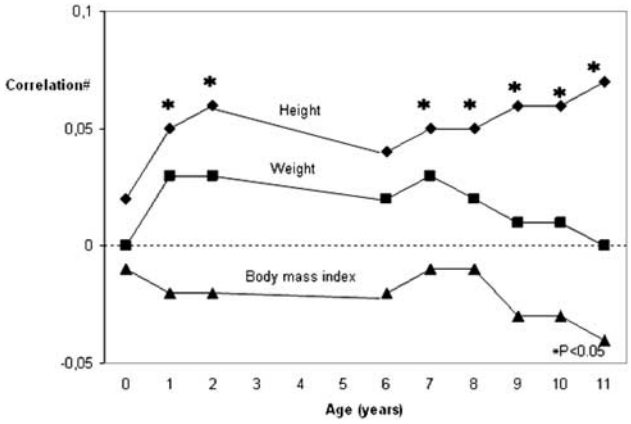

Figure 1. Intensity of Conditioning LTPA.

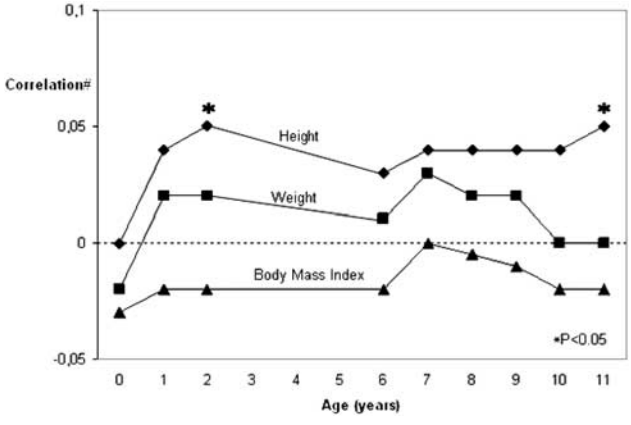

Figure 2. Energy Expenditure of Conditioning LTPA.

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P-10C-457

Very low birth weight and adult attachment

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Objective: The first attachment relationships a baby develops are important for his/her ability to form close relationships later in life. Severely preterm birth is a challenge to the development of early attachment. Studies show that individuals born severely preterm are less likely to start a family than their peers born at term; whether this is related to attachment behaviours is not known. Our aim was to examine potential differences in adult attachment between young adults with very low birth weight (VLBW, <1500 g) and those born at term. In addition, we examined the effects of being born VLBW and small for gestational age (SGA) on these differences.

Methods: Experiences in Close Relationships – Revised (ECR-R), a 36-item questionnaire was used to assess attachment related anxiety and avoidance in romantic relationships. Anxiety refers here to concern about being abandoned by a partner and avoidance refers to discomfort being close to others. The questionnaire was filled in by 162 young adults with VLBW (42.0% men, 32.7% SGA) and 172 control adults born at term (40.1% men, 0.0% SGA) at the mean age of 22.4 years (SD 2.2). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.

Results: Participants with VLBW had on average 8.9% lower scores in attachment anxiety (95% confidence interval (CI) 1.2% to 17.2%). There was no difference in attachment avoidance between the VLBW and control groups. The VLBW-SGA women, in turn, scored 16.0% higher than control women in attachment avoidance (95% CI 3.1% to 30.5%), while among men the corresponding groups did not differ from each other (95% CI -20.1% to 7.3%). All results were adjusted for sex, age and whether the participant was currently in a romantic relationship or not.

Conclusion: Our results suggest no uniform pattern of attachment behaviours in young adults born severely preterm. There is evidence for lower levels of attachment anxiety in VLBW adults contrasted to those born at term. However, the results also imply that VLBW-SGA women may be characterized by higher attachment avoidance.

P-10C-458

Gender differences in psychotropic drug consumption may indicate enhanced risk of pharmacotoxicologic programming in perinatal period

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Objective: Earlier we have shown clear gender differences for consumption of antidepressants (AD) and benzodiazepines (BZ) in a population of North-Western region, close to the town of Ijui, in the state of Rio Grande do Sul (RS), Brazil. In fact, female/male ratio was approximately 2:1 for consumption of the majority of psychotropic drugs in these two classes. Such evidence could indicate higher risk of pharmacotoxicologic programming/imprinting in perinatal period. However, in our previous studies the age dependency of drug consumption was not explored. Therefore, we have undertaken an investigation, in order to establish both gender differences and age dependency in psychotropic drug consumption.

Methods: As earlier, we have performed a collection of data in commercial pharmacy of Ijui – RS, using national brazilian system of special prescriptions for controlled psychotropic drugs. The period of data collection was during the second half of 2006, and patient gender was indicated by its name. As a whole, 76 and 125 prescriptions were analysed for AD and BZ respectively. In more than a half of them, patient age was identified by a pharmacist at the moment of drug dispensing.

Results: As in our previous studies, clear gender differences were established, demonstrating female predominance in consumption of the majority of psychotropic drugs explored. For example, female fractions in the quantities of AD consumption were 87.5%, 87.4%, 68.4% and 68% for fluoxetine, venlafaxine, paroxetine/imipramine and nortriptiline respectively, but only 42.2% for amitriptiline. On the other hand, female fractions in the quantities of BZ consumption were 87.3%, 77.8%, 69.4% and 66.7% for alprazolam, bromazepam, clonazepam and lorazepam respectively, but only 33.3% for diazepam. What for the number of prescriptions with patient's age identified, the majority of female and male consumers were more than 40 year-old, although for BZ this age difference was less expressive one, at least in females.

Conclusion: It seems to us that the risk of pharmacotoxicologic programming by psychotropic drugs is lower than could be supposed on the basis of gender differences only, since the great part of female consumer population is more than 40 year-old. Nevertheless, and especially for BZ, a risk of such programming really exists, considering not only the possibility of inadvertent drug use in a period close to conception, but also due to addiction potential for this class of psychotropic drugs. In conclusion, the research on gender differences, together with age dependency, for drug consumption should be continued and even amplified, including

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other classes of different drugs, for evaluating relative risk of pharmacotoxicologic programming/imprinting in perinatal period. However, the studies on these phenomena in experimental models of laboratory animals are highly encouraged also, in order to establish, e.g., the combined effects of psychotropic drugs and known agents with programming capacity, like glucocorticoids, as well as to clarify the molecular mechanisms of such effects.

P-10C-459

Synergistic role of nerve growth factor and breast milk fatty acids in mothers delivering low birth weight babies at term

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Objective: The successful progression of pregnancy depends on the complex interactions between numerous biological molecules within the uterine microenvironment. This involves an interaction of intracellular and extracellular factors including micronutrients, hormones, adhesion molecules, growth factors and immunomodulators that determine the fetal growth outcome. Nerve growth factor (NGF) is important for pre and post natal brain development. Low birth weight (LBW) is a key determinant of neonatal mortality, morbidity, subsequent growth and developmental retardation and early onset of adulthood diseases. Reports suggest that breastfeeding plays a role in preventing the neurological consequences of growth retarded babies. The present study therefore examines the association of circulating levels of NGF and breast milk fatty acids in women delivering normal birth weight babies (NBW) and LBW babies at term ($\geq$37 weeks gestation).

Methods: Singleton pregnant women delivering NBW babies ($\geq$2.5 kg; n = 61) and LBW babies ($<$2.5 kg; n = 32) at term without any pregnancy complications were recruited at Bharati hospital Pune, India. The long chain polyunsaturated fatty acids were estimated using the gas chromatograph. The omega 3 fatty acids included alpha linolenic acid, eicosapentaenoic acid and docosahexaenoic acid while omega 6 fatty acids included linoleic acid, gamma linolenic acid, dihomo-gammalinolenic acid, docosapentaenoic acid and arachidonic acid. The study was approved by institutional ethics committee and participants' informed consent was obtained. Maternal and cord plasma NGF levels were analyzed using promega kits.

Results: Maternal plasma NGF levels were significantly increased ($p < 0.01$) in LBW (359.71 ± 90.66 pg/ml) as compared to NBW (286.71 ± 110.25 pg/ml) group. Similar increase in cord NGF levels was seen in LBW (171.67 ± 114.89 pg/ml) as compared to NBW (125.85 ± 60.77 pg/ml) group. Breast milk docosahexaenoic acid concentrations were increased ($p < 0.01$) in mothers delivering LBW babies (0.29 ± 0.17 g/100 g fatty acids) as compared to NBW babies (0.20 ± 0.11 g/100 g fatty acids). Maternal plasma NGF levels showed a positive association ($r = 0.254$, $p = 0.023$, $n = 80$) with milk omega 6 fatty acids. Cord plasma NGF levels were negatively associated with baby weight ($n = 85$, $r = -0.240$, $p = 0.025$), head circumferences ($n = 84$, $r = -0.211$, $p = 0.05$) and chest circumferences ($n = 84$, $r = -0.258$, $p = 0.017$).

Conclusions: Increased levels of NGF together with the increased docosahexaenoic acid content in breast milk may have most important implication as the adaptive changes to spare/protect early brain development. Breast feeding LBW babies may be advantageous and help to prevent adverse neurological consequences in later life. Future animal studies need to be carried out to understand how NGF concentrations regulate breast milk LCPUFA composition.

P-10C-460

Dehydroepiandrosterone in newborn nails: a new tool for the biological assessment of fetal stress following maternal adversities during pregnancy?

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Objective: Early life factors may predispose individuals to certain diseases across life. Therefore, an understanding of fetal responses to such factors is of paramount importance. Several tissues, including fetal blood, amniotic fluid, and cord blood, have been sampled and analysed for fetal stress hormones in previous studies. However, difficulty of sample collection and storage, as well as decomposition of body fluids and single measurements limit the usefulness of these approaches. These limitations may best be dealt with by identifying an easily accessible tissue that can cumulatively indicate the fetal biological stress-response. Newborn nail is such an easily accessible tissue that may offer insight into fetal physiology. The detection of endogenous concentrations of cortisol and cortisone has been demonstrated in human hair, which is histologically comparable to the human nail. The biological basis for the measurement of stress hormones in the nail is that both nail growth and adrenal steroidogenesis start early during pregnancy. The aim of our work was two-fold: It

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was to study whether i) the major fetal adrenal steroids, dehydroepiandrosterone (DHEA) and its sulfated form DHEA-S, can be detected in the newborn nail and ii) maternal stress during pregnancy is related to their concentration in the newborn nail.
Methods: We determined in 80 infants the concentration of DHEA and DHEA-S in their fingernails (mean weight of nail samples = 4.9 mg (range: 1–29.4 mg)) with tandem mass spectroscopy. We then compared the concentrations of DHEA and DHEA-S in the offspring's nail between offspring of mothers reporting stress and offspring of mothers reporting no stress during pregnancy. Maternal stress was defined as having experienced at least one very severe life event during pregnancy assessed with the Inventory of Life Events (ILE). Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Both steroid hormones were detectable in nail substance (detection limit was 50 pg/mg nail for DHEA and 10 pg/mg nail for DHEA-S). Infants of mothers reporting stress during pregnancy showed increased concentrations of DHEA ($t(41) = -2.471$, $p = 0.018$), while there was no difference between groups in DHEA-S concentrations ($t(77) = -0.876$, $p = 0.384$). To control for confounding by factors that influence general maternal stress sensitivity, we repeated analyses after stratifying the mothers according to stress before pregnancy and did not observe any group difference ($t(41) = -0.098$, $p = 0.922$).
Conclusions: The major fetal steroids are detectable in newborn nails. Hence, they appear to be incorporated into nail tissue from systemic sources or synthesized locally. The concentration of DHEA in the nail seems to be associated with maternal stress during pregnancy. Hence, the measurement of DHEA in newborn nails is a potential, easily applicable tool to study stress biology in the fetus. As pituitary-adrenal activity plays a major role in mental health and disease processes, the method may provide a new approach to scrutinize the psychobiological mechanisms underlying the developmental origins of mental health and disease. Support source: This project was supported by the German National Academic Foundation (PhD scholarship to MT).

P-10C-461

Preeclampsia and depressive symptoms in the offspring at age 60 years: The Helsinki Birth Cohort Study

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Objective: Low birth weight and short length of gestation pose a risk for depressive symptoms later in life. Preeclampsia is one of the main factors underlying fetal growth and earlier delivery. Yet, the impact of preeclampsia on offspring mental health is not known. Our aim was to test whether preeclampsia predicts depressive symptoms in the offspring later in life.
Methods: Using mothers' blood pressure and urinary protein measurements at maternity clinics and birth hospitals, we defined preeclampsia according to current criteria for 981 women and men born at term (between 37 and 42 weeks of gestation) after primi- and multiparous pregnancies, and who participated in the Helsinki Birth Cohort 1934–44 Study. At ages 60 and 63 years they completed the Beck Depression Inventory (BDI). Linear and logistic regressions were used for the statistical analyses. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: Those born at term after a primiparous pregnancy complicated by preeclampsia had 36.9 (95% CI, 2.0 to 71.8, $P = 0.04$) and 52.2% (95% CI, 7.8 to 96.6, $P = 0.02$) higher depressive symptoms scores at ages 60 and 63 years, respectively, compared to those born at term and after primiparous normotensive pregnancies. When we applied the cut-off criterion for mild depressive symptom severity (BDI score $\geqslant$10), those born after primiparous pregnancies complicated by preeclampsia were 3.7-fold (95% CI, 1.2 to 11.3, $P = 0.02$) more likely to have at least mild depressive symptoms than were those born after primiparous normotensive pregnancies. These effects were unaffected by adjustment for mother's age and body mass index at delivery, childhood socioeconomic status, gender, age and educational attainment at testing, and birth weight adjusted for gestation. Preeclampsia and depression were not associated among those born at term and after multiparous pregnancies ($Ps > 0.22$).
Conclusions: Preeclampsia predicts depressive symptoms in adult offspring, at least among those born at term after a primiparous pregnancy. There was no evidence for this among subjects born from a multiparous pregnancy. Our results suggest that propensity to depression has its origins during fetal life and highlight the importance of identifying specific pregnancy disorders in birth cohort studies.

P-10C-462

Spectral analysis of EEG in violent offenders with diagnosis of alcohol dependence

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Objectives: To contribute to electrophysiological characterization of the offenders with alcohol and illegal drugs abuse.
Methods: The resting electroencephalogram was recorded in 12 alcohol and illegal drugs abuse offenders, evaluated for forensic psychiatric (Experimental Group). They were compared with 9 offenders without psychiatrics disorders (Control Group). The features at visual inspection of the Electroencephalogram and the use of frequency domain quantitative analysis techniques and time-frequency are described.
Results: 50% of experimental group had electroencephalographic abnormalities. The most frequent were the background activity organizational alterations, low amplitude electrogenesis, an attenuated alpha rhythm. Global increase of the delta-theta slows activities associated with a decrease of the alpha activity. The quantitative analysis showed differences between the frequency spectrums and between the broad band spectral measures from both groups and between experimental groups and the Cuban norms. The global delta-theta frequencies and beta activity in posterior temporal and parietal regions predominate in the experimental group.
Conclusion: A high incidence of electroencephalographic abnormalities was found in the experimental group. The most frequents were: electrogenesis alterations, attenuated alpha rhythm and global delta-theta slow activity and excess of beta activity in posterior regions. The quantitative analysis confirmed this finding. The results provides a strong lead for examining the electrophysiological differences between offender groups, the neurotoxic effect of drugs on the brain and to establish possible relations between the deficiency in information processing capacity of central nervous system how one of possible mechanisms related to increase the likelihood of criminal act in offenders with this diagnosis.

P-10C-463

Impact of a participatory intervention with women's groups on maternal depression and exclusive breastfeeding in tribal communities of Jharkhand and Orissa, India

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Objective: To examine (1) the impact of a participatory intervention with women's groups on maternal depression and on exclusive breastfeeding in tribal communities of Jharkhand and Orissa (India); (2) to determine whether the intervention improved infant feeding practices by reducing maternal depression. Maternal depression is prevalent in low and middle-income countries and has adverse consequences for infant development[1]. Interventions that reduce maternal depression therefore have the potential to improve long-term child health outcomes[2].
Methods: We analysed data from a cluster-randomised controlled trial in 36 clusters of 3 districts in Jharkhand and Orissa, eastern India (ISRCTN21817853). The trial tested a participatory intervention with women's groups: in intervention clusters, women facilitators convened monthly meetings to support participatory action and learning for 244 groups. Groups facilitated the development and implementation of strategies to address maternal and newborn health problems. We monitored birth outcomes, health-care seeking and home care practices for 19 030 births in a population of 228 186 over 3 years. Maternal depression was measured using the Kessler-10 item scale in the last 2 years of the trial. We compared maternal depression as a categorical outcome between intervention and control clusters. In addition, we will compare exclusive breastfeeding among infants whose mothers had no or mild depression, with breastfeeding among infants born to mothers with moderate or severe depression, and stratify the analysis by intervention and control group. Appropriate institutional ethics committee clearance and participants' informed consent were obtained.
Results: In the third year of the intervention, 150 of 3119 (4.8%) mothers in intervention areas had K10 scores of 16–30 indicating moderate depression compared with 292 of 2963 mothers (9.8%) in control areas (adjusted OR 0.39, 95% CI: 0.16–0.92). In addition, infants were more likely to have been exclusively breastfed in the first six of life in the intervention clusters (OR 1.47, 95%CI: 0.90–2.39). We will further test and report whether the intervention may have improved breastfeeding practices by reducing maternal depression.
Conclusions: A participatory intervention with women's groups reduced moderate depression by 61% in rural tribal communities of eastern India and improved exclusive breastfeeding. The impact of the intervention on exclusive breastfeeding may have been mediated by its effect on maternal depression. The reductions in maternal depression achieved through a participatory intervention with women's groups may have positive long-term consequences for child health outcomes by influencing infant feeding practices.
Support: The Health Foundation & The Big Lottery Fund.

1. P.L. Engle. *Am J Clin Nut*, 89:963S–966S, 2009.
2. WHO-UNFPA. Maternal mental health & child health and development in low and middle-income countries. World Health Organization, 2008.

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Knowledge, attitudes and practices around health research: a trainee physicians' perspective

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Objectives: Assessment of the level of knowledge, attitudes and practices towards research amongst a group of Post Graduate Medical Trainees (PGMTs') at Aga Khan University (AKU), Pakistan.
Methods: A cross sectional health research survey was carried out on all PGMTs' at AKU Pakistan. AKU is a tertiary care health facility which offers residency in 28 specialties and fellowship in 16 programmes. Knowledge, attitudes and practices related to health research were assessed using a pretested, structured and validated questionnaire. Health research related practices of the residents were examined using questions graded on Likert scale. The study was conducted in compliance with 'Ethical principles for medical research involving human subjects' of Helsinki Declaration. Informed consent taken from all participants and all measures were undertaken to ensure the confidentiality of each participant.
Results: Mean percentage score ± SD on the knowledge scale was 36.9% ± 20.2 and 47.19% ± 25.18 on the attitude scale. Of 104(55.6%) who had previously participated in research 28(26.9%) had been involved in basic science research only, 62(59.6%) in clinical research and 14(13.5%) had participated in both clinical and basic science research projects. 88(47.1%) planned to pursue a future research career. Those who planned to pursue a future research career had more positive health research attitudes $p < 0.001$. Limited time (45%), poor research infrastructure (20%) and inadequate research funding opportunities (20%) were the major hurdles faced by PGMTs' to pursue research.
Conclusion: PGMTs' demonstrate inadequate knowledge, while they have moderate attitudes towards health research. Residency training and research facilities at the institution need to undergo major transformation in order to encourage meaningful research by resident trainees.

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Education and mortality: Causality and selection – A twin approach

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Objectives: A strong inverse relationship between socioeconomic position (SEP) and mortality has been widely demonstrated[1]. There seems to be a general agreement that socioeconomic conditions have lifelong effects, and that social exposures accumulate over the life course[2]. Nevertheless, it is disputed whether all phases of life are equally important or if there are critical periods. Is the effect of social conditions on mortality most pronounced in childhood, or is it primarily in adulthood that SEP plays a role? In addition to a causal effect of SEP, the association may be confounded by background factors, e.g. genetic factors, that influence both obtained SEP and mortality[3]. To disentangle the complex network of mechanisms potentially driving the association between SEP and mortality, we investigated whether SEP, indicated by educational status in adulthood, has an additional impact on all-cause mortality above and beyond the influence of childhood environment and genetic constitution. Intra-pair twin analyses allow us to isolate the effect of educational status in adulthood by means of adjustment for genetic and environmental confounding per design, exploiting the fact that twins are partly or fully genetically identical and have similar socioeconomic exposures in childhood due to their common upbringing. If twin pairs discordant on SEP differ significantly with respect to mortality, it will suggest an independent effect of SEP in adulthood, whereas a lack of association will point to other explanations i.e. childhood environment and genetic confounding.
Methods: The study is register-based and includes data from the Danish Twin Registry and Statistics Denmark. Using Cox regression, we estimated the hazard ratios for mortality according to highest attained education among 5260 monozygotic (MZ) and 11,088 dizygotic same sex (DZSS) twin pairs born 1921–1950. Follow-up lasted from 1980–2006 and analyses were conducted separately for zygosity (MZ and DZSS), gender and birth cohorts (1921–1935 and 1936–1950). Intra-pair analyses were carried out by inclusion of a strata variable of twin-pair specific values in the model. Results from standard and intra-pair analyses were compared.
Results: Preliminary results show the expected social gradients in mortality in the standard analyses of all analysed subgroups. For all-cause mortality, risk estimates ranged from HR = 0.69 (CI 95% 0.60–80) to HR = 0.87 (CI 95% 0.78–0.97) for "long" compared to "short" education". In the intra-pair analyses, the association between education and mortality was generally attenuated and most estimates were close to one (HR = 0.93 (CI 95% (0.71–1.23) to HR = 0.96 (CI 95% 0.71–1.31)), except for men born 1921–35 who displayed estimates of the same magnitude as in the standard analysis. We found no significant differences in the estimates within MZ and DZSS twin pairs, respectively, but a tendency to a larger attenuation of the association within the MZ twins.
Conclusions: Since the association was attenuated in the intra-pair analyses, the results are most compatible with an effect of early environmental and genetic factors in explaining the educational inequality in mortality. Due to limited

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statistical power in the intra-pair analyses of the 15,000 twin pairs, results should, however, be interpreted with caution.

1. D. Black *et al.,* Inequalities in health: The Black report: The health divide. London, Penguin. Ref Type: Report, 1988.
2. G.D. Smith *et al., BMJ,* 316:1631–635, 1998.
3. M. Osler *et al., Int. J. Epidemiol.,* 36:77–83, 2007.

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Knowledge, Awareness and Practices Regarding Dengue Fever amongst the population Dengue hit Cosmopolitan and Implications for its' Children

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Objectives: Recent studies report the incidence of Dengue Fever (DF) in children in slum population of Karachi to be 185/100,000 population per year. No documented evidence exists in Pakistan which reveals the awareness and practices of the country's population regarding DF. This study was conducted to assess the level of knowledge, attitudes and practices regarding DF in people visiting tertiary care hospitals in Karachi.

Methods: A cross-sectional pilot study was conducted among people visiting two tertiary care hospitals in Karachi. Through convenience sampling, a pre-tested and structured questionnaire was administered to 447 visitors. The study was conducted in compliance with 'Ethical principles for medical research involving human subjects' of Helsinki Declaration. Confidentiality of each participant was ensured. The study was approved by the Ethical Review Committee, Community Health Sciences Department at The Aga Khan University.

Results: 89.9% of individuals had heard of DF and most (84%) believed the disease was infectious and transmissible. Fever (81.5%) and bleeding (41.9%) were the most commonly recognized symptoms. 51.1% were aware that dengue mosquito breeds in clean water and bites at sunset (57.5%) or sunrise (44%). People were more focused towards prevention of mosquito bites (78.3%) rather than mosquito eradication (17.3%) with use of mosquito sprays (48%) as commonest preventive measure. Sufficient knowledge was found in 38.5% of sample, with 66% in Aga Khan University Hospital and 33% in Civil Hospital. Significant associations were found between knowledge scores and education ($p < 0.001$), income ($p < 0.001$), and the hospital of interview ($p < 0.001$). Respondents with education up to (grade-6) were more ignorant compared to those with 10 years of schooling (48.4% versus 29.7%). Income and hospital-presented-to were independent predictors of knowledge. Television (62%) was considered as the most important source of information on the disease.

Conclusion: Dengue fever has been labeled endemic in squatter settlements of Karachi. Inadequate knowledge about the disease and preventive measures was seen amongst low socio economic and illiterate population which could account for this trend.

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