Proceedings of the Nutrition Society

Abstracts of Communications

A joint meeting of The Nutrition Society and The Association for the Study of Obesity was held at the Royal College of Physicians, London on 18 February 1998, when the following papers were presented.

All abstracts are prepared as camera-ready material by the authors.

The Editors of the Proceedings of the Nutrition Society *accept no responsibility for the abstracts of papers read at the Society's meetings for original communications.*

Consumption of yoghurt containing modified fat increases satiety and reduces subsequent food intake. By A.A. BURNS[1], L. LINDMARK[2], M.B.E. LIVINGSTONE[1], Ú. MULLANEY[1], I. ROWLAND[1] and R.W. WELCH[1], *[1]Northern Ireland Centre for Diet and Health, University of Ulster Coleraine, BT52 1SA and [2]Scotia LipidTeknik AB, Box 6686,11384 Stockholm, Sweden*

The effect of fat on satiety is not fully understood. Although fat relative to other macronutrients is considered to have the weakest effects on satiety (Blundell *et al.* 1996), infusion of fats into the small intestine has been shown to increase satiety in human subjects (Welch *et al.* 1988). It is possible that the type and form of fat may influence its effects on satiety. The aim of the present study was to compare the effects on satiety and food consumption of two yoghurts with the same macronutrient content, but which had different types of fat. Yoghurts were available in 200 g portions (800 kJ) and provided 6 g total fat. The control yoghurt contained only milk fat. In the test yoghurt 5 g milk fat was replaced with a defined fat emulsion (fractionated vegetable oil, acylglycerols in emulsified form; LipidTeknik, Stockholm).

In a double-blind study twenty-nine volunteers (14M and 15F; 19-32 years; BMI<30 kg/m^2; non-smokers) were given the same breakfast on two occasions (25 % of estimated total energy expenditure) at 09.00 hours. Following this only water was permitted until 13.00 hours when subjects were given 200 g of the control or test yoghurt in random order. Satiety ratings were evaluated (100 mm visual analogue scales) immediately before and after consumption of the yoghurt and hourly until 17.00 hours. At 17.00 hours volunteers were given *ad libitum* access to a range of foods. Intake was assessed by weighing the food before eating and then any leftovers, and macronutrient intake was determined using Compeat 4. Energy intakes, macronutrient intakes and satiety ratings were compared using paired t tests.

Food intake at evening meal	Energy (MJ)		Fat (g)		Protein (g)		Carbohydrate (g)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
M + F (*n* 29)								
Test	6.41**	0.49	70.4***	6.17	58.3*	4.75	177	12.6
Control	7.62	0.33	91.0	4.80	67.0	4.49	196	10.0
F (*n* 15)								
Test	5.26**	0.43	58.1**	5.90	48.8	5.14	142*	10.7
Control	6.74	0.35	80.0	5.01	58.0	3.99	174	13.6
M (*n* 14)								
Test	7.66	0.78	83.5*	10.2	68.5	7.39	214	19.3
Control	8.55	0.46	103	7.22	75.9	7.64	220	11.4

* Mean values were significantly different from control yoghurt, *P<0.05 ,** P<0.01, *** P<0.001(2-tail).

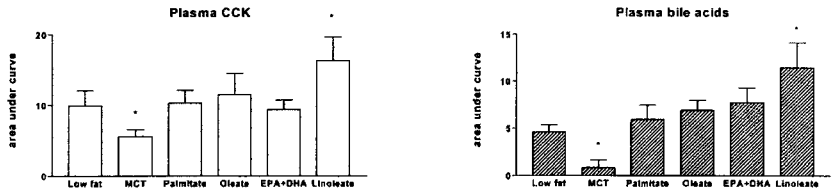
Satiety levels were higher with the test yoghurt from 13.00 to 17.00 hours, but these differences were only significant (P<0.05) at 16.00 and 17.00 hours. For the group as a whole, energy, fat and protein intakes were all significantly lower after the test yoghurt. While there was a significant suppression of energy, fat and carbohydrate intake in the females, only fat intake was significantly reduced in the males.

These results indicate that small amounts of defined fat can exert significant effects on satiety and subsequent food intake. These effects may be due to mechanisms similar to those observed for fat infusions into the small intestine (Welch *et al.* 1988). Further longer term studies will help to clarify potential effects in other meal patterns and male-female differences.

Blundell, J.E., Lawton, C.L., Cotton, J.R. & MacDiarmid, J.I. (1996). *Annual Review of Nutrition* **16**, 285-319.
Welch, I., Sepple, C.P. & Read, N.W. (1988). *Gut* **29**, 306-311.

Influence of dietary fat composition on postprandial plasma bile acid and cholecystokinin concentrations in premenopausal women. By VASSILIKI COSTARELLI[1], T.A.B SANDERS[1] and MARK JORDINSON[2], *[1]Nutrition, Food and Health Research Centre, King's College London, Campden Hill Road W8 7AH and [2]Department of Gastroenterology, Imperial College School of Medicine, Hammersmith Hospital, Ducane Road, London W12 0HS*

Plasma bile acids may influence risk of breast cancer by promoting the growth of mammary tumours (Baker *et al.* 1992). In the fasting state plasma bile acid concentrations are low but rise following a meal (Hofmann, 1977). Cholecystokinin (CCK) is the main stimulus for the secretion of bile acids and its secretion is stimulated by the intake of fat and protein (Mossner *et al.* 1992). The purpose of the current study was to investigate whether dietary fat composition influenced postprandial plasma bile acid and CCK concentrations. Twelve healthy premenopausal women consumed six isoenergetic test meals (4.9 MJ, 32 g protein, 5.1 g fibre), which consisted of a muffin and milk shake, in random order with one week between treatments. Five 50 g fat meals (20 g oleate, 20 g palmitate, 20 g linoleate, 20 g medium chain triglycerols (MCT) and 15 g Oleate + 5 g eicosapentaenoic acid (EPA) + docosahexanoic acid (DHA) were compared with a low fat meal (15 g high oleate sunflower seed oil, 12 g 18:1n-9, 2 g 18:2n-6, 0.6 g 16:0, 0.6 g 18:0) where the fat energy was replaced by an isoenergetic amount of polydextrose. The test meals were devised so that 30 g of the fat was held constant (approximately 20 g 18:1n-9, 6 g 18:2n-6, 2 g 16:0, 2 g 18:0) and 20 g consisted of the test fatty acids. Venous blood samples were obtained fasting and at 30, 60 and 180 min after consuming the test meal for measurements of plasma bile acid by GC - mass spectrometry and CCK by radioimmunoassay. The area under the curve (AUC) was calculated for CCK and bile acids and the results are presented in the following Figs.



*denotes significantly different from the low fat group P<0.05; Bonferroni's Multiple Comparison Test.

There were significant differences between test meals in the postprandial AUC for both plasma CCK and bile acids (both P<0.01). Compared with the low fat meal, the MCT rich meal suppressed the rise in plasma CCK and bile acid concentrations (P<0.05), whereas the linoleate rich meal increased plasma CCK and plasma bile acid concentrations (P<0.05). In rats MCT have been found to increase CCK more than other fatty acids (Douglas *et al.* 1990). The reasons for these differences warrant further investigation.

Baker, P.R., Wilton, J.C., Jones C.E., Stenzel D.J., Watson, N. & Smith, G.J. (1992). *British Journal of Cancer* **65**, 566-572.
Douglas, B.R., Jansen, J.B., de Jong, A.J., & Lamers, C.B. (1990). *Journal of Nutrition* **120**, 686-90.
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Reproducibility of lipaemic, insulinaemic and glycaemic responses to a high-fat, mixed meal. By JASON M.R. GILL and ADRIANNE E. HARDMAN, *Human Muscle Metabolism Research Group, Loughborough University, Leicestershire LE11 3TU*

High-fat meals, often containing carbohydrate as well as fat, are widely used as a means of measuring triacylglycerol (TAG) metabolic capacity but there are few reports of the reproducibility of the responses of plasma constituents. The purpose of the present study was, therefore, to evaluate the reproducibility of postprandial lipaemic, insulinaemic and glycaemic responses to a mixed, high-fat test meal.

Eight men aged 31-59 years, with BMI 26.3 (SD 2.1) kg/m^2, and body fat 27.9 (SD 7.2) % participated. They were studied on two occasions, with an interval of 1 week. Subjects arrived at the laboratory after a 12 h fast and blood samples were obtained by venous cannulation in the fasted state and 0.5, 1, 2, 3, 4, 5 and 6 h after consumption of the meal. This comprised cereal, coconut, nuts, chocolate, fruit and whipping cream (1.70 g fat, 1.65 g carbohydrate/kg fat-free body mass). Before each trial, subjects refrained from physical activity for 3 d, smoking for 2 d (only one subject was a smoker) and avoided alcohol for 1 d. In addition, they weighed and recorded all food and drink intake for 2 d before their first trial, replicating this diet before their second trial. Plasma was analysed for total cholesterol (TC) (fasted state only), HDL-cholesterol (HDL-C) (fasted state only), TAG and glucose, by enzymatic colorimetric methods (all Boehringer Mannheim Gmbh, U.K. Ltd). Serum was analysed for insulin by radioimmunoassay (COAT-A-COUNT Insulin, Diagnostic Products Corporation, Los Angeles, CA). Lipaemic, insulinaemic and glycaemic responses were determined as the areas under the plasma or serum concentration *v.* time curves. TAG and cholesterol concentrations were corrected for differences in plasma volume between trials (Dill & Costill, 1974). Results are shown in the Table:

	Fasting TAG (mmol/l)		Fasting TC (mmol/l)		Fasting HDL-C (mmol/l)		Lipaemic response (mmol/l.6h)		Insulinaemic response (μIU/ml.6h)		Glycaemic response (mmol/l.6h)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Trial 1	1.25	0.30	4.96	0.42	1.33	0.14	12.99	3.0	172.3	39.4	30.8	1.3
Trial 2	1.18	0.26	5.09*	0.43	1.35	0.13	12.75	3.1	178.4	38.4	31.4	1.3

* Significantly different from trial 1 values, $P<0.05$.

Paired t tests revealed no significant differences between trials for any index except fasting TC concentrations. The observed difference in TC, however, is probably of little clinical importance. Spearman rank order (Pearson product moment) correlations between the two trials were 0.95 (0.99) for the lipaemic responses, 0.81 (0.95) for the insulinaemic responses and 0.88 (0.94) for the glycaemic responses. A limits of agreement approach (Bland & Altman, 1986) revealed a mean difference of 0.24 mmol/l.6 h (1.9 %) in the lipaemic responses between trials 1 and 2 with a 95 % CI range for the difference from -2.61 mmol/l.6 h (-20.1 %) to 2.13 mmol/l.6 h (16.3 %). The mean differences between trials were 1.9 % (95 % CI -6.6 – 10.3 %) and 3.5 % (95 % CI -36.3 – 43.4 %) for the glycaemic and insulinaemic responses respectively. The findings indicate that with adequate prior control of lifestyle factors, the reproducibility of the lipaemic response to a high-fat mixed test meal is good. However, the intra-individual variability in the insulinaemic response to such a meal appears to be greater.

This research was supported by the British Heart Foundation.

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Dill, D.B. & Costill, D.L. (1974). *Journal of Applied Physiology* **37**, 247-248.

The effect of fish oil supplementation on fasting and postprandial lipid levels in individuals with an atherogenic lipoprotein phenotype (ALP). By A.M. MINIHANE[1], C. PATERSON[1], C. CHAPMAN[2], E.C. LEIGH-FAIRBANK[1], N. FURLONG[2], M.C. MURPHY[2], J.W. WRIGHT[2], B.A. GRIFFIN[2] and C.M. WILLIAMS[1], [1]*The Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading RG6 6AP;* [2]*Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH*

Accumulating evidence demonstrates a strong positive association between plasma triacylglycerol (TAG) levels and CHD risk. Elevated TAG-rich lipoproteins (TRL) are intimately associated with a decreased level of HDL-cholesterol (HDL-C) and a predominance of the small dense, 'atherogenic' LDL-3 particle. This combination of lipoprotein abnormalities, which has been defined as the atherogenic lipoprotein phenotype (ALP), is associated with a five fold increase in CHD risk. Numerous epidemiological and intervention studies have demonstrated the TAG-lowering potential of the fish-oil fatty acids as reviewed recently (Harris, 1997). However the biochemical mechanisms responsible for this hypotriacylglycerolaemic effect have not been determined.

In a randomized crossover double-blind placebo-controlled study, the effect of daily fish-oil supplementation on fasting and postprandial lipid, lipoprotein and hormone responses in middle-aged men with the ALP dyslipidaemia was investigated. The study consisted of two 6-week periods of test (2.8 g eicosapentaenoic acid + docosahexaenoic acid/d as 6 g fish oil) or 6 g olive oil supplementation, separated by a 12-week wash-out period. Fasting blood samples were collected at 0, 3 and 6 weeks. In addition, on the last day of the 6-week intervention period, after a 12 h overnight fast, postprandial responses were determined following a breakfast (t=0 h) and lunch (t=5.5 h) meal. Blood samples were collected at regular intervals throughout the day, up to 8 h after breakfast. Plasma samples were analysed for total cholesterol (TC), HDL-C, TAG, non-esterified fatty acids (NEFA), glucose, LDL-3 and apolipoprotein B.

	Age (years)		BMI (kg/m^2)		TAG (mmol/l)		TC (mmol/l)		HDL-C (mmol/l)		Glucose (mmol/l)		LDL-£ (%)	
n	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
22	54	2	28.4	0.8	2.66	0.22	6.61	0.18	0.90	0.05	5.7	0.2	72	2

Results presented here are preliminary data from a larger study (n 80). The Table shows the screening variables of the participants. Fish-oil supplementation resulted in a significant 25 % decrease in fasting TAG levels ($P<0.05$) from 2.65 (SE 0.81) to 1.99 (SE 0.78) mmol/l over the 6-week period. A marked decrease in postprandial lipaemia was also evident with the incremental area under the curve for TAG being 23 % less following the fish oil period ($P<0.05$). No significant change in fasting or postprandial glucose or TC was evident, although fasting TC did decrease by 5% whilst on fish oils. A lower postprandial NEFA response following the lunch meal was evident after the fish oil supplemented period, however this trend failed to reach significance at the 5 % level ($P=0.07$).

Fish oil supplementation had a significant effect on plasma TAG in our ALP group. Further work measuring plasma acylation stimulating protein levels and plasma and adipose tissue levels and gene expression of lipoprotein lipase will be carried out in this study, which will help us to gain a better understanding of the biochemical basis of the observed antiatherogenic benefits of fish oils.

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The effects of soyabean isoflavones on plasma HDL concentrations in healthy male and female subjects. By TRACEY S. DEAN[1], J. O'REILLY[1], ELIZABETH BOWEY[2], HELEN WISEMAN[1] IAN ROWLAND[2] and T.A.B. SANDERS[1], *[1]Nutrition, Food & Health Research Centre, King's College London, Campden Hill Road, London W8 7AH, [2]BIBRA International, Woodmanstern Road, Carshalton, Surrey, SM5 4DS*

A meta-analysis of the effects of consuming soyabean protein suggests a favourable change in plasma lipoprotein concentrations (Anderson *et al.* 1995). Soyabeans contain isoflavones that have some oestrogenic effects. As oestrogens are known to increase the expression of LDL receptors and increase HDL concentrations, we postulated that soyabean products with isoflavones would have a beneficial effect on plasma lipids. The aim of the present study was to test the hypothesis that isoflavones present in soyabean protein isolates influence plasma HDL concentrations. A randomized crossover trial of two types of soyabean product (only difference being low or high in isoflavones) of 2 weeks duration with a 2 week wash-out period between treatments was conducted in twenty-two subjects (five male, seventeen female, mean age 30 years). The treatments were either rich (56 mg/d) or low (2 mg/d) in isoflavones. Subjects were also asked to avoid foods (Reinli & Block, 1996) known to be rich in isoflavones during the entire study period. Fasting venous blood samples were obtained on the last 2 d of each treatment phase for lipid and lipoprotein analyses. The results are shown in the Table.

	High-isoflavone soyabean product (*n* 22)		Low-isoflavone soyabean product (*n* 22)	
	Mean	SE	Mean	SE
HDL cholesterol (mmol/l)	1.50**	0.07	1.44	0.07
Apolipoprotein AI (g/l)	1.60**	0.06	1.51	0.06
Apolipoprotein AII (g/l)	0.46	0.01	0.46	0.01

Mean values were significantly different from low isoflavone group, ** $P<0.01$ (paired t test)

Plasma total cholesterol and apoprotein B concentrations were unaffected by the isoflavone content. However, plasma HDL and apolipoprotein AI concentrations were significantly greater on the high-isoflavone diet. Studies in primates have reported that soyabean isoflavones increase plasma HDL and lower LDL cholesterol concentrations (Anthony *et al.* 1996). The results of the present study suggest that isoflavones at concentrations present in human diets can exert effects on plasma lipoproteins. However, we failed to find any influence on LDL concentrations. This may be because the subjects were young and mostly female and, therefore, would have upregulated LDL receptors. Further studies in older subjects are warranted.

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The influence of dietary isoflavones on markers of lipid peroxidation in healthy male and female volunteers. By JAMES O'REILLY[1], TRACEY S. DEAN[1], ELIZABETH BOWEY[2], IAN ROWLAND[2] T.A.B SANDERS[1] and HELEN WISEMAN[1], *[1]Nutrition, Food and Health Research Centre, King's College London, Campden Hill Road, Kensington, London W8 7AH and [2]BIBRA International, Woodmansterne Road, Carshalton, Surrey SM5 4DS*

Epidemiological evidence suggests that consumption of soyabean products is protective against atherosclerosis and some forms of cancer (Adlercreutz, 1990). Soyabeans contain isoflavones, principally genistin and daidzin which may act as antioxidants *in vivo*. Lipid peroxidation in LDL leading to oxidative modification is widely believed to be a key step in atherogenesis. We have shown previously that genistein (the aglycone of genistin) inhibits lipid peroxidation *in vitro* (Wiseman & O'Reilly, 1995). Hitherto, there has been little evidence that isoflavones have antioxidant activity *in vivo*. The aim of the present study was to investigate the influence of isoflavones on markers of *in vivo* lipid peroxidation. A randomized crossover study was carried out in which a diet high in isoflavones (56 mg/d) and a diet low in isoflavones (2 mg/d) was consumed for 17 days separated by a 21 day washout period. Fasting venous blood samples were obtained at the end of each dietary period. 8-Epi-$PGF_{2\alpha}$, a prostaglandin F_2-like compound (F_2-isoprostane), is formed *in vivo* from the peroxidation of arachidonate. This species was extracted from plasma by a solid-phase procedure which was followed by separation using gas chromatography and analysis by negative-ion chemical ionization mass spectrometry (Nourooz-Zadeh *et al.* 1995). LDL was isolated from plasma preserved using sucrose (10%, w/v) by density gradient ultracentrifugation (O'Reilly *et al.* 1997). The resistance of LDL to cupric ion-catalysed oxidation was determined from the length of the lag phase before diene formation (Esterbauer *et al.* 1989).

	Plasma 8-epi-$PGF_{2\alpha}$ (pg /ml) (*n* 18)		LDL[a] lag phase (min) (*n* 18)	
	Mean	SE	Mean	SE
Low-isoflavone diet	405	50	44	1.9
High-isoflavone diet	326*	32	48*	2.3

Mean values were significantly different from low-isoflavone diet,* $P < 0.05$ (paired *t*-test)
[a] LDL protein concentration was 0.1 mg/ml

The Table shows the mean length of the LDL lag phase and the mean concentration of 8-epi-$PGF_{2\alpha}$ in plasma obtained from volunteers after consumption of both diets. After consumption of the diet high in isoflavones the length of the LDL lag phase was 48 min compared with 44 min for LDL obtained after the low-isoflavone diet. Thus, LDL isolated after isoflavone consumption was more resistant to cupric ion-induced oxidation. The concentration of 8-epi-$PGF_{2\alpha}$ was approximately 20% lower in plasma obtained after consumption of the high-isoflavone diet compared with the low-isoflavone control diet. The findings of this study suggest that soyabean isoflavones inhibit lipid peroxidation *in vivo*. This may be one mechanism by which soyabean products appear to be protective against certain diseases.

We thank MAFF and EC (FAIR-CT-95-0894) for financial support.

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Wiseman, H & O'Reilly, J. (1995). *Biochemical Society Transactions* **25**, 107S.

The effect of chronic acipimox administration on nutrient selection and energy balance following surgery. By A.R. BOSAGH ZADEH and P.W. EMERY, *Department of Nutrition and Dietetics, King's College London, Campden Hill Road, London W8 7AH*

The metabolic response to surgical trauma is characterized by increased glucose turnover, increased energy expenditure and loss of body fat. We have previously tried to quantify the increase in glucose utilization by offering rats a choice between a high-carbohydrate diet and a high-fat diet after surgery, but their carbohydrate intake did not increase (AR Bosagh Zadeh and PW Emery, unpublished results). However there was a profound decrease in fat intake which may have been caused by an obligatory increase in lipolysis. To test this hypothesis we have, therefore, determined whether chronic administration of acipimox, an inhibitor of lipolysis, would increase fat intake and improve energy balance in the postoperative period. Thirty-six adult female Sprague-Dawley rats were offered a choice between two diets containing 20 % energy from protein and either 10 % or 50 % energy from fat. Four rats were killed for measurement of initial carcass composition and half the remaining rats underwent hysterectomy under halothane-anaesthesia. The operated rats were offered the same choice of diets *ad libitum* after surgery while the non-operated controls were individually pair-fed using the same diets. Half the rats in each group had 10 mg acipimox/g added to the diet throughout the postoperative period. The rats were killed 4 d after surgery, body composition was measured and energy expenditure was calculated from the difference between energy intake and energy stored.

	Normal				Acipimox			
	Surgical		Control		Surgical		Control	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fat intake (g/4 d)	7.2	0.5	7.2	0.5	9.1	0.6	9.1	0.6
Body protein (g)	41.7	1.5	42.5	0.6	41.0	0.9	42.7	0.8
Body fat (g)	18.9*	1.6	24.0	1.2	22.9	1.7	23.9	1.5
Energy expenditure (kJ/4 d)	971*	58	793	35	887	40	769	58

Significantly different from corresponding controls: * $P<0.05$.

Before surgery the rats chose to consume 42 % energy as fat and 38 % energy as carbohydrate. After surgery the proportion of energy from carbohydrate rose to 47 %, but the total energy intake fell so that the amount of carbohydrate consumed by the normal surgical rats actually fell from 6.5 g/d to 5.8 g/d, and the amount of fat consumed fell from 3.2 to 1.8 g/d. Administration of acipimox increased the amount of fat consumed by 25 % ($P<0.05$). Surgery caused a significant loss of body fat in the normal rats, and this was almost completely suppressed by acipimox. The calculated value for energy expenditure was significantly increased in the normal surgical rats in comparison with pair-fed controls, whereas in the acipimox-treated rats this increase was 33 % smaller and not statistically significant. These results suggest that lipolysis may be an important factor in the increase in metabolic rate following surgery.

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Impact of maternal nutrient restriction in early to mid gestation on lamb size and insulin-like growth factor-1 (IGF-1) status at term. By LINDSAY HEASMAN, TERENCE STEPHENSON and MICHAEL E. SYMONDS, *Division of Child Health, School of Human Development, University Hospital, Nottingham NG7 2UH*

It is established that alterations in maternal feed intake at different stages of gestation have differential effects on placental and fetal growth (Godfrey *et al.* 1996). We have shown that maternal nutrient restriction over the period of maximal placental growth (30-80 d) initially results in a smaller placenta at 80 d of gestation (Clarke *et al.* 1998), but a larger placenta close to term (Symonds *et al.* 1998). The present study, therefore, aimed to investigate the impact of maternal nutrient restriction in early to mid gestation on lamb size and plasma IGF-1 concentrations in cord samples taken close to term.

Forty-seven singleton bearing ewes were fed to meet either half their maintenance energy requirements between 28 and 77 d of gestation (450 (SE 50) g hay and 106 (SE 11) g concentrate; nutrient restricted; NR; *n* 28) or twice their requirement (965 (SE 68) g hay and 220 (SE 15) g concentrate; controls; C; *n* 19). All ewes were then fed to requirements for the remainder of gestation. At 145 d all lambs were delivered by Caesarean section, an umbilical venous blood sample was taken for measurement of plasma IGF-1 concentration, and lamb dimensions were measured. Plasma IGF-1 was measured using the ELISA OCTEIA® kit no. AC-27F1 (IDS, Boldon, Tyne and Wear, UK).

Lambs born to nutrient-restricted ewes tended to be heavier (NR: 3.91 (SE 0.11); C: 3.67 (SE 0.13) kg; (ANOVA)), have a longer crown-rump length (NR: 504 (SE 4); C: 482 (SE 6) mm), a larger thoracic circumference (NR: 368 (SE 3); C: 358 (SE 5) mm), and were taller (NR: 436 (SE 5); C: 422 (SE 5) mm), than controls. There was no difference in plasma IGF-1 concentrations between groups (NR: 102.5 (SE 7.1); C: 95.8 (SE 10.6) μg/l). In controls, however, all body dimensions were significantly correlated with plasma IGF-1 concentration (r^2 0.58-0.87; $p<0.001$), a relationship that was not observed in lambs born to nutrient-restricted ewes (r^2 0.02-0.25).

Nutrient-restriction in early to mid gestation followed by feeding to requirements for the remainder of gestation significantly alters the relationship between fetal bone growth and IGF-1. This may be related to altered nutrient flux across the placenta and provides strong evidence of reprogramming of the IGF-1 growth axis *in utero*.

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Prevalence of overweight and underweight in Chinese adolescent girls. By XUEQIN DU[1], HEATHER GREENFIELD[1], DAVID R. FRASER[2] and KEYOU GE,[3] *[1]University of New South Wales, Australia, [2]University of Sydney, Australia, [3]Chinese Academy of Preventive Medicine, China*

As part of a cross-sectional study on the nutritional status and bone health of Beijing adolescent girls, anthropometry measurements were conducted to assess growth and development and to investigate associated environmental factors. A random sample of 1200 girls aged 12-14 years was selected from schools in the Beijing area. Weight and height were measured in September and October, 1995, and BMI was calculated (kg/m^2). Stepwise regression analysis of BMI on potential associated factors was performed, including nutrient intakes derived from a semi-quantitative food frequency questionnaire (Du *et al.* 1997a), physical activity, age, bone age determined from hand-wrist X-ray and pubertal status (Du *et al.* 1997b). School Physical Activity Score (SPAS) was used as an indicator of physical activity level (1 – 4, where 1 = most active) at school over the previous 12 months.

Nutritional status*	BMI (kg/m^2)	No. of subjects	%
Underweight	< 18	391	32.4
Acceptable weight	18 - 21	573	47.5
Overweight	> 21	93	7.7
Obese	> 23	149	12.4

* Modified from Ministry of Public Health and National Education Committee (1993), for girls aged 13 years.

Prevalence rates of overweight and obesity were 7.7% and 12.4%, respectively, using the Chinese reference standards for adolescent girls, while underweight was very high at 32%. Applying the Australian 95th BMI percentile for adolescent girls as the cut-off for overweight in this population, 8.7% of the girls would be classified as overweight (Lazarus *et al.* 1995). A small proportion of the girls (0.7%) had a BMI of >30. Inadequate nutrient intakes may contribute to the problem of underweight. The mean total energy intake of the study girls was about 6500 kJ/d which was only 68% of the Chinese RDA for this group, while energy intakes were even lower, by 6%, among the underweight girls. Protein intake was about 50 g/d against the Chinese recommendation of 80 g/d for this group if consuming a plant-based diet.

Bivariate correlation showed that BMI was positively correlated with menarcheal status (P<0.0001), bone age (P<0.0001), SPAS (P<0.01), total energy intake (P<0.05) and protein intake (P<0.05). The preliminary results of multiple linear regression analysis showed that all these factors except for total energy intake were predictors of BMI, though including the SPAS and protein intake only increased R^2 by 1%. The model explained 10% of the variation in BMI. In summary, underweight was the major problem in this population of Beijing adolescent girls while overweight was apparently beginning to emerge as a problem, associated with lower physical activity and a higher rate of maturation.

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Obesity and hyperleptinaemia in metallothionein-null mice. By JOHN H. BEATTIE, ANNE M. WOOD, JACKIE S. DUNCAN, IAN BREMNER and PAUL TRAYHURN, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB.*

Metallothionein (MT) is a low molecular weight metal-binding protein which has proposed roles in metal detoxification, Zn and Cu homeostasis, free-radical scavenging, and resistance to stress. Nevertheless, mice with targeted disruption of MT-I and -II genes (MT-null mice) are reported to have no phenotypic abnormalities and show normal reproduction and development (Michalska & Choo, 1993; Masters *et al.* 1994). A survey of MT-null mice maintained at the Rowett indicated that male animals were significantly heavier than male C57BL/6J (MT-normal) mice of the same age. Indeed, over 20 % of the male MT-null mice aged 22-39 weeks had a body weight of 46-59 g, with an average for the colony of 40.3 (SE 0.9 n 58) g. The heavier mice had noticeably large depots of white adipose tissue and fatty livers. In growth studies from weaning to 14 weeks of age, we found that MT-null mice grew at a significantly faster rate than MT-normal mice and that the weight divergence was most marked between age 5 and 7 weeks. From 8 to 14 weeks, the growth rate of MT-null mice paralleled that of the MT-normal mice, thus maintaining a weight difference of about 6 g.

To further investigate this phenotype, three groups of six MT-null mice, aged 22-39 weeks, were selected so that one group (Lean group) was weight-matched with the MT-normal mice, a second group (Average group) had a mean weight equivalent to the mean for the MT-null male colony aged 22-39 weeks and a third group was representative of the larger mice (Obese group). All animals were of similar mean age and age variance. Epididymal white adipose tissue (eWAT):body weight ratio was found to be significantly greater in the Average and the Obese groups, indicating that the mice in both groups were relatively obese (see Table). eWAT *ob* gene expression was significantly elevated in the Average and Obese MT-null groups, compared with the Lean and MT-normal groups (see Table). More strikingly, the mean plasma leptin concentration in the Obese group was over 80 ng/ml, which was 25-fold higher than the levels in the MT-normal group. The Average group mice were, by comparison, only moderately obese, but plasma leptin levels were significantly elevated (see Table).

	MT-normal		MT-null					
			Lean		Average		Obese	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
eWAT/body weight (mg/g)	16	1	15	2	33**	3	48**	2
ob mRNA (% 18S rRNA)	18	4.4	2.3	0.9	43.9*	2	49.2*	7.1
Plasma leptin (ng/ml)	3.2	0.5	9.8	4.9	21.5*	4.3	81.0**	24.2

Mean values were significantly different from those for MT-normal mice, *P < 0.05, **P < 0.001 (unpaired t test using a pooled estimate of error)

Food intake of 15-week-old MT-normal and MT-null mice was monitored over a period of 17 d and was found to be 4.5 (SE 0.1) g/mouse and 6.1 (SE 0.4) g/mouse respectively, a difference that was statistically significant (unpaired t test: P < 0.001). Thus, the MT-null mice actually consumed significantly more food than the MT-normal mice, indicating leptin insensitivity and a mechanism for the accretion of fat. A role for MT in the regulation of energy balance is implied but possible alternative explanations, such as perturbation of genes neighbouring the MT locus by targeted disruption of MT, have yet to be excluded.

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Increased circulating leptin levels after muscle gene transfer in normal mice. By AMELIA MARTI[1], FRANCISCO J. NOVO[2], EDUARDO MARTINEZ-ANSO[1] and J. ALFREDO MARTINEZ[1], *[1]Department of Physiology and Nutrition, [2]Department of Genetics, University of Navarra, 31080 Pamplona, Spain*

Leptin is a hormone produced by fat cells and placenta, which is involved in body weight and food intake regulation, but also in other peripheral actions such as reproduction, immunity, lipolysis, etc. (Frühbeck *et al.* 1997). In this context, new support is given to the genetic hypothesis of obesity and the possibility of developing new therapeutic strategies to treat obese individuals. The aim of the present communication is to report the potential usefulness of muscle gene transfer to increase the *in vivo* production of leptin in normal mice. A plasmid expression vector containing mouse leptin cDNA under the regulation of a cytomegalovirus immediate early promoter and myosin light chain enhancer (Novo *et al.* 1997) was injected in the tibialis anterior muscle of 5-week-old Balb/c mice. Sham-injected animals were included as the control group. Circulating plasma leptin levels as well as other metabolic indicators (body weight, plasma glucose and insulin) were assayed 5 d after injection. Fat tissue O_2 consumption was also measured *ex vivo* with a Clarke electrode (YSI Model 5300 biological O_2 monitor).

	Control (n=5)		Leptin (n=5)	
	Mean	SE	Mean	SE
Plasma Glucose (mmol/l)	6.9	0.65	8.2	0.69
Plasma Insulin (μU/ml)	28.4	3.1	26.3	2.6
Plasma Leptin (U/ml)	2.32	0.48	5.52*	1.08

* Significantly different from the control group, $P<0.05$ (2-tail t test).

Muscle gene transfer of a leptin-expressing plasmid vector induced a two fold increase in leptin levels in normal immunocompetent non-obese mice. However, no significant changes were found in plasma glucose or insulin. In addition, body-weight gain was similar in both experimental groups during the experimental (5 d) although an increase in fat O_2 consumption was detected (+20 %) which was not statistically significant.

The data suggest that leptin can be produced in muscle and released into the bloodstream, without modifying either glycaemia or insulinaemia in normal mice, which is in contrast to previous studies in genetically obese animals (Muzzin *et al.* 1996). Our results may be explained by the short-term experimental period and the low circulating leptin levels achieved by this approach, but raise new possibilities concerning gene therapy in obese individuals.

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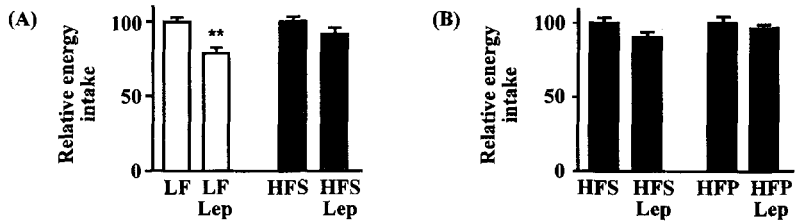
Leptin insensitivity in C57BL/6J mice fed on high-fat diets of differing composition. By D.V. RAYNER, HELEN WALBANK and PAUL TRAYHURN, *Division of Biomedical Science, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB*

Our understanding of the regulation of energy balance is changing rapidly following the identification of the *ob* gene. This gene is expressed primarily in white adipose tissue (Zhang *et al.* 1994), encoding a secreted hormone, leptin, which affects energy balance by decreasing food intake and stimulating energy expenditure (Pellymounter *et al.* 1995). High-fat diets are considered to predispose to obesity, and although the basis for this has not always been evident, such diets have recently been reported to reduce leptin sensitivity in mice (Van Heek *et al.* 1997). It is not, however, clear whether this relative leptin resistance, which relates to the inhibitory effects of the hormone on food intake, is a general effect of high-fat diets or if it is dependent on the nature of the dietary lipid. In the present study we have examined the effects of feeding two high-fat diets of differing composition (saturated/mono-unsaturated and polyunsaturated) on the decrease in food intake induced in mice by leptin.

C57BL/6J mice (males, aged 6-7 weeks) were housed individually and fed on either a low fat (LF; 12 % of energy from maize oil) or a high fat (HFS; 47 % of energy as fat, predominately from lard) semi-purified diet (190 g lactalbumin/kg as protein source) for 3 weeks. Half the mice in each group were then treated with recombinant mouse leptin (Peprotech; 1.25 μg/g intraperitoneally, twice daily) or vehicle, for 7 days. Food intake and body weight were measured daily during the 4 weeks of the study. In the second experiment, maize oil was substituted for the lard to compare the effects of a high polyunsaturated fatty acid diet (HFP) with the HFS diet.



During 7 d of treatment with vehicle, gross energy intakes on the LF and HFS diets were not significantly different ($P>0.05$, n 8). Energy intake was significantly ($P<0.01$) reduced in the leptin treated (Lep) group on the LF diet, by 19 % of that of the animals injected with vehicle (Fig. 1A; all data in Fig. 1 shown as mean values and SE, n 8). In contrast, the reduction in intake in the mice fed on the HFS diet was only 7 %, and this decrease was not significant. In the second experiment, energy intakes were not significantly different on the two high-fat diets, and neither group exhibited a significant fall in intake during leptin treatment (Fig. 1B).

This study shows that leptin significantly decreases food intake in mice fed on a LF diet, but not in mice fed on either of two high-fat diets. It is concluded that leptin insensitivity is induced by high-fat diets and that this occurs with diets of widely differing fatty acid composition. Dysregulation of the leptin system induced by high-fat diets may be a factor in the development of obesity.

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Functional phenotypes: increased leptin and BMR as adaptive responses to a habitual high-fat (high energy) diet. By JOHN COOLING[1], JULIAN BARTH[2], and JOHN BLUNDELL[1], *[1]Biopsychology Group, School of Psychology, University of Leeds, Leeds, LS2 9JT [2]Department of Chemical Pathology and Immunology, Leeds General Infirmary, Leeds LS1 3EX*

It has been argued that body weight is maintained over time by current environmental conditions and by an individual's behavioural and metabolic characteristics (functional phenotypes) (Hill *et al.* 1995). We have identified habitual fat consumption as a meaningful phenotype (Macdiarmid *et al.* 1996) which affects behavioural processes concerning appetite control (Cooling & Blundell, 1998). This line of investigation has been extended to examine certain physiological processes which may characterize those with habitual high-fat dietary intakes (HF) and low-fat intakes (LF). Evidence from the Dietary and Nutritional Survey of British Adults has indicated that some individuals with a high fat (high energy) intake remain lean (Macdiarmid *et al.* 1996) suggesting some behavioural or physiological protection against weight gain and obesity. We report here two separate studies in which different cohorts of HF and LF were compared.

In study 1 BMR and other metabolic variables were measured by indirect calorimetry (SensorMedics Vmax 29) in eight HF and eight LF males (mean % energy as fat 44.3 and 32.0 respectively). In study 2 fasting plasma leptin and other variables were measured in ten HF and nine LF males (mean % energy as fat 45.4 and 31.8 respectively). HF and LF did not differ significantly in BMI (mean HF 22.5, mean LF 22.4 kg/m^{-2}), percentage body fat (measured by impedance procedure, Spacelabs BC-300) (mean HF 11.4 %, mean LF 10.2 %) or age (mean HF 22.2, mean LF 21.7 years) for both studies combined.

	Study 1			
	HF		LF	
	Mean	SE	Mean	SE
BMR (MJ/day)	6.8*	0.15	6.1	0.27
Resting heart rate (beats/min)	66.1*	2.5	57.1	2.5
Resting RQ	0.84*	0.008	0.89	0.002
	Study 2			
Plasma leptin (ng/ml)	2.92*	0.43	1.79	0.15
Plasma glucose (mmol/l)	4.88*	0.08	5.18	0.11
Plasma triacylglycerol (mmol/l)	0.91	0.10	0.87	0.08

Mean values were significantly different from LF , *P<0.05 (2-tail)

Study 1 indicated that HF and LF differed significantly in BMR and resting heart rate despite having almost identical mean BMI, percentage body fat, body weight and age. In study 2 HF and LF groups had significantly different plasma leptin levels. Leptin values correlated significantly with percentage body fat (r 0.72, P<0.001), with fat mass (r 0.82, P<0.001) and with dietary fat intake (r 0.52, P<0.02), but not with total energy intake (r 0.32, P=0.18) and inversely with % carbohydrate intake (r -0.53, P<0.02). Taken together these findings suggest the possibility that metabolic adjustments (BMR, RQ and plasma leptin) may offer protection against the potential weight-gain effects of a high fat diet in habitual high-fat consumers (HF phenotype).

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Studies on the leptin system in adult human subjects from an Aberdeen population. By L. THOMAS[1], J. BROOM[2], S. HEYS[3] and P. TRAYHURN[1], *[1]Division of Biomedical Science, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB; [2]Research & Development Office, Aberdeen Royal Infirmary, Foresterhill, Aberdeen, AB25 2ZD; [3]Department of Surgery, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD*

The aetiology of obesity has been extensively investigated, but there is continuing uncertainty as to the main determinants of the disease. Considerable interest has been generated by the recent identification of the *ob* gene and its protein product, leptin. Leptin is secreted by mature adipocytes and is thought to function in part as a satiety signal regulating the size of fat depots (Zhang *et al.* 1994). While a number of factors which regulate *ob* gene expression and the production of leptin have been identified in animals, as yet little is known of the regulation and functions of the hormone in man. However, it is now clear that there is an increase in the expression of the *ob* gene and elevated circulating leptin levels in obese compared with lean subjects; indeed there is a positive correlation between BMI and circulating leptin (Considine *et al.* 1996*a*). The present study has examined the relationship between circulating leptin and BMI in an Aberdeen population, together with the expression of the *ob* and leptin receptor genes in fat biopsies from patients undergoing surgery.

Serum was obtained from patients (n 34; 17 male, 17 female; age 57.5 (SE 2.9)) attending the lipid and diabetic clinics at Aberdeen Royal Infirmary. Circulating leptin levels were analysed by a sensitive human-specific ELISA (Hardie *et al.* 1996). In the second part of the study serum samples (n 7) and subcutaneous adipose tissue biopsies (n 11) were obtained from patients (both sexes) undergoing surgery for gall-bladder or inguinal hernias. One group was fasted before surgery (n 7) while the second was fed intravenously (n 4). Reverse transcription polymerase chain reaction (RT-PCR) was carried out on adipose tissue biopsies to investigate expression of the *ob* gene and of the leptin receptor gene (Ob-R and Ob-Rb splice variants).

In the group of 34 subjects, leptin levels ranged from <0.1 to 106.8 (28.8 (SE 4.4)) ng/ml and BMI varied from 18.6 to 44.5 (30.0 (SE 1.2)) kg/m^2. There was a positive correlation between circulating leptin level and BMI in this group (r 0.47, P=0.004). In the second part of the study, leptin levels (n 7) ranged from 0.5 to 232 (65.7 (SE 29.5)) ng/ml, and the BMI from 23.2 to 46.3 (28.5 (SE 2.2)) kg/m^2. There was no apparent effect of nutritional state on plasma leptin. The RT-PCR analysis showed that *ob* gene expression was evident in all biopsy samples. In addition, each sample exhibited expression of the leptin receptor gene when employing primers for all the known receptor splice variants (Ob-R). Expression of Ob-Rb, the long form splice variant containing an intracellular signaling domain, was also evident in a number of samples. To date, there has been no previous report of Ob-Rb mRNA in human adipose tissue, although Ob-R expression has been described in human hypothalamus (Considine *et al.* 1996*b*).

In summary, a positive correlation between BMI and plasma leptin was evident in an Aberdeen group of subjects with a wide range of BMI, similar to findings from studies on other populations. Fat biopsies showed clear evidence of expression of the *ob* gene, and importantly of both Ob-R and Ob-Rb receptor mRNA. Leptin is believed to be involved in a feedback loop to adipocytes, and the expression of the Ob-Rb receptor splice variant in fat samples is therefore potentially important, implying a paracrine role for the hormone in adipose tissue.

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