Proceedings of the Nutrition Society

Abstracts of Communications

A Scientific Meeting was held at the University of Newcastle on Wednesday–Friday, 9–11 July 1997, when the following papers were presented.

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

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Differential effects of supplementation with a vitamin E analogue on antioxidant enzyme expression in human skin fibroblasts exposed to ultraviolet B. By SANDRA A. JONES, CATHERINE I. A. JACK and MALCOLM J. JACKSON, *Department of Medicine, University of Liverpool L69 3GA*

Ultraviolet (u.v.) light is known to generate free radicals and ROS, causing premature ageing and proliferative changes in skin (Halliwell, 1992; Garmyn *et al.* 1995). The role of free radical species and the potential protective effect of antioxidants in these processes is unknown. Many of the modifications associated with u.v.-induced ageing of the skin are related to changes in gene expression by fibroblasts (Moysan *et al.* 1993). Therefore, we have developed a model system using cultured human skin fibroblasts to examine the role of free radicals in ultraviolet B (u.v.B) induced changes in gene expression and the ability of supplemental antioxidants to modify this.

Human skin fibroblasts obtained from American Type Culture Collection (ATCC) were cultured in DMEM medium plus 100ml fetal calf serum/l and 5 x 10^4 IU penicillin/l, 50mg streptomycin/l, 25μg amphotericin B /l , 25mg gentamicin/l. Following u.v.B exposure at 80 mJ/cm^2, the cells were harvested at specified times and analysed for changes in the activity of key antioxidant enzymes, catalase (E.C.1.11.1.6; CAT) and superoxide dismutase (E.C.1.15.1.1; SOD). Further fibroblast cultures were treated with Trolox, a water-soluble vitamin E analogue at concentrations of 0-50 mg/l, 14 days before study.

	CAT (U/mg)		SOD (U/mg)		Cell viability (%)	
	mean	SE	mean	SE	mean	SE
non-exposed	0.48	0.05	0.16	0.01	100	1.50
u.v.B exposed only	1.21	0.11	0.36	0.04	97	1.49
u.v.B exposed, trolox (50mg/l)	0.56	0.10	0.23	0.02	88	1.61

Exposure to u.v.B induced a rapid rise in the activity of both CAT and SOD six hours post exposure. Cultured fibroblasts supplemented with 50mg trolox /l prior to u.v.B exposure, showed a significant decrease in the rise of CAT activity compared with the non-supplemented, exposed fibroblasts. There was no significant effect of trolox on SOD activity. These data (see above table), indicate that u.v.B-induced change in CAT expression may be induced by free radicals and potentially modified by antioxidant supplementation, although similar mechanisms may not underly the change in SOD.

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Increased cooking stability of all-*trans*-β-carotene in red palm oil using *Murraya koenigii* or *Cnidoscolus aconitifolius* leaves. By G. LIETZ[1], C.J.K. HENRY[1] and A.M. TOMKINS[2], [1]*Oxford Brookes University, School of Biological and Molecular Sciences, Gipsy Lane, Headington, Oxford OX3 0BP,* [2]*Centre for International Child Health, 30 Guildford Street, London WC1N 1EH*

Vitamin A deficiency has been recognized as a major public health problem in the developing world. Since pre-formed vitamin A in meat and dairy products is out of reach for the economically deprived, the main source of vitamin A in the diet of the rural communities is carotenoids from vegetables and fruits. Red palm oil is one of the richest sources of β-carotene. Before the initiation of an intervention trial, using red palm oil as a dietary supplement in pregnant women in Tanzania, we studied the kinetics of decay of all-*trans*-β-carotene under deep-fat frying conditions. Since minor food constituents (i.e. phenylalanine) are known to alter the degradation of all-*trans*-β-carotene (Papadopoulou & Ames, 1994), we studied the effect of leaves traditionally used in frying operations in less industrialized countries. The two leaves chosen were *Murraya koenigii* (curry leaf) and *Cnidoscolus aconitifolius* (chaya leaf) which are reputed to contain "antioxidants".

The effect of these "antioxidant"-rich leaves on the degradation of all-*trans*-β-carotene in red palm oil was investigated under controlled conditions at 170°c for 30 min, with samples analysed every 5 min. A 0.1 g portion of either leaf was added to 1 g red palm oil. All-*trans*-β-carotene was analysed using a recently modified HPLC procedure (Lietz & Henry, 1997).

Table 1. *Degradation of all-trans-β-carotene in red palm oil at 170°c in the presence of air with and without the addition of either* Murraya koenigii *or* Cnidoscolus aconitifolius

Treatment	All-*trans*-β-carotene (% initial concentration)													
	0 min	SD	5 min	SD	10 min	SD	15 min	SD	20 min	SD	25 min	SD	30 min	SD
Oil alone	100	1.5	48.1	2.1	30.2	2.8	17.2	0.9	1.9	0.3	0.9	0.9	0.6	0.7
Murraya koenigii (1 mg/g)	100	1.6	58.7	1.0	38.0	1.6	35.8	1.5	18.5	2.6	19.8	2.4	18.3*	4.0
Cnidoscolus aconitifolius (1 mg/g)	100	1.4	65.5	0.7	46.0	4.0	40.4	2.8	26.0	2.3	13.1	3.7	10.9*	2.3

*Mean values were significantly different $P<0.05$ (ANOVA).

The degradation of all-*trans*-β-carotene was significantly lower with the addition of either curry leaf or chaya leaf (Table 1). For the first 15 min of frying, chaya leaf afforded a better protection of all-*trans*-β-carotene than the curry leaf. In contrast, at the end of 30 min the curry leaf appeared to be more protective. The concentrations of lutein, all-*trans*-β-carotene and α-tocopherol were very similar in both leaves.

It is concluded that the use of traditional leaves as a source of "antioxidants" may be a useful means of improving the stability of all-*trans*-β-carotene in red palm oil during cooking in the less industrialized countries.

Supported by Oxford Brookes University, and the Overseas Development Administration.

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Plasma total homocysteine in 972 people aged 65 years and over, representative of Britain, in a National Diet and Nutrition Survey. By C.J. BATES[1], M.A. MANSOOR[2], J.C. VAN DER POLS[1], A PRENTICE[1], T.J. COLE[1] and S. FINCH[3], *[1]MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ, [2]Division of Clinical Chemistry, Central Hospital in Rogaland, Stavanger, Norway and [3]Social Community Planning and Research, 35 Northampton Square, London EC1V 0ED*

Raised concentrations of plasma total homocysteine (tHcy) can predict increased risk of vascular diseases, and provide functional evidence of low B-vitamin status. Older people have higher tHcy than young adults, and may also have lower intakes and status for critical B-vitamins, but few data exist for Britain. The present study measured tHcy in participants in the National Diet and Nutrition Survey of People Aged 65 Years or Over (Finch *et al.* 1997), a representative sample of mainland Britain. Fieldwork, during 1994-5, included an interview, a 4 d weighed dietary record, anthropometry, health measurements, and fasting blood for haematology and biochemistry. The blood was chilled and transported to a local hospital within 3-4 h for immediate separation. tHcy was measured in Stavanger, Norway by a method based on HPLC (Mansoor *et al.* 1992). Of 2624 people for whom participation was possible, 2059 completed the interview; 1276 gave a blood sample, and 972 had tHcy measured. One fifth of the participants were in long-stay institutions such as nursing homes; the remainder were at private addresses.

Plasma total homocysteine (μmol/l)	Males			Females		
	Median	Mean	SD (n)	Median	Mean	SD (n)
Free-living						
65-74 years	13.80	14.88	5.46 (163)	12.50	13.45	4.83 (149)
75-84 years	15.90	17.12	8.92 (151)	14.65	15.69	6.57 (136)
85+ years	16.40	18.16	7.22 (61)	15.20	17.43	7.14 (86)
All ages	15.20	16.35	7.43 (375)	13.85	15.24	6.29 (373)
Institutions						
65-74 years	16.05	17.49	6.52 (24)	15.60	16.73	4.09 (8)
75-84 years	18.20	20.11	7.53 (47)	17.45	21.00	12.55 (38)
85+ years	18.00	20.03	10.77 (50)	19.80	21.58	9.30 (57)
All ages	17.65	19.55	8.87 (121)	19.30	21.00	10.31 (104)

All groups had higher tHcy than Britons aged 40-59 years (Perry *et al.* 1995) or Swedes aged 35-61 years (Mansoor *et al.* 1995) and 51.4% of values were greater than 15μmol/l, a suggested upper limit of the normal range. tHcy increased with age, was generally greater in males than in females, and greater in institutionalized than in free-living people. tHcy was directly correlated with plasma cysteine, cysteinyl-glycine, urea, creatinine, α_1-antichymotrypsin and calcium, and inversely correlated with blood measures of folate, vitamin B_{12}, B_2, zinc and lutein adequacy. This dataset, from a large and representative sample of people aged 65 or over, provides reference data for tHcy in Britain.

The authors acknowledge financial support from the Department of Health, the British Heart Foundation and the European Commission.

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Folic acid status and uracil misincorporation in human DNA. By SUSAN J. DUTHIE and PAUL McMILLAN, *Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB*

Fruit and vegetables are a major source of folic (pteroylglutamic) acid and there is evidence that low folate status may be involved in the pathology of several malignancies, notably cancer of the cervix, lung and colon. Folate is essential in the synthesis of purines and the pyrimidine nucleoside thymidine. Deoxyuridine monophosphate is converted to thymidine monophosphate by the methyl donor, 5,10, methylene tetrahydrofolate. Poor folate status may block normal methylation and lead to misincorporation of uracil into DNA in the place of thymidine. Imbalances in these DNA precursors may decrease DNA stability and increase the risk of cancer (Reidy, 1987).

To study the relationship between folate deficiency and DNA instability we have modified the comet assay (Collins *et al.* 1993) by inclusion of the bacterial DNA repair enzyme, uracil DNA glycosylase, to detect uracil misincorporation specifically in both normal human lymphocytes and cultured human cervical cells (HeLa).

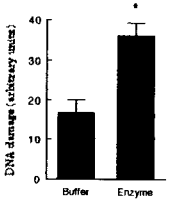

Fig. 1. Detection of uracil in human DNA. Lymphocytes incubated in the presence of uracil DNA glycosylase show increased strand breakage owing to excision of misincorporated uracil compared with cells incubated in buffer alone. Results are means with their standard errors * $P < 0.05$ for $n = 4$.

HeLa cells and lymphocytes, incubated for 60 min at 37° with 1 unit of uracil DNA glycosylase per μg DNA (enzyme), contained detectable levels of uracil in their DNA (Fig. 1), estimated as a few hundred per cell using X-ray calibration (Collins *et al.*, 1996). Lymphocytes cultured for 8 d in folate-deficient (F-) medium grew less rapidly and showed significantly more DNA damage revealed by uracil DNA glycosylase than cells grown in folate-replete (F+) control medium (Table 1).

Table 1 The effect of folate depletion on misincorporated uracil in human lymphocyte DNA

Cell number (10^5/ml)				DNA damage (arbitrary units)			
F-		F+		F-		F+	
Mean	SE	Mean	SE	Mean	SE	Mean	SE
0.51*	0.12	6.38	0.93	234.0*	29.6	114.3	15.6

* Significantly different from cells grown in control medium, $P < 0.05$ (for $n = 4$).

Incubating HeLa cells with 100 μM-deoxyuridine for up to 3 d did not affect growth (results not shown). However, the number of DNA breaks detected after incubation with uracil DNA glycosylase in cells grown in deoxyuridine for 2 d was increased 2-fold compared with cells grown in medium alone. The mean level of damage (arbitrary units) in the deoxyuridine-treated group was 201.7 (SE 28.8) compared with 101.8 (SE 8.7) in the untreated group ($P< 0.01$, for $n = 6$).

In conclusion, we can specifically detect misincorporated uracil in both normal human lymphocyte and transformed epithelial cell DNA. This assay should prove valuable in determining the role of folic acid status in DNA instability and human health.

Funded by the Scottish Office Agriculture, Environment and Fisheries Department.

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Dietary flavonoids and DNA damage in isolated human lymphocytes: genotoxicity and cytoprotection. By SUSAN J. DUTHIE and VICTORIA L. DOBSON, *Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB*

Reactive oxygen species may be involved in the aetiology of a number of human diseases such as cancer. Diets rich in fruit and vegetables are protective due to their content of antioxidants such as vitamin C, vitamin E and β-carotene. However, dietary components such as flavonoids may also be important.

The present study investigated the toxic and protective effects of the flavonoids quercetin, myricetin and silymarin in isolated human lymphocytes. DNA damage (strand breakage) was measured using the comet assay (Duthie *et al.* 1996). Inhibition of cell growth was also determined. Comet analysis was performed either on lymphocytes incubated with flavonoid for 30 min at 37° (toxicity) or on lymphocytes pretreated with flavonoid before exposure to 200 μM - H_2O_2 for 5 mins on ice (cytoprotection).

Lymphocytes showed different concentration-dependent susceptibilities to the flavonoids; quercetin induced the most DNA strand breakage. Quercetin was also growth inhibitory (Table 1; mean values with their standard errors for at least three determinations).

Table 1. The effect of flavonoids on DNA damage and inhibition of growth in human lymphocytes

	DNA damage (arbitrary units)						Inhibition of growth (% untreated)					
Conc	Quercetin		Myricetin		Silymarin		Quercetin		Myricetin		Silymarin	
(μM)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	20.9	5.3	23.3	4.8	17.0	3.1						
10	24.5	12.1	38.7	6.4	18.8	3.7	72.5	12.6	111.8	11.8	105	11.6
100	199.5	6.7	62.5	21.4	61.9	13.6	38.8	3.4	97.4	9.7	62.4	4.5

Quercetin significantly inhibited H_2O_2-induced DNA strand breakage (46 %). Myricetin also decreased oxidant-induced DNA damage but this was significant only at 100 μM (results not shown). Silymarin was not cytoprotective (Table 2; mean values with their standard errors for at least three determinations).

Table 2. The effect of flavonoids on hydrogen peroxide-induced DNA damage (arbitrary units)

Treatment	Quercetin		Myricetin		Silymarin	
	Mean	SE	Mean	SE	Mean	SE
Untreated	22.9	4.7	22.7	3.4	17.7	5.4
Flavonoid (50 μM)	49.3	16.2	42.0	8.8	11.0	5.5
H_2O_2 (200 μM)	184	10.3	156.2	9.1	166.0	11.0
Flavonoid + H_2O_2	99.8	3.1*	109.2	19.4	179.3	15.4

* Significantly different from H_2O_2-treated lymphocytes $P < 0.01$.

In conclusion, at high concentrations flavonoids induce DNA damage in human lymphocytes. At physiological concentrations, quercetin protects against oxidative DNA damage.

Funded by the Scottish Office Agriculture, Environment and Fisheries Department and The Ministry of Agriculture, Fisheries and Food.

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Features of the iron binding antioxidant potential of preterm baby plasma. By A. LOBAN[1], S.C. YONG[2], A.T. GIBSON[2] and H.J. POWERS[1], *[1]University Department of Paediatrics, Sheffield Children's Hospital, The University of Sheffield, S10 2TH and [2]The Neonatal Intensive Care Unit, The Jessop Hospital for Women, Sheffield S3 7RE*

A major antioxidant defence of human plasma is to prevent metal ions participating in free-radical reactions. Plasma transferrin can bind Fe in a form that will not stimulate free-radical reactions. Plasma proteins albumin and bilirubin, as well as uric acid, can also bind Fe (Davies *et al.* 1986; Hulea *et al.* 1995). Non transferrin-bound Fe, present in the plasma of some preterm babies (Kime *et al.* 1996), may confer particular importance on the Fe-binding activity of these plasma constituents. The importance of these plasma constituents as antioxidants was investigated using a liposome system.

Plasma was collected from nineteen babies born between 25 and 34 weeks gestation, on the day of birth, and from twenty-three healthy adults. The ability of these plasma samples to inhibit liposome oxidation was assessed. Liposomes were prepared (New, 1990) and their oxidation was assessed by measuring the production of thiobarbituric acid-reactive products after a 1 h incubation at 37°. 'Maximum' liposome oxidation was induced by 100 μmol/l ascorbic acid which is presumed to act by reducing endogenous trace Fe. Ascorbic acid was included in all the reaction mixtures; the ability of plasma samples to protect liposomes from oxidation was expressed as a percentage inhibition. Transferrin, caeruloplasmin, albumin, bilirubin and uric acid were also measured in these samples.

	Adults (*n* 23)		Preterm babies (<24 h old; *n* 19)	
	Mean	SD	Mean	SD
Transferrin (g/l)	2.4	0.48	1.1***	0.26
Caeruloplasmin (mg/l)	340.5	41.07	113.6***	27.67
Albumin (g/l)	41.0	5.19	23.7***	3.56
Bilirubin (mg/l)	6.8	2.37	39.5***	16.53
Uric acid (mg/l)	37.8	13.12	60.8***	17.56
% Inhibition	58.9	12.62	55.1	11.74

***Preterm group significantly different from adult group, P<0.001

As shown in the Table, plasma from preterm babies is as efficient at inhibiting oxidation of liposomes as plasma from adults. On the day of birth, babies born preterm had significantly lower concentrations of plasma transferrin, caeruloplasmin and albumin than adults. The antioxidant activity in adults was positively correlated with concentrations of albumin ($p<0.01$) and transferrin ($p<0.05$). In contrast, preterm babies had significantly higher plasma concentrations of bilirubin and uric acid than adults, which may compensate for the relative lack of albumin, transferrin and caeruloplasmin.

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A method to measure haemoglobin turnover rate. By J.M. HIBBERT[1], P.S. SWERDLOW[2], D.C. GORE[1], L.WOLFE[1], F. JAHOOR[3] AND A.A. JACKSON[4]. *Departments of [1]Surgery and [2]Hematology, Medical College of Virginia, Richmond, USA, [3]Children's Nutrition Research Center, Baylor College of Medicine , Houston, USA and [4]Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX*

The synthesis of haemoglobin in erythrocytes (RBC) is thought to account for 5 % of total body protein synthesis but has never been measured directly, although the life span of RBC and the rate of haemoglobin synthesis have been estimated (Callender *et al.* 1945; Shemin & Rittenberg, 1946; London *et al.* 1949). We measured the rate of haem synthesis, *in vivo*, an index of haemoglobin turnover, in six normal adults.

Glycine is the sole nitrogenous precursor for protoporphyrin, from which haem is formed. A tracer dose of [^{15}N]glycine, 800 mg, was given over 12 h to label the haemoglobin *in vivo*. A baseline sample of blood was collected at zero time and at 12 h (by which time isotopic enrichment had been achieved in the RBC free glycine pool) and 24 h (by which time labelled RBC had been released into the circulation). Enrichment of free glycine was determined from a TCA extract of 3 ml RBC, which was esterified and derivatized, before being measured with selective ion monitoring at m/z 272 to 273 by positive chemical ionization gas chromatography mass spectrometry. Haem was isolated from 1 ml haemolysed RBC and enrichment measured by combustion isotope ratio mass spectrometry. With the enrichment in RBC free glycine being taken as the precursor pool, the fractional rate of haem formation (fHbT, %/d), for a defined period of 12 h, was calculated according to the equation:

fHbT = change in enrichment of haem/ change in enrichment of free glycine pool.

From this a value for whole-body haemoglobin turnover rate (HbT, mg/kg per d) was derived.

Sex	Age (years)	Weight (kg)	fHbT (%/d)	HbT (mg/kg per d)
F	41	50	1.2	110
F	25	66	2.8	272
F	22	46	0.72	62
M	26	66	0.73	82
M	25	91	0.96	107
M	25	83	0.88	99

HbT was very high in one subject, but for the others the mean rate was 92 mg/kg per d, with about 20% variability. The theoretical value derived by London *et al.* (1949) was 90 mg/kg per d. The variability among individuals is of the order found for most measures of protein kinetics in normal people.

There is the need to determine the extent to which the enrichment of free glycine in the circulating RBC is a reasonable approximation for the enrichment in the precursor pool at the site of haem formation, and the extent to which nutritional, life-style and other factors might contribute to the variability within and among individuals.

This work was supported in part by NIH Clinical Research Center Grant M01 RR00065.

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Association between present dietary intake and bone health in post-menopausal and elderly Scottish women. By S. A. NEW[1], J. A. TREDGER[1], R. SMITH[2], M. C. GREENACRE[1], D. A. GRUBB[3] and D. M. REID[2], *[1]Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH, [2]Osteoporosis Research Unit, University of Aberdeen, Aberdeen AB9 1BQ, [3]Computing Department, Rowett Research Unit, Aberdeen AB2 9SB*

Adequate intakes of Ca and vitamin D are considered to be important for bone health of elderly women. Suprisingly, however, little is known about the influence of other micronutrients. This is remarkable considering that a diet which is low in Ca is unlikely to provide adequate quantities of other nutrients. In two recent cross-sectional studies, our group has identified intakes of K, Mg, Zn, fibre & vitamin C as being important to bone health of pre-menopausal women (New *et al.* 1996, 1997). The aim of the present study was to see if we could confirm our previous findings in post-menopausal & elderly women.

As part of a collaborative European study, a total of 200 Scottish women aged 55-87 years were randomly selected and invited to attend for a bone mineral density (BMD) scan of their lumbar spine (LS) and left hip (femoral neck FN) using dual X-ray absorptiometry. Women were asked to complete our validated food frequency questionnaire (FFQ) (New & Bolton-Smith, 1993). Weight, height, age of menarche, years since menopause, medication use and history of fracture were also recorded.

A total of 177 women completed the FFQ (88% response rate). Results are presented for 164 subjects since thirteen FFQ were rejected due to incorrect completion. Macro and micronutrients were similar to the reference nutrient intake for the UK population. Positive correlations were found between intakes of protein, Ca, P, K, Mg and fibre and LS BMD but remained significant only for K after adjustment for confounding factors including energy, weight and height. Nutrient intakes were grouped into quartiles and the mean BMD at the LS and FN were calculated. Significant differences were found at the LS and FN BMD for intakes of protein, Ca, P, K, Mg and Zn but only remained significant for intakes of protein, P and K after adjustment for confounding factors as shown in the Table below.

		Quartiles of nutrient intake							
		Protein				Potassium			
		1	2	3	4	1	2	3	4
LS BMD (g/cm^2)	Mean	0.806^a	0.841^{ab}	0.856^{ab}	0.953^b	0.806^a	0.820^{ab}	0.878^{ab}	0.953^b
	SD	0.164	0.162	0.154	0.157	0.151	0.162	0.153	0.163
FN BMD (g/cm^2)	Mean	0.674^a	0.685^{ab}	0.709^{ab}	0.811^b	0.672^a	0.691^{ab}	0.728^{ab}	0.784^b
	SD	0.135	0.127	0.132	0.129	0.124	0.129	0.127	0.121

^{a,b} Values with unlike superscripts within a category were significantly different, ANCOVA, $P<0.05$.

To determine whether nutrients were independent predictors of BMD, energy, nutrient intake and confounding factors were entered into a regression model. K intake was found to account for 9% of variation in LS BMD (being more important than age {6%}or wt{2%}), and 5% of variation in FN BMD.

The study is limited as data are cross-sectional and thus only associations and not relationships between nutrition and BMD can be determined. However, these results suggest that nutrients other than Ca, and in particular K, may also be important to bone health in older women. Further analysis of past dietary intakes in the critical stages of peak bone mass development would be helpful.

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Glucose tolerance is affected by previous consumption of a high-fat meal. By R.A. HENDERSON, G.E. VIST and R.D.E. RUMSEY, *Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN*

The effect of the fat content of an evening meal on the glycaemic response to oral glucose and oral fat tolerance tests the following morning was studied. With the approval of the local Ethics Committee, twelve healthy male subjects were provided with a high-fat meal (62% energy as fat) and a low-fat meal (13% energy as fat) on two occasions each. Meals comprised a pasta-based main course (vegetable lasagne, Heinz Weightwatchers) followed by a dessert (Safeways Delight, made with either skimmed milk or 2/3 double cream and 1/3 single cream); a high-energy (carbohydrate) drink was given with the low-fat meal so that the two meals were isoenergetic (3520 kJ). Thus, meals were identical other than the fat:carbohydrate ratio with respect to other macronutients and the total energy ingested. Subjects consumed nothing other than water until the next morning. An oral glucose tolerance test (OGTT, 100 g glucose; HyCal SmithKline Beecham) was undertaken, 12 h after the evening meal, once after the high-fat meal and once after the low-fat meal. Similarly, an oral fat tolerance test (OFTT, 84 ml dairy cream containing 40 g fat) was undertaken once after the high-fat meal and once after the low-fat meal. Treatments were performed in a randomized order. Arterialized blood samples were collected before and at intervals after ingestion of the test drinks and analysed for concentration of glucose. Statistical analysis was by repeated measures ANOVA and paired *t* tests where appropriate.

There were no differences between fasted blood glucose concentrations in the morning after the high- and the low-fat meals (P=0.184).

There were highly significant differences between glycaemic responses after the four tests (P<0.001). With respect to the OFTT, subsequent to the high-fat meal the blood glucose levels did not change significantly from the fasted concentration (P=0.38), but blood glucose fell in response to OFTT subsequent to the low-fat meal (P=0.04).

	Oral glucose tolerance test			
	Low-fat premeal		High-fat premeal	
	Mean	SD	Mean	SD
Peak value (mmol/l)	7.9	1.4	8.8*	1.7
Time to peak (min)	31.3	13.0	38.3	16.4
Slope value (mmol/l per min)	0.14	0.07	0.15	0.06
Area under curve (mmol.min per l)	1767	160	1719	205

* Mean value was significantly different from low-fat premeal, P=0.009.

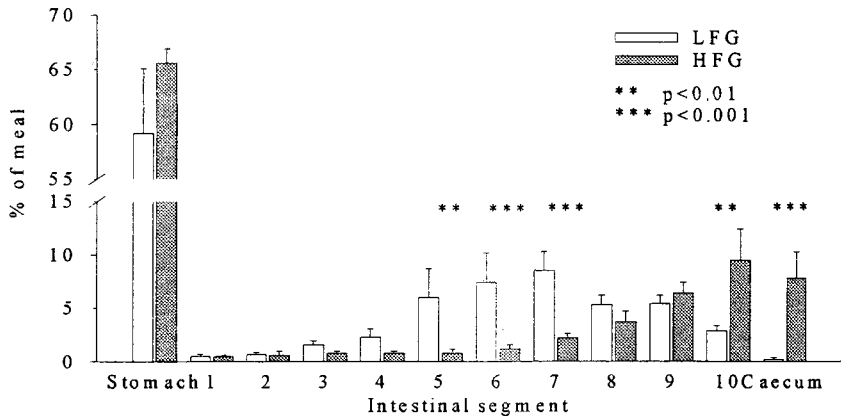
The high-fat evening meal also resulted in a significantly higher peak blood glucose concentration during OGTT but there were no differences in time to peak, slope value or areas under the curves (table). Similarities in time to peak and slope value suggest no differences in transit or absorption due to the premeal and metabolic factors are the likely cause of the increased blood glucose concentrations.

These results demonstrate that ingestion of a single high-fat meal has an effect on glucose tolerance the next morning. Consumption of a single high-fat meal results in a higher peak blood glucose concentration during OGTT and elimination of a fall in blood glucose during OFTT. In addition to the importance of these results in the consideration of glycaemic balance, there are obvious clinical and research implications for the need to control pretest meals.

Gastrointestinal transit adaptation to force-feeding high-fat meals in adult rats. By A. SHAFAT and R.D.E. RUMSEY, *Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN*

Fat content of a meal increases stomach-to caecum transit time in a dose-dependent manner in rats (Murray *et al.* 1987). The present study was designed to investigate whether chronic feeding of rats with high-fat meals would alter the delay in gastrointestinal (GI) transit of a high-fat meal.

Ten Sprague-Dawley female rats were allocated to a low-fat group (LFG) or a high-fat group (HFG). The LFG was intubated with 3 ml complan–water mixture (50:50, w/w), twice daily, for 6 d, while in the HFG olive oil was substituted for water. Food intake and body weight were measured daily. After 6 d, the rats were fasted for 18 h and then intubated with a semi-liquid, high-fat meal (300g olive oil/kg, 700g washed and liquidized baked beans/kg) labelled with ^{99}Tc-tin colloid. At 4 h after intubation animals were killed by chloroform inhalation and the GI tract from oesophagus to anus dissected. The activity along the excised gut was recorded using a gamma-counter, dedicated software and computer (Brown *et al.* 1987).



The head of the meal was still in the ileum in the LFG, whereas in the HFG animals 8% had reached the caecum (P=0.008). The different meal profiles show different transit responses to the meal in the two dietary groups. Although there were no differences in weight gain (11.2 (SE 2.1g) *v.* 11.0 (SE 1.3 g); LFG *v.* HFG), HFG intubated rats only partially compensated for the high-energy intubation by reducing their chow intake (190 (SE 5 kJ/d per rat) *v.* 238 (SE 13 kJ/d per rat); LFG *v.* HFG, P=0.003). These data demonstrate reduced delay of intestinal transit in response to fat in animals chronically intubated with high-fat meals.

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A comparison of lipoprotein lipase activity and gene expression in human and rat adipose tissue.
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Lipoprotein lipase (*EC* 3.1.1.34; LPL) is the rate-limiting enzyme in the removal of dietary triacylglycerol (TAG) from chylomicrons and VLDL postprandially. Hence the size and duration of postprandial lipaemia is largely determined by the rate of clearance of dietary lipids by LPL following a meal. Raised postprandial lipaemia has been implicated in an increased risk of CHD. The measurement of LPL gene expression and activity therefore gives an estimate of an individual's potential to clear dietary fat postprandially and hence risk of CHD.

The work presented here describes part of the ongoing work studying LPL activity and gene expression in rat (epididymal) and human (abdominal) adipose tissue. Human adipose tissue biopsies for analysis of LPL gene expression and activity were obtained from the anterior abdominal wall under local anaesthetic. Biopsies from human subjects were obtained five hours postprandially and the rats were sacrificed at 09.00 hours having been fed normal rat chow *ad libitum*. LPL activity was measured in acetone-ether powders of adipose tissue or as heparin-releasable activity in explants of adipose tissue. LPL activity was measured by the detection of free fatty acids from a ^{3}H-labelled triolein substrate emulsion (Nilsson-Ehle & Schotz, 1976). LPL gene expression was measured by Northern Blot Analysis and employed a ^{32}P-labelled human cDNA probe specific for LPL (Murphy *et al.* 1993). The results are shown in the Table; LPL gene expression is expressed as a ratio of the density (A_{550}) of the LPL signal to 10 μg RNA standard.

	Heparin-releasable LPL activity (pmol oleate released/min per g wet weight)		LPL activity in acetone-ether powders (pmol oleate released/min per g wet weight)		LPL gene expression (absorbance at 550 nm)	
	Mean (*n* 28)	SEM	Mean (*n* 9)	SEM	Mean (*n* 12)	SD
Human	5.44	0.84	167	49	0.18	0.03
Rat	17.87 ***	1.44	725 ***	149	1.29 ***	0.38

*** Mean values were significantly different from values for humans, $P < 0.001$.

In all cases measurements of human LPL were significantly lower than in the rat ($P < 0.001$). Heparin-releasable LPL activity in explants of human adipose tissue was only 30% of the activity measured in rat epididymal adipose tissue. Similarly, LPL activities in acetone-ether powders of human abdominal adipose tissue were 23% of those found in the rat and human LPL gene expression was calculated to be 23% of the levels of rat expression.

The fact that the differences seen in the three methods of LPL measurement correlate so closely suggests that the interspecies differences occur throughout LPL production. These inter-species differences explain the increased sensitivity needed to accurately measure LPL activity and gene expression in samples of human adipose tissue. An explanation for these inter-species differences is as yet unclear and the cause deserves further investigation.

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Influence of increased long-chain *n*-3 polyunsaturated fatty acid (PUFA) intakes on the insulin sensitivity of glucose disposal in middle-aged men.
BY A.C. FEREDAY, E. AH-SING, A. IRVINE, L.M. MORGAN, K.A. SLEVIN, C.M. WILLIAMS, J. WRIGHT and D.J. MILLWARD, *Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey GU2 5XH*

Insulin resistance, present in 25% of the normal UK population, and in a higher proportion of subjects with moderate obesity, a family history of non-insulin-dependent diabetes mellitus (NIDDM) and in South Asians, is associated with increased risk of cardiovascular disease. Some dietary intervention studies have suggested that low-fat, high-carbohydrate diets improve insulin sensitivity, while dietary supplementation with *n*-3 PUFA has been shown to decrease postabsorptive insulin levels in both healthy men and NIDDM subjects. We are investigating the dietary regulation of insulin action on postprandial macronutrient disposal and report here initial results of a dietary intervention with long-chain *n*-3 fatty acids on the insulin resistance of glucose disposal.

Non-smoking and non-exercising middle-aged men were screened with blood tests, anthropometry and a 4 d diet diary. Twelve were selected into the trial on the basis of fasting insulin levels, minimal oily fish consumption and a waist:hip ratio > 0.8. The study was a single blind, randomised crossover design of 10 weeks feeding with a 12-week washout interval. The *n*-3 intervention involved a combination of specifically manufactured *n*-3-enriched savoury and sweet food items, a spread (MD Foods Ltd) and fish-oil capsules (Pikasol, LUBE Ltd) and provided between 2 and 3 g long chain *n*-3 fatty acids/d. Food intake was monitored by food diaries and by personal interview, and compliance was measured by analysis of erythrocyte plasma lipid profiles. Eleven subjects completed the dietary supplementation. Insulin sensitivity was measured by the short intravenous insulin tolerance test involving injection of 0.1 U insulin/kg in subjects after an overnight fast following a standardized meal at 20.00 hours. Blood was sampled every minute from venous blood "arterialized" by hand warming. The test was terminated at 15 min with a glucose drink.

The fractional rate constant for glucose disappearance was calculated as the slope of the log glucose-time plot between 4 and 15 min. There was a wide range of insulin sensitivity with rate constants varying from -0.027 to -0.005/min, mean -0.0144 (SD 0.007)/min. There was a variable response to *n*-3 supplementation according to the degree of insulin resistance (r 0.75, P=0.008 for the relationship between initial insulin resistance and the magnitude of the response to *n*-3 fatty acids). The seven most insulin-resistant subjects all increased insulin sensitivity from 0.0089 (SD 0.0037) to 0.0153 (SD 0.0042), P=0.0098, n 7, whilst the least insulin-resistant subjects showed no overall response as a group: i.e. K glucose values of 0.022 (SD 0.004)/min control and 0.014 (SD 0.008)/min intervention, P=0.11, n 4.

These results demonstrate that in markedly insulin-resistant adults dietary supplementation with 2-3 g long chain *n*-3 PUFA/d, predominantly from food, improves insulin sensitivity whilst having no effect on more insulin-sensitive subjects.

This study was supported by the Ministry of Agriculture, Fisheries and Food, MD Foods Ltd, LUBE Ltd and Waltham

Influence of increased long-chain *n*-3 polyunsaturated fatty acid (PUFA) intakes on postprandial lipaemia in middle-aged men. By K.A. SLEVIN, A.C. FEREDAY, E.AH-SING, A. IRVINE, L.M. MORGAN, C.M. WILLIAMS, J.WRIGHT and D.J.MILLWARD, *Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

It is becoming increasingly clear that lipid intolerance, as indicated by postprandial lipaemia (PPL), is a risk factor for cardiovascular disease and an appropriate target for intervention. Furthermore pharmacological doses of fish oil are known to reduce plasma triacylglycerol (TAG) levels. The present study aimed to determine the effectiveness of increased intakes of *n*-3 PUFA delivered mainly in food on PPL within a larger investigation of the insulin sensitivity of postprandial macronutrient utilization in normal middle aged men.

Twelve non-smoking, non-exercising middle-aged men were recruited into the trial on the basis of fasting insulin levels, minimal oily fish consumption and a waist:hip ratio > 0.8. The study was a single blind, randomized crossover design of 10 weeks feeding with a 12-week washout interval. The *n*-3 intervention involved a combination of specifically manufactured *n*-3-enriched savoury and sweet food items, a spread (MD foods) and fish-oil capsules (Pikasol, LUBE Ltd) and provided between 2 and 3 g long-chain *n*-3 fatty acids/d. Food intake was monitored by food diaries and by personal interview and compliance was measured by analysis of erythrocyte plasma lipid profiles. Eleven subjects completed the dietary supplementation.

Postprandial lipaemia was studied after a standardized high fat breakfast providing 4.48 MJ (fat 82.7 g, protein 20.3 g, carbohydrate 72.5 g) given to subjects after an overnight fast following a standardized meal at 20.00 hours. Alcohol was restricted for 24 h prior to the study. Blood samples were taken every 30 min for 2 h and hourly for the following 7 h, for the measurement of TAG and other variables. We report here preliminary measurements of overall PPL in terms of total TAG levels.

PPL, assessed as the area under the TAG curve between 0 and 9 h, (TAG_{AUC}), was influenced by the fish-oil diet, although the magnitude of the response varied between subjects according to the fasting TAG level at the start of the intervention. Thus the extent of reduction in PPL (ratio fish oil:control TAG_{AUC}) was inversely correlated with the initial fasting TAG (r 0.66, P=0.025, n 11; r 0.88, P =<0.001, n 10). Because of this the level of significance for the reduction for the entire group was low, (TAG_{AUC} 782 (SD 239) control, 685 (SD 205) intervention, P=0.16, n 11). However removal of one or two non-responding subjects with the lowest initial fasting TAG levels resulted in significant responses: i.e. for 10/11 subjects (TAG_{AUC} 793 (SD 248) control, 636 (SD 129) intervention, P=0.037, n 10) and more so for 9/11 (TAG_{AUC} 824 (SD 241) control, 627 (SD 133) intervention, P=0.014, n 9). Insulin responses to the meal, assessed as the area under the insulin curve, increased with fish oil, P=0.04, n 11, as did the insulin sensitivity of TAG clearance, assessed as the ratio postprandial TAG (AUC) : postprandial insulin (AUC), P=0.004, n 11

It is clear therefore that in lipid-intolerant subjects identified in terms of fasting TAG levels, dietary supplementation with 2-3 g long chain *n*-3 PUFA given mainly in the form of manufactured foods improves lipid tolerance and reduces postprandial lipaemia. Further analysis is being conducted to investigate the role of increased insulin action on lipid clearance.

This study was supported by the Ministry of Agriculture, Fisheries and Food, MD Foods Ltd and LUBE Ltd.

Enrichment of chylomicron-triacylglycerol during the postprandial period following oral administration of [1-^{13}C]palmitic acid, [1-^{13}C]stearic acid and [1-^{13}C]oleic acid. By S.A. WOOTTON, A.E. JONES, M. STOLINSKI, A. HOUNSLOW and J.L. MURPHY, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

Diets rich in saturated fatty acids (SFA), particularly those with chain lengths 12-16 C atoms, have been shown to be hypercholesterolaemic in human subjects (Keys *et al.* 1965). However diets rich in stearic acid appear to be neutral with respect to plasma cholesterol, similar to the effects of diets rich in monounsaturated fatty acids (Bonanome & Grundy, 1988). These effects on circulating lipids may arise from differences in the movement of these fatty acids within the chylomicron and lipoprotein pools during the postprandial period and the possible role that the intestine or liver may play in the desaturation of stearic acid to oleic acid (Rhee *et al.* 1997). The present study examined the changes in ^{13}C-enrichment of fatty acids isolated from chylomicron-triacylglycerol (TAG) during the postprandial period following the oral administration of ^{13}C-labelled fatty acids.

Following an overnight fast three groups of healthy, normal-weight women (BMI 18.4-24.0 kg/m^2) ingested either [1-^{13}C]palmitic acid, [1-^{13}C]stearic acid or [1-^{13}C]oleic acid (10 mg/kg body weight) prepared as a casein-glucose-sucrose emulsion as part of a test meal (3 MJ; 30 g lipid). Venous blood samples were collected from an indwelling cannula before and at hourly intervals for 8 h following label administration. A chylomicron-rich fraction (Sf>400) was separated by discontinuous-gradient ultracentrifugation, lipids extracted and the TAG component isolated by TLC (Stolinski *et al.* 1997). The fatty acid profile of the chylomicron-TAG and the ^{13}C-enrichment of the fatty acid methyl esters were determined by gas chromatography-isotope ratio mass spectrometry (Orchid GC-IRMS). In addition the total TAG concentration of plasma was determined enzymically. As the label was primarily restricted to the same fatty acid species as that ingested, the Table shows the concentration of ^{13}C-labelled fatty acid within chylomicron-TAG at time points 1 - 4 h and area under the curve (AUC) over the 8 h postprandial period.

		^{13}C-labelled fatty acid (mg/l plasma)									
		1 h		2 h		3 h		4 h		AUC (0 - 8 h)	
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
[1-^{13}C]palmitic acid	6	3.8	0.7	13.0	3.6	3.6	1.2	2.1	0.7	26.6	5.8
[1-^{13}C]stearic acid	6	2.9	0.4	3.5*	0.3	2.6	0.4	1.6	0.3	13.7*	0.7
[1-^{13}C]oleic acid	5	2.1	0.7	2.2*	0.5	0.8*†	0.1	0.7*†	0.1	7.3*†	1.2

* Significantly different from [1-^{13}C]palmitic acid; P<0.05.
† Significantly different from [1-^{13}C]stearic acid; P<0.05.

Whilst the time-course and magnitude of the increases in plasma TAG were similar for all three trials, marked differences in the handling of the labelled fatty acids were observed within chylomicron-TAG even though comparable amounts of label were ingested within identical test meals. The greatest levels of enrichment were observed for palmitic acid and the least for oleic acid; stearic acid behaved more like oleic acid than palmitic acid. Small increases in enrichment were also observed in oleic acid after 5 h during the stearic acid trial (equivalent to <1% of administered dose). These results suggest that stearic acid within the meal was handled within circulating chylomicrons more like oleic acid than palmitic acid and that some desaturation of stearic acid occurred late in the postprandial period. Such differences between these SFA may contribute to the apparent neutral effect of stearic acid on plasma lipids.

This research is supported by the Ministry of Agriculture, Fisheries and Food.

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The gastrointestinal handling and postprandial metabolism of [1-^{13}C]stearic acid in healthy women. By A.E. JONES, A.R. PEARCE, M. STOLINSKI, A. HOUNSLOW, J.L. MURPHY and S.A. WOOTTON, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

Tracer studies would suggest that when administered as the free acid, saturated fatty acids (SFA) are poorly absorbed and may be oxidized to a lesser extent than unsaturated fatty acids (Jones *et al.* 1985; Murphy *et al.* 1995), however these differences may simply reflect the physicochemical properties of the crystalline form of SFA rather than a true metabolic difference. Emulsifying the free acids before administration appeared to overcome the problems associated with the physicochemical properties of SFA so that the absorption and subsequent oxidation of emulsified [1-^{13}C]palmitic acid was similar to that of [1-^{13}C]oleic acid (Jones *et al.* 1997). As there is increasing evidence that stearic acid is handled differently from palmitic acid within the circulation (Emken, 1994) the present study utilized stable-isotope tracer methodologies to examine the gastrointestinal (GI) handling and postprandial metabolism of [1-^{13}C]stearic acid presented in an emulsified form.

Following an overnight fast six healthy, normal-weight women ingested [1-^{13}C]stearic acid (10 mg/kg body weight) prepared as a casein-glucose-sucrose emulsion as part of a test meal (3 MJ; 30 g lipid). Breath tests and indirect calorimetry measurements were performed before and at hourly intervals for 10 h following label administration and again at 15 and 24 h. A baseline stool and all stools passed over a 5 d period following label administration were collected. The total ^{13}C-enrichment of breath and stool samples was analysed by continuous flow-isotope ratio mass spectrometry. In addition the nature of the species bearing the ^{13}C-label within the stool was determined by gas chromatography-isotope ratio mass spectrometry (Stolinski *et al.* 1997). The results are shown in the Table for the excretion of ^{13}C in stool expressed as a percentage of administered dose and on breath as $^{13}CO_2$ as a percentage of absorbed dose which takes into account stool losses of ^{13}C.

	Stool ^{13}C (% administered dose)		Breath $^{13}CO_2$ (% absorbed dose)	
	Median	Range	Median	Range
[1-^{13}C]stearic acid	10.5*†	3.2 - 14.2	23.9†	21.4 - 24.6
[1-^{13}C]palmitic acid‡	1.1	0.2 - 2.4	24.8	20.9 - 32.0
[1-^{13}C]oleic acid‡	0.7	0.0 - 5.5	25.9	23.0 - 32.4

* Significantly different from [1-^{13}C]palmitic acid (Mann-Whitney U); $P<0.05$.

† Significantly different from [1-^{13}C]oleic acid (Mann-Whitney U); $P<0.05$.

‡ Taken from Jones *et al.* (1997).

The excretion of ^{13}C within stool following [1-^{13}C]stearic acid was greater than that observed for [1-^{13}C]palmitic and oleic acids indicating poorer absorption of stearic acid (85.8 - 96.8%). Approximately 87% of the ^{13}C-label within stool was identified as [^{13}C]stearic acid equivalent to 1.7 - 12.1% of administered dose with the remainder as [^{13}C]palmitic acid equivalent to 0.5 - 1.4% of administered dose. This would suggest that there was chain shortening of [1-^{13}C]stearic acid within the GI tract resulting in the appearance of labelled palmitic acid. The excretion of $^{13}CO_2$ on breath following administration of [1-^{13}C]stearic acid was not different to that observed for [1-^{13}C]palmitic acid but was less than that observed for [1-^{13}C]oleic acid. This difference in oxidation was apparent from 6 h postprandially. These results would suggest that when presented in an emulsified form the GI handling of stearic acid is different from palmitic and oleic acids, although the subsequent oxidation appears to be similar.

This research is supported by the Ministry of Agriculture, Fisheries and Food.

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An *in vitro* study of glycerol-3-phosphate dehydrogenase (*E.C.* 1.1.1.8; GPDH) activity as a marker of triacylglycerol synthesis in ovine adipocytes taken from animals at different stages of development. By R.H. Bennett[1], R.G. Vernon[2], and B. Soret[2]. [1]*Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne NE1 7RU and* [2]*Hannah Research Institute, Ayr KA6 5HL*

Meat fat and its fatty acid composition have become important considerations to consumers as they attempt to follow national guidelines to reduce intake of total fat, and particularly of saturated fatty acids. As a result, consumption of ruminant meat products has declined, making the ability to manipulate adipose tissue content and composition of potential importance to the ruminant livestock industry. The work to be discussed is an attempt to improve our understanding of factors controlling sheep adipose tissue development and to investigate the potential to manipulate its development and composition in the young lamb, particularly during fetal and sucking periods.

Subcutaneous and perirenal adipose tissue samples were taken from five lamb fetuses at approximately 110 d gestation, and five lambs aged approximately 10 weeks, and treated according to the method of Adams *et al.* (1992) to obtain the stromovascular cell fraction. Culture in Medium 199 containing Earles salts, 200 ml new-born calf serum/L, and supplemented with 2 mM-acetate, 4.8 mM-L-glutamine and antibiotics allowed the preadipocytes to grow to confluence. Cells were then changed to DMEM F-12 medium, which promoted preadipocyte differentiation, and exposed to triiodothyronine (T_3; 200 mg/ml) and Excyte (E; 10 μl/ml), a commercial lipid supplement used for cell culture, plus differing combinations of the factors under investigation: insulin (I; 500 μg/ml), growth hormone (GH; 100 ng/ml), dexamethasone (D; 1 μl of 10 nM solution/ml) and fibroblast growth factor (FGF; 100 ng/ml). Cells differentiated and accumulated lipid for 10 d in each treatment group and then harvested and assayed for the activity of GPDH, an enzyme instrumental in the production of triacylglycerols and used as an indicator of lipid deposition. A protein assay (Bradford, 1976) on each sample allowed enzyme activity to be expressed as units/g protein. There were no significant differences in GPDH activity in cells derived from perirenal or subcutaneous tissue from either fetal or lamb tissue. Presence of I significantly increased ($P<0.05$) GPDH activity compared with cultures lacking in I, but presence of both I and GH reduced the activity compared with I alone ($P<0.05$). In fetal adipocytes, GH for 5 of the 10 d differentiation significantly increased ($P<0.05$) GPDH activity compared with cells exposed to GH for 10 d, but there were no similar effects in lamb adipocytes. Presence of FGF or D with I and GH significantly ($P<0.05$) increased activity in lamb adipocytes, being greater than with I alone, but had no effect in fetal adipocytes. FGF and GH with I, resulted in significantly increased activity ($P<0.05$) in cells from both fetal and lamb tissue compared with D and GH with I.

This study demonstrated stimulatory effects of I and FGF, and to a lesser extent, D on cell differentiation in fetal and lamb tissues, whereas responses to GH depended on the age of the animal, and in the case of fetal tissues, the duration of exposure to GH.

We would like to acknowledge the Ministry of Agriculture, Fisheries and Food for funding this project.

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Fish oil supplementation does not affect plasma antioxidant status. By PARVEEN YAQOOB[1], HICRAN S. PALA[1], MARIO CORTINA-BORJA[2], ERIC A. NEWSHOLME[1] and PHILIP C. CALDER[3], *[1]Department of Biochemistry and [2]Department of Statistics, Oxford University, Oxford, OX1 3QU and [3]Division of Human Nutrition, Southampton University, Southampton, SO16 7PX*

The protective effect of vitamin E against oxidative damage to long-chain polyunsaturated fatty acids (PUFA) has been well documented. However, vitamin E is only one of a number of antioxidant defence systems and to date there is little perception of the relationship between dietary intake of PUFA and total antioxidant status. The purpose of the present study was therefore to investigate the effects of supplementation of the diet with encapsulated oils, containing a dose of vitamin E, on the plasma concentrations of vitamin E and on total plasma antioxidant activity in healthy volunteers.

Healthy volunteers aged 21-60 years consumed capsules containing either a mixture of fractionated coconut oil and soyabean oil (placebo; PL), olive oil (OO), sunflower-seed oil (SO), evening primrose oil (EPO), fish oil (FO) or evening primrose oil plus fish oil (EPO/FO; ratio 4:1) for 12 weeks at a dosage of 9 g oil/d (*n* 8 per group). Each capsule (1 g oil) also contained 22.9 (SEM 0.4) mg vitamin E. Blood samples were obtained at baseline, after 8 and 12 weeks of supplementation and after an 8-week washout period. Plasma vitamin E concentration was determined by HPLC. Plasma antioxidant activity was analysed as described by Miller *et al.* (1993). Statistical analysis was performed using repeat measures ANOVA.

		Baseline		8 weeks		12 weeks		Washout	
	Capsule	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Vitamin E	PL	25.7	2.6	50.1	3.3	46.3	4.1	28.3	2.6
(μM)	OO	25.6	2.8	40.4	3.6	43.1	4.4	29.3	4.4
	SO	29.3	2.0	42.9	3.0	41.3	3.5	26.0	2.2
	EPO	28.2	1.5	45.0	4.3	45.2	3.4	30.9	1.9
	EPO/FO	25.2	2.6	38.0	2.2	35.2	2.9	26.2	2.1
	FO	26.1	2.1	38.4	2.6	36.9	2.5	25.1	1.6
Antioxidant	PL	1.39	0.02	1.43	0.02	1.42	0.03	1.42	0.02
activity	OO	1.40	0.02	1.40	0.03	1.42	0.02	1.42	0.02
(mM)	SO	1.42	0.02	1.42	0.02	1.42	0.02	1.42	0.02
	EPO	1.40	0.02	1.41	0.02	1.42	0.02	1.43	0.03
	EPO/FO	1.38	0.02	1.40	0.02	1.38	0.02	1.43	0.02
	FO	1.42	0.02	1.44	0.02	1.43	0.02	1.44	0.02

Plasma concentrations of vitamin E were significantly elevated during supplementation in all subjects and returned to baseline values after an 8 week washout period. Although plasma vitamin E levels in subjects taking FO or EPO/FO appeared to be lower, they were not significantly different from those in the other groups. Plasma total antioxidant activity was also unaffected by supplementation, indicating that the supply of this level of *n*-3 PUFA and vitamin E can be fully compensated for by antioxidant systems.

This work was supported by the Ministry of Agriculture, Fisheries and Food (Grant No. AN0215)

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Amino acid and peptide absorption across the ovine gastrointestinal tract: a dietary comparison. By L. BERNARD[1], J. C. MacRAE[2], D. L. WILSON[2], L. BRUCE[2] and F. R. C. BACKWELL[2], *[1]SNRH, INRA-Theix, Clermont-Ferrand, France, [2]Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB*

Differences in the reported magnitude of gastrointestinal peptide absorption in ruminants have been attributed to differences in methodology, diet or species. We have previously shown (Bernard *et al.* 1997) that two different methods used for the measurement of small peptides (1500-3000 Da) in blood, gave similar circulating concentrations, but no detectable intestinal absorption when applied to arterial and mesenteric blood samples from sheep. The present study was designed, therefore, to examine the effect of different diets on intestinal amino acid and peptide absorption and on whole-body amino acid kinetics.

Three sheep received 800 g/d of (i) a pelleted lucerne ration (200 g crude protein (CP)/kg; diet I) and (ii) a mixture of orchard-grass hay (153 g CP/kg) and soyabean meal (519 g CP/kg) in the ratio 80:20 (diet II). On experimental days each animal received jugular vein infusion of [d_3]leucine (150 μmol/h) and jejunal infusion of [^{13}C]leucine (150 μmol/h). p-Aminohippuric acid (PAH) and sodium heparinate were infused into the mesenteric vein to allow, respectively, blood-flow measurement and continuous withdrawal of four 1 hourly integrated blood samples from arterial and portal catheters.

Peptide concentrations in the arterial and portal blood samples as determined by the methods of Backwell *et al.* (1996) and Bernard & Rémond (1995) were similar to those previously reported (Bernard *et al.* (1997). They did not differ between diets, neither were there any portal-arterial differences in peptide concentrations detected by either method on either ration, indicating that all amino acid was absorbed in free form.

	PDV leu flux (mmol/d)		WBLF (mmol/d)		ILS_{art} (mmol/d)	
Diet	Mean	SD	Mean	SD	Mean	SD
I Lucerne	51	16	146	16	49	7
II Orchard-grass hay/soyabean meal	17	(±2)	110	(±8)	37	(±10)

Values in parentheses represent the range of two diets.

Represented in the Table are preliminary data on portal-drained viscera (PDV) flux, whole-body leucine flux (WBLF) and the rate of intestinal leucine sequestration from arterial blood (ILS_{art}) determined from the leucine kinetics of three sheep given diet I and two given diet II. PDVF was 3-fold higher and WBLF was 50% higher on the lucerne ration, but ILS_{art} as a proportion of WBLF was similar on both diets.

This experiment confirms our earlier findings that there is no absorption of peptides across the ovine intestine as determined by different methods, even on diets which differ considerably in composition and quality.

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Is crypt cell proliferation a reliable bio-marker of intestinal cancer risk? By J.C. MATHERS[1], M. KOOSHKGHAZI[1], J. COAKER[1], S. WILLIAMSON[2], A. KARTHEUSER[3], J. BURN[4] and R. FODDE[5]. *[1]Department of Biological and Nutritional Sciences, [2]Department of Pathology, [4]Department of Human Genetics, University of Newcastle, Newcastle upon Tyne, NE1 7RU, [3]Department of Surgery, Université Catholique de Louvain, Brussels, Belgium and [5]Department of Human Genetics, University of Leiden, The Netherlands*

Mutations in the *apc* gene are an early event in the majority of human colorectal cancers. The development of *apc* gene knockout mice (Fodde *et al.* 1994) which yield intestinal tumours spontaneously has provided a relevant and sensitive model for investigations of diet and large-bowel cancer. The present study was designed to test the hypothesis that increased mucosal cell proliferation, which is readily altered by dietary manipulation (Goodlad *et al.* 1987), is a reliable biomarker of colorectal cancer risk.

For 5 months from weaning, F9 *Apc* 1638N mice (Fodde *et al.* 1994) were fed on commercial rodent chow (RM3(E), SDS, Witham, Essex) or a semi-purified 'Western' diet containing (g/kg): casein 280, gelatin 20, lard 250, sucrose 150, maize starch 250 and mineral and vitamin mix 50. Wild type C57/B16 mice were also fed on the 'Western' diet. Crypt cell proliferation (CCP; arrested cells/crypt per 2 h) was measured by the vincristine metaphase arrest method (Mathers *et al.* 1993).

Genotype...	*Apc* 1638N		Wild type	SEM
Diet...	Chow	'Western'	'Western'	(n 20)
CCP: Duodenum	14.2	14.5	13.2	1.2
Caecum	14.2	6.9	9.9	1.0
Colon	4.4	3.3	3.8	0.5
Tumours/mouse	2.0	1.7	0	0.3

CCP was similar for both wild type and *Apc* 1638 N mice fed on the 'Western' diet but only the *Apc* 1638N animals developed tumours. The NSP-rich chow diet resulted in increased CCP in the caecum ($P<0.001$) but intestinal tumour numbers were unaffected. In addition the distribution of mitotic cells within the crypt was unaffected by mutation of one *apc* allele. We conclude that CCP may be an unreliable bio-marker of cancer risk.

This work is supported by the World Cancer Research Fund and Cancer Research Campaign.

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Acarbose increases caecal short-chain fatty acids (SCFA) and uptake into portal vein. By M. DEHGHAN KOOSHKGHAZI and J.C. MATHERS, *Human Nutrition Research Centre, Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne NE1 7RU*

Acarbose (BAY g 5421) is a pseudotetrasaccharide which inhibits α-glucosidases thus slowing the rate of digestion in the small bowel and increasing flow of starch and gluco-oligomers to the colon (Hiele *et al.* 1992). It is used in the treatment of non-insulin-dependent diabetes mellitus. The objective of the present study was to determine the effects of acarbose treatment on large-bowel fermentation.

Eighteen male Wistar rats (six per treatment) with initial weight approximately 90g, were fed on semi-purified diets containing 0, 250 or 500 mg acarbose/kg diet for 3 weeks. Portal blood was collected by venepuncture under anaesthesia 2-4 h after feeding. Blood and caecal contents were assayed for short-chain fatty acids (SCFA) concentration.

	Acarbose (mg/kg diet)				Probability of
	0	250	500	SEM	acarbose effect
Caecal SCFA pool size (μ mol/rat)					
Acetic acid	90	460	430	45	P<0.001
Propionic acid	36	194	207	27	P<0.001
Butyric acid	10	130	91	16	P<0.001
Portal blood SCFA (μ M)					
Acetic acid	1443	1449	1305	126	P=0.69
Propionic acid	119	276	422	44	P<0.05
Butyric acid	15	112	83	14	P<0.001
Caecum weight (g)	1.9	13	13.3	0.32	P<0.001

Acarbose treatment increased caecal mass 6.8 times and caecal SCFA pool size 5.6-fold. This increase was especially marked for propionic and butyric acids and was reflected in significant increases in portal blood concentration for both. In conclusion acarbose treatment, which increases supply of fermentation substrate to the large bowel, enhances portal uptake of propionate and butyrate which may contribute to the metabolic effects of this drug on liver and colonic tissue.

Mrs Dehghan Kooshkghazi holds a scholarship from the Ministry of Health and Medical Education, Iran

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Expression of the amino acid transporter GLYT1 in human small bowel and the intestinal epithelial cell line Caco-2. By G.R. CHRISTIE, D. WALKER and B.H. HIRST. *Department of Physiological Sciences, University of Newcastle upon Tyne, Medical School, Newcastle upon Tyne NE2 4HH*

Amino acid absorption across epithelia is mediated by a number of different amino acid transport systems. Identification of the complement of amino acid transporters expressed in gastrointestinal tissue is important in understanding normal dietary protein assimilation. Recently a number of amino acid transport proteins and corresponding cDNA clones have been identified and grouped into gene families. GLYT transporters are expressed in the brain and spinal cord where they mediate high-affinity Na^+/Cl^--dependent glycine transport. Two independent genes, termed GLYT1 and GLYT2, have been identified to date. As a result of differential RNA splicing, transcription of the GLYT1 gene gives three different isoforms, GLYT1a, GLYT1b and GLYT1c (Malandro & Kilberg, 1996).

Here we provide evidence for the expression of GLYT1 in human small bowel, as well as in the human cultured cell line Caco-2. Caco-2 cells are derived from a human colonic adenocarcinoma. When grown in culture, Caco-2 cells undergo spontaneous enterocytic differentiation, and once fully differentiated have been demonstrated to possess many morphological and physiological attributes characteristic of enterocytes *in vivo*, including amino acid absorption.

Oligonucleotide primers were designed to a region of the rat brain GLYT1 sequence (Guastella *et al.* 1992). mRNA was isolated from both human small bowel, obtained from surgically resected specimens taken for diagnostic purposes, and Caco-2 cells. cDNA coding for the designated region of GLYT1 was amplified by reverse transcription-polymerase chain reaction (RT-PCR). GLYT1-specific PCR products were identified in both human small bowel and Caco-2 cells. Amplified fragments were subcloned into the vector pTAg, followed by subsequent automated sequencing. Alignment of the sequence of the Caco-2 RT-PCR product revealed 100 % identity with human GLYT1c (Fig. 1).

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Caco2:     1 TCACACTACTTCCCATCTCCCCGCTGTGGTCTCTGCTCTTCTTCTTCATGCTTATCCTGC 60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Brain:  1372 TCACACTACTTCCCATCTCCCCGCTGTGGTCTCTGCTCTTCTTCTTCATGCTTATCCTGC 1431

Caco2:    61 TGGGGCTGGGCACTCAGTTCTGCCTCCTGGAGACGCTGGTCACAGCCATTGTGGATGAGGTGGGGAATGAGT 132
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Brain:  1432 TGGGGCTGGGCACTCAGTTCTGCCTCCTGGAGACGCTGGTCACAGCCATTGTGGATGAGGTGGGGAATGAGT 1503
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Fig. 1. Comparison of Caco-2 (**Caco2**) sequence and human glycine transporter type 1c cloned from substantia nigra (**Brain**).

We believe that this is the first report of the expression of GLYT1 in the intestine. The amplification of a GLYT1 sequence from cultured human Caco-2 cells indicates that epithelial cells, in addition to neural tissues, express GLYT1 and suggests that this transporter may be involved in nutrient absorption.

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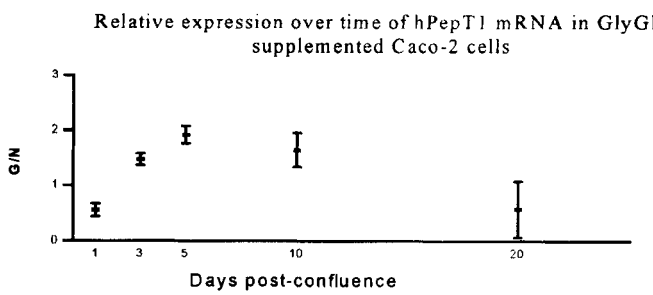
The positive feedback effect of the dipeptide GlyGln on its own absorption in the gastrointestinal tract by the carrier protein hPepT1 By J.D.PICKER[1], D.WALKER[2], B.H. HIRST[2] and J.HALL[1]. [1]*Departments of Biological & Nutritional Sciences and* [2]*Physiological Sciences, University of Newcastle upon Tyne NE1 7RU*

Dietary proteins are digested to amino acids and short oligopeptides which are then absorbed from the gastrointestinal (GI) tract by different mechanisms. This has been shown in protein-energy malnutrition states where oligopeptide absorption remains relatively unaffected compared with amino acid absorption (Adibi, 1974). This difference has important implications with regard to the management of such malabsorptive conditions.

Oligopeptides are transported from the GI lumen intracellularly via hPepT1a H+-coupled oligopeptide cotransporter. Nutrient regulation of absorptive processes is a well recognised phenomenon, including oligopeptide transport (Ferraris & Diamond, 1985)

In the present study, the effect of the dipeptide glycyl glutamine (GlyGln) on expression of hPepT1 mRNA was investigated in human Caco-2 cells *in vitro*. These cells exhibit the morphological and biochemical features of enterocytes and thus offer a good tissue culture model. Cells were grown in MEM based media (including non essential amino acids, fetal calf serum and gentamicin) with or without dipeptide. At defined times post-confluence the cells were harvested and the total mRNA was extracted. This was then electrophoresed on a formaldehyde gel, northern blotted and probed. The results were densitometrically quantified and analysed. The factors investigated were the effects of : variable GlyGln concentrations (0-20 mM); time of cell harvesting (0-20 d. post-confluence) and specificity of effect on brush border membrane protein mRNA levels.

Results (corrected for loading variability by comparison with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene transcript) demonstrated that hPepT1 mRNA expression was upregulated in the presence of GlyGln. This upregulation was greatest (mean 1.47 times ; $P<0.02$) at 4mM GlyGln and when the cells were harvested at 5 d. post-confluence. This upregulation appears to be relatively specific as there was no transcriptional upregulation of the brush-border membrane proteins, sucrase-isomaltase and dipeptydyl peptidase IV.



G/N : ratio of hPepT1 mRNA in GlyGln supplemented over unsupplemented cells (corrected for GAPDH)

These results confirm that the dipeptide GlyGln leads to an increase in Caco-2 hPepT1 mRNA levels compared with when the medium is supplemented with the constituent amino acids only. Transcriptional upregulation of the hPepT1 gene probably contributes to this effect. Currently only structural sequence data are known and present work is dedicated to identifying the genomic sequence wherein the promoter/regulator elements of hPepT1 can be identified and further investigated.

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APC gene controls crypt fission and not cell division in mice with multiple intestinal neoplasia (MIN). by H. WASAN[1], N. MANDIR[2], H.S.. PARK[2], N.A.. WRIGHT[2], W. BODMER[1] & R. A. GOODLAD[2]. [1]*Imperial Cancer Research Fund - Cancer Genetics & Immunogenetics Laboratory &* [2]*Histopathology Unit, 44 Lincoln's Inn Fields, London, WC2A 3PX*

A nonsense mutation in the mouse adenomatous polyposis coli (*Apc*) gene leads to a phenotype - MIN analogous to human familial adenomatous polyposis (FAP). Heterozygous mice invariably develop numerous polyps from an early age. The number and size of these polyps can be modified by diet and by other agents. 'Protective' diets may also be those which reduce cell proliferative rates, as increased cell proliferation is often associated with cancer risk. The effects of this mutation on cell division and also on crypt fission, were investigated by microdissection in the normal (non-neoplastic) intestine of 100 d old MIN mice and their wild-type littermates.

Contrary to expectations, no significant changes in intestinal epithelial cell proliferative rates were seen at five matched intestinal sites of MIN mice. However, there was a 75% increase in crypt fission in the colon of MIN mice (P<0.001).

Crypt fission index in the gut of MIN mice (the sites are defined by their percentage of length)

Small intestine						Colon					
10%		50%		90%		10%		50%		90%	
mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
6.50	1.00	3.23	0.24	1.01	0.26	1.13	0.18	0.65	0.24	0.33	0.11
10.81	1.30	5.43	0.12	1.13	0.23	2.23.	0.29	0.57	0.12	0.76	0.20

Two-way analysis of variance, small intestine, effect of site, P<0.001, MIN P<0.001 interaction P<0.001
Two-way analysis of variance, colon, effect of site, P<0.001, MIN P<0.01 interaction P<0.01

These increases in fission showed a correlation with final tumour size, but not actual tumour numbers. Human FAP data concurs with these findings showing an even larger (thirteen fold) increase in crypt fission. The mean crypt fission index in the normal mucosa of FAP was 3.77 (SEM 0.46)%; (range 0.0 - 23.81) as compared with 0.31 (SEM 0.05) % (range 0-2.24%) for the controls. Again there was no indication of increased cell proliferation.

The absence of an increase in intestinal cell division implies that mutated *Apc* deregulates intestinal differentiation specifically through perturbing the intestinal crypt cycle, leading to an increase in crypt fission. We conclude that *in vivo*, the major defect in pre-neoplastic intestinal mucosa harbouring *Apc* mutations, results in elevated rates of crypt fission, and propose that this is also the mode by which micro-adenomas enlarge.

Dietary fibre and the intestinal microflora, effects on intestinal morphometry and crypt branching. By J.S. MCCULLOUGH, B. RATCLIFFE[1], N. MANDIR[2], K.E. CARR & R.A. GOODLAD[2]. *The Queens University of Belfast, Anatomy Department Medical Biology Centre, Belfast.* [1]*The Robert Gordon University, Kepplestone, Aberdeen.* [2]*Imperial Cancer Research Fund, Histopathology Unit, 35-43 Lincoln's Inn Fields, London WC2A 3PN*

Conventional and germ-free rats were fed for two weeks on fibre-free or fibre supplemented (30%) diets. Fibre had a direct (bacteria independent) effect on the number of goblet cells in the small intestine (SI) and a similar effect on the goblet cells in the colon, which was attenuated by the presence of bacteria. Furthermore, there was also a marked decline in the entero-endocrine cell counts in the small intestine of the germ-free animals.

Morphometry indicated hypertrophy of the muscle layer in the colon. However, the changes in muscle were not of a sufficient magnitude to explain the differences in tissue weight observed. A possible explanation for this was provided by the observation that the number of crypts per circumference in the proximal colon and number of branched crypts (expressed as percentage of total crypts) were also increased by fibre in both the germ-free and conventional rats.

Crypt branching Index (%)

	Germ-Free				Conventional				Two-way AOVO Effect of		
	Fibre-free		+Fibre		Fibre-free		+Fibre		Diet	Flora	Interaction
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM			
Proximal SI	2.189	0.17	3.59	0.61	2.14	0.38	3.16	0.48	-	**	-
Mid SI	4.74	0.92	3.73	0.75	4.03	0.73	4.66	0.66	-	-	-
Distal SI	11.85	0.68	18.92	1.76	8.40	0.89	10.79	0.45	***	***	*
Proximal Colon	52.93	3.56	72.09	2.96	60.56	5.34	65.40	5.09	-	**	
Mid Colon	32.38	9.52	35.98	7.35	7.60	1.67	9.10	1.65	***	-	-
Distal Colon	7.30	1.72	8.78	4.10	5.77	1.43	7.148	0.76	-	-	-

Crypt branching can lead to the production of new crypts -if they go on to fission. However, the situation is more complicated as there was also a large increase (240%) in the number of branched crypts in the mid colon of the germ free rats with little effect of fibre being observed suggesting that not all crypts have to undergo crypt fission, and that crypt branching does not always equate with crypt fission. Nevertheless, the lengthening of the colon observed in the fibre treated groups and the increased number of crypts per circumference indicates that fibre has indeed augmented the number of crypts present, and that another, potentially very important effect of fibre is to increase crypt fission in the colon.

Keratinocyte growth factor (KGF) and gut growth, cytoprotection cell migration. By R.J. PLAYFORD[1], N. MANDIR[4], T. MARCHBANK[1], A. HIGHAM[3], K. MEERAN[2], M.A. GHATEI[2], S.R. BLOOM[2], R.A. GOODLAD[4] *[1]Department of Medicine and Therapeutics, University of Leicester, [2]Department of Medicine, Royal Postgraduate Medical School, London. [3]Department of Physiology, University of Liverpool [4]Histopathology Unit, Imperial Cancer Research Fund, London WC2A 3PN*

KGF is found throughout the gastrointestinal tract but its role in maintaining gastrointestinal integrity is unclear. We examined the effect of recombinant KGF on gut growth and repair using a variety of *in vivo* and *in vitro* models. Rats receiving total parenteral nutrition (TPN) had co-infusions of KGF or control for 6 d. Effects of KGF on gastric repair and acid secretion in rats were determined using an indomethacin (20 mg/kg) restraint model and animals fitted with chronic gastric fistulas. Effects on cell migration were assessed using wounded monolayers of the human carcinoma cell line HT29.

Table 1 The effects of TPN and TPN supplemented with KGF on the weight of the gastrointestinal tract, weights expressed as percentage of total body weight Statistics refer to AOVO on TPN groups

	Stomach		Small Intestine		Caecum		Colon	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TPN	0.566	0.026	1.849	0.028	0.373	0.022	0.422	0.017
KGF 0.1 mg/kg	0.722	0.017	2.244	0.071	0.333	0.003	0.472	0.015
KGF 0.3 mg/kg	1.330	0.063	2.618	0.054	0.438	0.048	0.700	0.042
KGF 1.0 mg/kg	1.076	0.068	3.073	0.128	0.466	0.012	0.783	0.029
Orally fed	0.570	0.020	3.488	0.187	0.553	0.023	0.752	0.041
	***		***		**		***	

Table 2 The effects of TPN and TPN supplemented with KGF on the 2 hour accumulation of vincristine metaphases, expressed as metaphases per gastric gland or per crypt

	Antrum		50% small intestine		10% Colon		50% Colon		90% Colon	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TPN	1.38	0.17	22.72	1.37	11.14	2.01	11.23	1.61	13.23	1.25
KGF 0.1 mg/kg	7.28	0.27	26.08	0.90	13.81	4.23	9.88	1.30	12.21	1.86
KGF 0.3 mg/kg	7.60	0.24	33.56	1.93	11.74	1.00	8.26	1.11	11.99	0.99
KGF 1.0 mg/kg	6.56	0.38	39.02	1.51	18.58	3.93	9.08	0.94	9.76	0.97
Orally fed	2.93	0.24	43.48	2.00	19.37	1.50	19.99	2.43	23.18	3.35
	***		***		NS		NS		NS	

Table 3 The effects of TPN and TPN supplemented with KGF on plasma hormone levels (pmol/l)

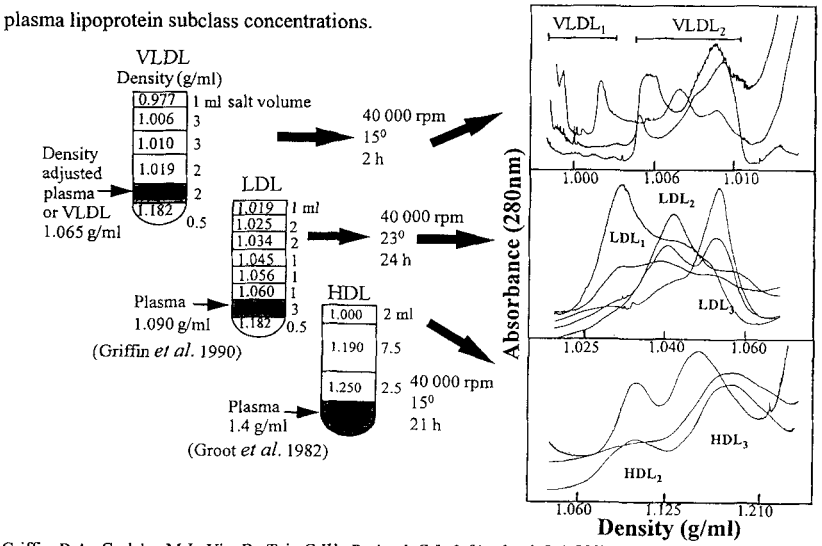
	Gastrin		Insulin		Enteroglucagon		PYY		GLP-I	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TPN	10.82	2.20	1109.8	12.55	28.25	5.31	28.80	7.12	5.77	1.20
KGF 0.1 mg/kg	34.57	5.67	807.8	133.25	69.33	19.10	41.27	8.36	18.72	3.56
KGF 0.3 mg/kg	55.07	11.02	744.7	120.97	77.08	20.02	98.60	10.96	32.77	6.16
KGF 1.0 mg/kg	41.02	4.40	729.5	143.38	101.98	12.11	177.15	28.64	29.28	6.76
Orally fed	82.44	1.89	307.9	234.40	189.44	31.17	73.56	11.88	50.46	10.03
	***		**		***		***		**	

KGF at 0.1, 1 and 3 mg/kg increased the wet weight of all the regions of the gastrointestinal tract, but only increased proliferation in the stomach and small intestine. Plasma gastrin, PYY enteroglucagon and GLP-1 were all increased whereas insulin was lowered by KGF (all $p < 0.01$). KGF was ineffective in reducing indomethacin-induced gastric damage but caused a reduction in basal acid secretion of about 35% and 50% when administered at 0.2 or 5mg/kg ($P < 0.05$). KGF (1-50mM) did not influence the rate of cell migration of HT29 cells.

KGF is therefore unlikely to be important in stimulating the early restitutive phase of gastrointestinal repair but may be important in the later stages of increased proliferation and remodelling.

Separation of human plasma lipoprotein subclasses. By BRUCE A. GRIFFIN and N. P. FURLONGER, *Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

Expanding interest in the relationship between dietary polyunsaturated fats and CHD has placed increased emphasis on the role of serum triacylglycerol as a progenitor of lipid-mediated CHD risk. For the majority of healthy, free-living individuals, a dietary-induced predisposition to CHD risk arises, not from raised serum cholesterol *per se*, but from the development of abnormalities in the distribution of VLDL, LDL and HDL subclasses which originate from a defect in triacylglycerol metabolism. Thus, the measurement of lipoprotein subclasses has a major application in studies designed to assess the impact of diet on CHD risk. Lipoprotein subclasses are operationally defined by their flotation properties on optimized density salt (NaBr) gradients, which resolve visibly discrete lipoprotein heterogeneity. This study describes the application of two previously published density gradients (LDL, HDL) together with a new method for the separation of VLDL subclasses. The physical displacement of the gradient and continuous u.v. monitoring (280nm), facilitates the generation of lipoprotein spectra, quantitation and isolation of individual subclasses. All the gradients illustrated below are centrifuged under the prescribed conditions in a Beckman SW40 rotor. The gradients are eluted by infusion pump connected to a Beckman fraction recovery system. This is coupled to DU650 spectrophotometer equipped with adapted Beckman 'Gel Scan' software. The area beneath individual lipoprotein subclasses is used to proportionate either total lipoprotein or apoprotein mass to yield plasma lipoprotein subclass concentrations.



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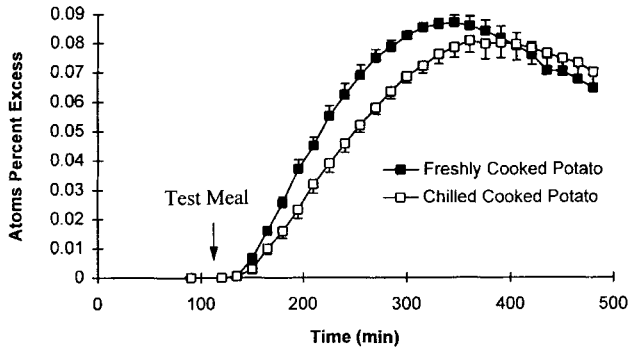
Acute metabolic effects of freshly cooked *v.* cooked and chilled potatoes. By MARK DALY[1], MICHELLE TOWNSLEY[2], CATHERINE VALE[2], ALISON LITTLEFIELD[2], K.G.G.M. ALBERTI[1], MARK WALKER[1], GEOFF LIVESEY[3] AND JOHN MATHERS[2], [1]Department of Medicine and [2]Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne NE1 7RU and [3]Institute of Food Research, Norwich NR4 7UA.

It is well established that cooked starchy foods undergo retrogradation when allowed to cool (Asp *et al*, 1996), and that this alters the rate and extent of starch digestion. In the present study the metabolic effects of freshly cooked *v.* partially retrograded starch were investigated using potatoes labelled by growing them in a $^{13}CO_2$ -enriched environment. Following an overnight fast, eight healthy volunteers consumed test meals containing either hot mashed potato or cooked, chilled potato (338 g) in a randomised, crossover design. In each case the rest of the meal was cold turkey (100 g) and skimmed milk (200 ml). Blood glucose and insulin concentrations and breath CO_2 enrichment (Leijssen & Elia, 1996) were measured at frequent intervals up to 6 h after the meal.

Fig : Atoms Percent Excess (APE) enrichment of expired CO_2 with $^{13}CO_2$ (mean values with their standard errors)



Blood glucose and insulin concentrations peaked earlier and were higher with the freshly cooked potato. This was reflected in a significantly more rapid rise in breath $^{13}CO_2$ enrichment suggesting that the oxidation rate of the absorbed glucose was also faster.

In conclusion, keeping cooked potato at 4° for 16 h before eating reduced post-prandial glycaemia and retarded glucose oxidation.

This study was supported by MAFF grant ANO 0309.

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Time course of the effects of fish oil feeding on blood cholesterol concentrations in the rat. By N.M. JEFFERY[1], E.A. NEWSHOLME[1] and P.C. CALDER[2], [1]*Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU and* [2]*Division of Human Nutrition, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

Fish oil (FO) has been shown to result in lower blood concentrations of cholesterol and triacylglycerol when included in the diet of both laboratory animals and free-living human subjects (see Harris, 1996 for a review). We have reported that feeding weanling rats for 10-12 weeks on a diet containing 200 g/kg FO results in lower serum cholesterol concentrations than if the rats are fed on a low-fat (LF; 25 g/kg) diet or diets containing 200 g/kg hydrogenated coconut oil, olive oil, safflower oil (SO) or evening primrose oil (Yaqoob *et al.* 1995). It was of interest to determine the duration of FO feeding required to elicit this cholesterol-lowering effect.

Weanling male Lewis rats (*n* 3 per diet) were fed for 1 - 8 weeks on a LF diet or on diets containing 200 g/kg SO or FO (menhaden oil); the levels of all other components of the diet (apart from that of non-nutritive bulk which was higher in the LF diet) were identical. Rats were killed in the fed state by an overdose of CO_2. Serum was prepared and free and total cholesterol concentrations measured using enzymic assays; cholesterol ester concentrations were determined by the differences between total and free cholesterol concentrations.

		Total cholesterol (mg/ml)		Free cholesterol (mg/ml)		Cholesterol ester (mg/ml)	
Duration of feeding (weeks)	Diet	Mean	SE	Mean	SE	Mean	SE
1	LF	1.30	0.19	0.46	0.01	0.84	0.01
	SO	1.29	0.05	0.58	0.03	0.71	0.01
	FO	1.33	0.04	0.54	0.03	0.79	0.01
2	LF	1.16	0.04	0.21	0.03	0.95	0.01
	SO	1.22	0.02	0.26	0.03	0.93	0.01
	FO	1.00†	0.02	0.19	0.02	0.81†	0.03
4	LF	1.11	0.04	0.26	0.02	0.85	0.04
	SO	1.46	0.13	0.31	0.01	1.15	0.13
	FO	0.79*†	0.08	0.18*†	0.02	0.62*†	0.09
8	LF	1.28	0.01	0.27	0.01	1.04	0.03
	SO	1.30	0.03	0.29	0.02	0.99	0.06
	FO	0.97*†	0.05	0.19*†	0.01	0.79*†	0.04

Mean values were significantly different from: *LF; †SO ($P<0.05$; ANOVA).

At 1 week post-weaning approximately 60% of serum cholesterol was present as cholesterol ester irrespective of the diet fed, but by 2 weeks post-weaning 80% of cholesterol was in the esterified form; again this proportion was unaffected by diet (see Table). There were no differences in the free, esterified or total cholesterol concentrations between animals fed on the LF and SO diets at any time point (see Table). However, after 4 weeks of feeding the FO diet the concentrations of free, esterified and total cholesterol were significantly lower than those in rats fed on either of the other two diets (see Table). These data indicate that the cholesterol-lowering effect of dietary FO in rats begins to be exerted within 2 weeks of the onset of feeding and is established within 4 weeks of the onset of feeding.

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Breakfast composition, waist:hip ratio (WHR) and cardiovascular (CVD) risk factors in middle-aged men. By D.L. FRAPE[1], N.R. WILLIAMS[1], JAYSHRI RAJPUT-WILLIAMS[1], B.W. MAITLAND[1], C. MARSHALL[1], C.R. PALMER[2], R.J. FLETCHER[3] and EITHNE CAHILL[3], *[1]Pathology Department Papworth Hospital, Cambridge CB3 8RE; [2]University of Cambridge, Institute of Public Health, Robinson Way, Cambridge CB2 2SR; [3]The Kellogg Company of Great Britain Ltd, The Kellogg Building, Talbot Road, Manchester M16 0PU*

Abdominal obesity is considered to be a risk factor for diabetes mellitus and cardiovascular disease. An experiment was conducted with twenty-four middle-aged men who were given either a cereal, semi-skimmed milk, orange juice breakfast, containing 5.5 g fat (low fat, L), or a Cornish pastie breakfast containing 25.8 g fat (moderate fat, M) of similar fatty acid composition and of similar energy and protein contents for twenty-eight consecutive days. By analysis the L breakfast contained 6.5 % more metabolizable energy than the M breakfast, and 57 g more carbohydrate. Other daily meals were not strictly controlled and the fat content of the lunch and evening meals was slightly higher, amongst those receiving the L breakfast.

The subjects were allocated to treatment from groups of similar WHR and paired according to body weight. Plasma insulin was measured by radio-immune assay, plasma plasminogen activator inhibitor-1 antigen (PAI-1) and tissue plasminogen activator (t-PA) by ELISA assay and blood platelets with a PAP4 aggregometer. Body measurement changes over the experiment are shown in the table:

	Body weight (kg)		BMI (kg/m^2)		Waist:hip	
	Mean	SD	Mean	SD	Mean	SD
L	+0.358	0.867	+0.112	0.273	-0.015	0.038
M	-0.158	1.267	-0.048	0.423	+0.016	0.028
P	0.26		0.28		0.03	

WHR was reduced by treatment L and the ratio was positively correlated with increased risk factors for CVD: pre-dose serum insulin (r 0.45, P = 0.001), LDL-cholesterol (r 0.50, P = 0.015) and platelet aggregation score (r 0.35, P = 0.06). The population was bimodal for a tendency of platelets to disaggregate, once aggregated, which reduces thrombosis risk, as found in the Caerphilly Collaborative Heart Disease Study (Elwood *et al.* 1991). Fasting insulin was correlated with fasting PAI-1 concentration (r 0.53, P = 0.001) and diurnal t-PA increased only in treatment L (P = 0.0008). Fasting serum LDL-cholesterol and platelet aggregation lag time in the presence of collagen were correlated (r -0.66, P = 0.001). Fasting serum triacylglycerol concentration was related (P = 0.01) to other haemostatic factors in the presence of collagen; positively to platelet maximum aggregation and negatively to lag time.

Abdominal fat is more readily mobilized than gluteofemoral fat (Landin *et al.* 1990) with the formation of circulating non-esterified fatty acids (NEFA). Serum NEFA concentration from after breakfast until late afternoon was considerably greater in treatment M than in L (P = 0.00001) and it is associated with reduced hepatic insulin binding and clearance (Svedberg *et al.* 1990). Treatment L improved glucose tolerance as measured by serum incremental areas under the curve: glucose (P= 0.007), ln insulin (P = 0.002), ln insulin:glucose ratio (P = 0.014) and ln C-peptide (P = 0.0025). Small differences in fat intake at breakfast may be important in the control of risk factors of atherosclerosis and thrombosis.

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The effects of two commercially available spreads on plasma lipoproteins in human subjects. By SARAH E. SCHENKER[1], JENNIFER M. GARRY[1], JACQUELINE C. BROWN[1], ALLAN GAW[2], DAVID A. HUGHES[1] and JOHN C. STANLEY[1], *[1]Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA and [2]Department of Pathological Biochemistry, Glasgow G31 2ER*

In previous intervention studies a high dietary intake of *trans* fatty acids (TFA) has been shown to increase plasma LDL-cholesterol and decrease HDL-cholesterol (Zock & Mensink, 1996). Both changes are associated with an increased risk of cardiovascular disease. As part of a larger study investigating the relationship between TFA and plasma lipoproteins, a pilot study was conducted to investigate the effects of low- and high-TFA spreads on human plasma lipoproteins. A nutrient database was used to identify the lowest and highest TFA spreads commercially available. Seventeen participants from the local population completed the study. Each participant completed a 7 day weighed intake. The average normal dietary intake of the participants was 2.5% energy as TFA which is in line with the average intake of the UK diet. Baseline blood samples were taken and the subjects were asked to eat one of two diets calculated to contain either less than 1% TFA or 10% TFA as total energy. The diets were made up using prepared meals of known fatty acid composition, the commercially available spreads, and cakes baked using the spreads. The participants consumed the diets for 3 weeks during which they completed a 5 day weighed intake. At the end of the dietary period a final blood sample was taken. The fatty acid composition of the spreads was analysed for all *cis*- and *trans*- C18:1 isomers by GC using a 30 m BPX 70 column.

	Low TFA diet (n 7)						High TFA diet (n 10)					
	0 weeks		3 weeks		Difference		0 weeks		3 weeks		Difference	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total cholesterol	4.99	0.12	4.57**	0.14-	0.42	0.03	4.52	0.40	4.86*	0.32	0.34	0.14
VLDL-cholesterol	1.55	0.43	1.22	0.25	-0.33	0.23	0.99	0.10	1.19	0.20	0.20	0.14
LDL-cholesterol	3.10	0.12	2.94	0.12	-0.16	0.11	2.96	0.31	3.18	0.24	0.22	0.14
HDL-cholesterol	1.09	0.15	1.04	0.10	-0.06	0.06	1.19	0.10	1.23	0.09	0.04	0.05
Triacylglcerol	0.80	0.20	0.60	0.11	-0.20	0.14	0.52	0.06	0.54	0.13	0.03	0.12

NB: There was no significant difference (P>0.05) between groups at baseline.
Significantly different from 0 weeks (baseline):P<0.05 ** P<0.01.

The fatty acid composition of the high TFA spread by analysis was 15.7% total TFA which was markedly different from that given in the nutrient database (28.8%). The change in lipoprotein concentrations (Table 1) were probably due to differences not only in TFA but also linoleic acid and fatty acids of C20 or greater. This emphasizes the dangers of accepting values for the fatty acid composition of foods given in databases and underlines the value of pilot studies in human nutrition.

This work was sponsored by the Ministry of Agriculture, Fisheries and Food.

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Effects of two types of dietary fish oil on blood lipid levels and lymphocyte proliferation in the rat. By N.M. JEFFERY[1], P. SANDERSON[1], P. YAQOOB[1], E.A. NEWSHOLME[1] and P.C. CALDER[2], [1]*Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU and* [2]*Division of Human Nutrition, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

The basis of recent recommendations to increase the consumption of oily fish is the link between intake of the long chain *n*-3 polyunsaturated fatty acids (PUFA) found in fish-body oils and a lowered incidence of CHD. Part of the reason for the beneficial effects of *n*-3 PUFA may be their ability to lower blood triacylglycerol (TAG) levels in man (see review by Harris, 1996); there are conflicting reports regarding the effects of *n*-3 PUFA on blood cholesterol levels in man (see Harris, 1996). In addition to protective effects towards CHD, there is evidence that dietary *n*-3 PUFA afford some protection against inflammatory diseases. The basis of this may be the potent effects of *n*-3 PUFA on the production of inflammatory mediators and on the functions of cells involved in the inflammatory and immune responses (see Calder, 1996 for a review). Most fish oils used in experimental situations, and many of those commercially available for human consumption, originate from the bodies of oily fish. However, fish-liver oils, such as that from cod liver, are also readily available to consumers. Oils from both the body and liver of fish are rich in *n*-3 PUFA (e.g. menhaden oil (MO) and cod liver oil (CLO) each contain approximately 25 g *n*-3 PUFA/100 g total fatty acids). However, fish-liver oils contain higher levels of some components including vitamins A and D (e.g. MO contains approximately 500 IU vitamin A and 100 IU vitamin D per g whereas CLO contains up to 1500 IU vitamin A and 300 IU vitamin D per g), which themselves exert potent biological effects. Therefore, it seemed important to compare the effects of fish body and liver oils upon blood lipid levels and lymphocyte functions.

Male weanling Lewis rats (*n* 6 per diet) were fed for 12 weeks on a low-fat (LF; 25 g/kg maize oil) diet or on diets containing 200 g/kg MO or CLO. At death blood was collected into heparin, diluted and cultured in the presence of the T-cell mitogen concanavalin A (Con A; 37.5 μg/ml). Spleen lymphocytes were prepared and cultured in the presence of Con A (5 μg/ml) and 25 ml/l autologous serum. Whole blood and spleen lymphocyte proliferation were measured as [^{3}H]thymidine incorporation over the final 18 h of a 66 h culture period. Serum cholesterol and TAG concentrations were measured by enzymic techniques.

	Serum TAG (mM)		Serum cholesterol (mg/ml)		[^{3}H]thymidine incorporation (dpm/well) Whole blood		Spleen lymphocytes	
Diet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LF	1.71	0.28	1.01	0.07	11767	2179	129545	2109
MO	0.61*	0.08	0.57*	0.05	6742*	1028	108090*	5488
CLO	0.59*	0.06	0.67*	0.06	5572*	1332	104638*	5671

* Mean values were significantly different from LF (P<0.05; Student's t-test).

Serum TAG and cholesterol concentrations and T-cell proliferation in both whole blood and purified spleen lymphocyte cultures were very similar for both MO- and CLO-fed rats. In all cases these values were significantly less than those observed for LF-fed rats. Thus, this study indicates that the blood lipid-lowering and immunomodulatory effects of fish body and fish liver oils are the same.

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Effect of lactose induced diarrhoea on macronutrients digestibility and immune function in well-nourished and undernourished rats. By ANNA M. CIOCCIA, ESTER L. ARCINIEGAS and PATRICIO HEVIA, *Laboratorio de Nutrición, Universidad Simón Bolivar, Caracas, Venezuela 89000.*

Diarrhoea is a very important cause of death in young children in underdeveloped countries and a key factor in infant malnutrition. A vicious cycle has been proposed which indicates that diarrhoea causes nutrient malabsorption, which favours malnutrition. This in turn curtails the immune defence mechanisms, facilitating gastroenteritis and other infections. Diarrhoea in malnourished children is difficult to control and often degenerates into an intractable state. Since this is a relatively frequent occurrence in Venezuela, we compared the availability of nutrients in a balanced diet offered to young well-nourished and undernourished Sprague Dawley rats, with and without diarrhoea. Malnutrition was induced by restricting food intake (50%) in one half of the rats for 2 weeks and diarrhoea was induced by including 45 % lactose in the diet after malnutrition had been established . During the experiment which lasted 8 d the animals were kept on the same feeding protocol but one half of the nourished and one half of the undernourished received lactose to induce diarrhoea. Therefore during the experiment there were well nourished rats (Nou) with and without lactose and undernourished rats (U.Nou) with and without lactose.

	Nou		Nou + Lac		U.Nou		U.Nou + Lac	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Food intake (g/8 d)	164.5a	17.44	110.5b	22.23	75.11c	0.04	74.53c	2.62
Growth (g/8 d)	61.0a	15.12	39.4b	10.83	30.7b	4.02	31.2b	4.61
Faecal mass (g/3 d)	2.02a	0.43	19.14b	6.44	1.04a	0.22	8.07c	1.82
Apparent Digestibility (%)								
Whole Diet	97.3a	0.39	87.1b	6.15	97.5a	0.35	92.1c	1.25
Nitrogen	94.9a	0.83	83.9b	3.07	94.4a	1.29	80.2b	5.41
Fat	89.5a	2.26	74.3b	11.93	92.2a	1.33	87.6a	1.58
Energy	96.9a	0.49	86.5b	6.08	97.4a	0.37	92.8c	1.18
Apparent Retention (%)								
Nitrogen	79.3a	5.05	54.9b	19.44	76.3a	1.68	53.9b	7.50
Energy	96.1a	0.52	75.3b	13.37	96.5a	0.52	86.17c	3.14
Packed cell volume (%)	49.8a	4.34	47.4ab	4.48	43.0b	4.50	45.4ab	8.09
Leukocytes (Cells x 10^4 / ml)	471.9a	97.7	443.6a	66.8	285.8b	23.4	278.6b	42.1
Thymus weight (g)	0.74a	0.163	0.54b	0.115	0.32c	0.069	0.25c	0.046
Serum Ig G (mg/L)	5369a	1295.3	3875b	1349.6	4206ab	1074.9	4219ab	1329.1

Entries are mean and standard deviation of eight rats. a,b,c Mean values within a row not sharing a common letter were significantly different, P< 0.05 (Anova and Duncan's multiple range test).

The results showed that the inclusion of lactose at 45% in the diet caused a severe diarrhoea both in the nourished and undernourished rats. This diarrhoea however, resulted in a reduction in food intake and growth only in the well-nourished rats. In the rats with diarrhoea the apparent digestibility of the diet and of its macronutrients decreased compared with the animals without diarrhoea but this reduction was less apparent in the undernourished rats. Similar results were obtained in relation to the retention of N and energy. In this case, diarrhoea was associated with retentions which were lower than those seen in the rats without diarrhoea but the undernourished rats with diarrhoea retained more energy than the well-nourished rats with diarrhoea. Malnutrition resulted in lower packed cell volume, leukocyte count and thymus weight but diarrhoea in the malnourished rats did not cause a further reduction in these variables as it did in the well-nourished animals. In general, these results indicate that in well-nourished rats, diarrhoea had a negative effect whereas in the undernourished group it did not. It appears that the undernourished rats compensated their nutrient utilization so that diarrhoea did not worsen their undernourished condition.

Funded by CONICIT Grant S1-2265.

γ-Linolenic acid inhibits the expression of the major histocompatibility complex (MHC) class II molecule, HLA-DR, on human monocytes *in vitro*. By DAVID A. HUGHES[1], ELIZABETH S. GLASSCOCK[2] and ANDREW C. PINDER[1], *[1]Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA; [2]School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ*

Polyunsaturated fatty acid (PUFA)-rich diets are associated with suppression of cell mediated immune responses and there is growing evidence that dietary PUFA supplementation is of value in the treatment of disorders involving an overreactive immune response, such as rheumatoid arthritis (RA). Specific immune responses are initiated by the presentation of antigen to helper T-lymphocytes on the surface of an antigen-presenting cell (APC). A pre-requisite for this function of APC is the cell surface expression of MHC class II molecules, aided by the presence of adhesion molecules. We have previously shown that the *n*-3 PUFA, eicosapentaenoic acid and docosahexaenoic acid, found in fish oil, can inhibit the cell surface expression of MHC class II molecules and adhesion molecules on normal human blood monocytes *in vitro* (Hughes *et al.* 1996). Since there is also evidence that the *n*-6 PUFA, γ-linolenic acid (GLA), can be beneficial in the treatment of RA (Zurier *et al.* 1996), we investigated the possibility that this fatty acid may also inhibit the expression of functionally associated surface molecules.

Purified monocytes, obtained from eight healthy volunteers, were incubated with or without GLA (15 μg/ml) in culture medium supplemented with 50 ml fetal calf serum/l for 48 h at 37°. In addition, parallel cultures were performed in the presence of interferon-γ (IFN-γ; 400 U/ml) to stimulate upregulation of MHC class II molecule expression by the monocytes. Following incubation, the monocytes were immunofluorescently stained with monoclonal antibodies raised against the major MHC class II molecule, HLA-DR, and the adhesion molecules intercellular adhesion molecule-1 and leucocyte function associated antigen-3. Both the percentage of monocytes expressing each of these molecules and the intensity of expression of each molecule were quantified by flow cytometry.

In the presence of GLA alone, compared with controls, there was no significant difference in the percentages of cells expressing each of the surface molecules studied. However, in the presence of both GLA and IFN-γ there was a significant reduction in the percentage of monocytes expressing HLA-DR and the intensity of expression of this molecule was reduced, compared with monocytes cultured in the presence of IFN- γ alone ($P<0.05$ in both cases). There were no significant changes in the expression of the adhesion molecules studied.

Since it has been shown that the percentage of MHC class II-positive monocytes and the density of these molecules on the cell surface can alter the degree of immune responsiveness of an individual (Janeway *et al.* 1984), these findings suggest that GLA may depress immune reactivity in a similar manner to *n*-3 PUFA, by inhibiting antigen-presenting cell function. The inhibition of MHC class II molecule expression observed in the presence of IFN-γ, supports the possibility that GLA might be useful in the treatment of RA, a disorder associated with elevated expression of HLA-DR on synovial fluid monocytes. The concentration of GLA used in this study was the same as that achieved in the plasma of RA patients given GLA supplements in a clinical trial which showed a beneficial effect (Zurier *et al.* 1996). The possibility that GLA might inhibit the expression of HLA-DR on synovial monocytes in RA patients remains to be explored.

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Dietary fish oil decreases the production of pro-inflammatory mediators by murine macrophages. By F.A. WALLACE, S.J. NEELY, E.A. MILES and P.C. CALDER, *Division of Human Nutrition, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

Despite a number of studies, the effects of dietary fish oil on the production of pro-inflammatory cytokines remain controversial with many contradictory reports in the literature (see Calder 1996 for a review). To investigate the effect of fish oil and other lipids further, we fed male C57Bl6 mice for 12 weeks upon a low-fat (LF) diet (25 g/kg maize oil) or on diets containing 200 g/kg coconut oil (CO), olive oil (OO), safflower oil (SO) or fish oil (FO). Peritoneal macrophages were elicited with thioglycollate and were cultured for 24 h at a density of 5×10^5 cells/ml (total volume 2 ml) in the presence of 10 μg/ml lipopolysaccharide and 50 ml/l fetal calf serum. The concentrations of prostaglandin E_2 (PGE_2), interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α (ng/ml) in the medium were measured by ELISA and the concentration of nitrite (an indicator of nitric oxide production; μM) was measured by a colorimetric assay (see Yaqoob and Calder, 1995 for methods).

	Concentration in medium									
	Nitrite		PGE_2		IL-1β		IL-6		TNF-α	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
LF	24.4[a]	2.3	74.7[a]	6.1	0.15[a]	0.03	0.85[c]	0.01	3.1[a]	0.6
CO	11.6[b]	3.0	57.8[b]	13.6	0.13[a]	0.01	0.86[c]	0.02	2.0[ab]	0.3
OO	20.5[a]	3.4	50.8[b]	3.7	0.11[a]	0.01	0.88[c]	0.03	1.3[bc]	0.4
SO	19.8[a]	6.2	50.4[b]	4.1	0.11[a]	0.02	0.95[b]	0.05	0.70[cd]	0.07
FO	24.6[a]	3.0	13.4[c]	6.2	0.057[b]	0.004	0.83[a]	0.02	0.12[d]	0.02

Data are mean values with their standard errors from four to six animals fed on each diet.
[a,b,c,d]Mean values within a column not sharing a common superscript letter were significantly different ($P<0.05$; ANOVA).

Nitrite production was significantly lower by cells from animals fed on coconut oil. As expected, fish oil feeding caused a significant decrease in PGE_2 production. The production of two pro-inflammatory cytokines (IL-1β and TNF-α) was markedly reduced if the mice were fed on fish oil; IL-6 production was slightly lower following fish oil feeding. TNF-α production was also reduced by feeding the olive or safflower oil diets; IL-6 production was enhanced by safflower oil feeding. Thus, this study demonstrates that fish oil feeding greatly diminishes the ability of macrophages to produce some inflammatory mediators. This effect may be, at least partly, responsible for the beneficial effects of dietary fish oil in acute and chronic inflammatory conditions.

This research is supported by the BBSRC (Grant No. 51/FO5696). FAW holds a Rank Prize Fund Studentship.

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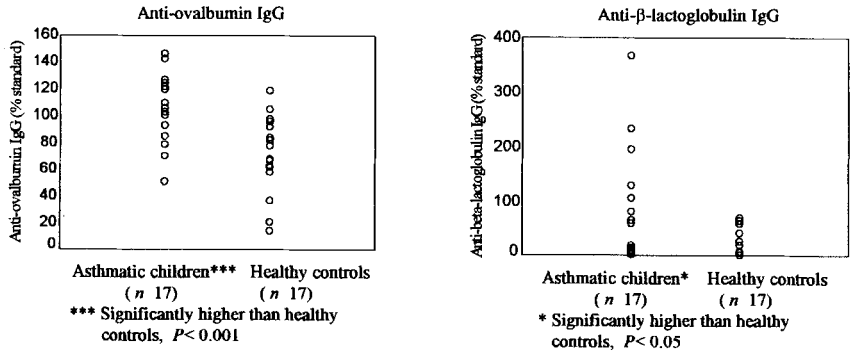
Differences in specific food antibody levels in asthmatic and healthy children. By NOOR AINI M. YUSOFF[1], S.M. HAMPTON[1], A.S. MALLIK[2] and JANE MORGAN[1], *[1]School of Biological Sciences, University of Surrey, Guildford GU2 5XH and [2]Frimley Park Hospital, Frimley GU16 5UJ*

The prevalence of asthma is increasing in children worldwide and in young children aged 6-7 years it is estimated to range from 3.5-32.1% (Koning *et al.* 1996). Asthmatic episodes can be triggered by a wide variety of agents. These include antigens, viral infections, exercise, exposure to fumes and other irritants, certain drugs, food, drink and food additives (Weeke, 1992). The specific antigen in a particular individual may be suggested from the clinical history. However sometimes the clinical history does not point to the specific antigen. Our understanding of the role of foods in asthmatic children is still limited. The aim of the present study was to determine food antibody levels, specifically anti-ovalbumin immunoglobulin G (IgG) and anti-β-lactoglobulin IgG in asthmatic and healthy children.

Seventeen volunteers (thirteen male, four female) aged between 2 and 15 years, clinically diagnosed with mild to moderate asthma and seventeen healthy controls matched for sex and age with no personal or family history of atopy and/or adverse reactions to food allergens were recruited to the study. A casual blood sample was collected and serum analysed by indirect ELISA for the determination of the anti-ovalbumin IgG and anti-β-lactoglobulin IgG (Hampton *et al.* 1990). The results are shown below.



Results show that there were significantly higher levels ($P<0.05$) of the anti-ovalbumin IgG and anti-β-lactoglobulin IgG in asthmatic children compared with healthy controls. The mean anti-ovalbumin IgG level in asthmatic children was 105.4% of standard compared with 71.5% in healthy controls. The mean anti-β-lactoglobulin IgG level was 83% of standard in asthmatic children compared with 22.7% in healthy controls. Eight of seventeen asthmatic children had adverse reactions to foods such as eggs, milk and/or peanuts as reported by questionnaire.

In conclusion, this study suggests that asthmatic children have higher food antibody IgG levels compared with healthy children. Findings in this study also indicate the possibility that adverse reactions to foods take place in asthmatic children of which parents are unaware.

This research was funded by The Malaysian Government. We acknowledge the assistance of Frimley Children Centre, Frimley Park Hospital and The Royal Surrey County Hospital.

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Fluoride intake in 4-year-old Iranian children. By FATEMEH V. ZOHOURI and ANDREW J. RUGG-GUNN, *Department of Child Dental Heath, The Dental School, Newcastle University, Newcastle upon Tyne NE2 4BW*

It is very well accepted that dental caries, which is one of the most prevalent diseases, can be prevented by the appropriate use of fluoride. Knowing the amount of fluoride ingested by children would help to determine the relative importance of the various sources. The purpose of the present study was to measure the daily fluoride intake from diet and toothpaste by a sample of Iranian children of aged 4 years. To achieve this purpose the silicon-facilitated diffusion method in unashed samples (Venkateswarlu, 1992) with some modification was employed to measure the acid-diffusable fluoride of 117 food samples. The average daily dietary fluoride intakes of 103 children were estimated from a 3 d dietary diary in summer and winter to allow for any seasonal variation in fluoride intake. This was calculated in high-, medium- and low-fluoride areas with about 4.0, 0.6, and <0.3 mg fluoride/l in drinking water respectively. Ingestion of fluoride from toothpaste per day was measured for each child by (a) recording the type of toothpaste used (if any), (b) determining the fluoride content of this toothpaste, (c) recording the frequency of brushing, and (d) estimating the weight of fluoride ingested per brushing by subtracting the weight of fluoride in expectorated toothpaste, water and saliva after brushing from the weight of fluoride in the toothpaste on the brush before brushing (Hargreaves *et al.* 1972). Toothpastes containing Na-monofluorophosphate (SMFP) were incubated with acid phosphatase (EC 3.1.3.2) at 37 ° for 3 h before use of the F-ion selective electrode.

The overall mean recovery of fluoride added to sixty food samples before diffusion, was 98%, and the mean recovery of SMFP added to all toothpaste samples was 96 (SD 5)%. The fluoride blank from the method employed was 0.02 μg fluoride/g, which was subtracted from each reading.

		Daily dietary fluoride intake (including water)			
		mg		mg/kg body weight	
Area	*n**	Mean	SD	Mean	SD
Low-fluoride area, <0.3 mg/l (LF)	85	0.388	0.126	0.028	0.009
Medium-fluoride area, 0.6 mg/l (MF)	9	0.634	0.144	0.048	0.007
High-fluoride area, 4.0 mg/l (HF)	9	3.050	0.899	0.246	0.052

* Number of children.

The Table shows the mean daily dietary fluoride intake of children expressed as mg and on a body weight basis. About 65 - 85% of total daily dietary fluoride intake came from all sources of water, and tap water (drinking water) contributed from 34 to 47% of total daily dietary fluoride intake, in all areas.

The average fluoride ingestion from toothpaste in the thirty (29%) children who brushed their teeth regularly was 0.10 (SD 0.07) mg. The mean dietary daily intake of fluoride in the 4-year-old children in the LF area was slightly more than half of the optimum level of 0.05 mg/kg body weight; in the MF area, it was about the optimum level, and in the HF area, the fluoride intake was five times more than the optimal. Consumption of more than 600 ml tap water and tea (with F concentration of 4.5 μg/ml) was the main reason for the high F intake in the HF area. In the HF area, a mild dental fluorosis was observed in these children.

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Similar recovery of $^{13}CO_2$ and $^{14}CO_2$ in breath during infusion of bicarbonate labelled with ^{13}C and ^{14}C simultaneously. By N.J. FULLER, R.M. McDEVITT, M. HARDING, W.A. COWARD and M. ELIA, *MRC Dunn Clinical Nutrition Centre, Cambridge CB2 2DH*

Accurate assessment of the oxidation rates of labelled substrates or energy expenditure using labelled bicarbonate depends on the validity of values assigned to the amount of label recovered in breath CO_2. However, a comprehensive review of the literature by Leijssen & Elia (1996) has suggested that there are inconsistencies in recoveries of administered ^{13}C- and ^{14}C-labelled bicarbonate, and that these may be biological or methodological in origin. Since bioequivalence of the ^{13}C and ^{14}C isotopes has never been determined directly, the present study was undertaken to assess recovery of $^{13}CO_2$ and $^{14}CO_2$ from a continuous infusion of bicarbonate labelled simultaneously with both isotopes.

Six healthy males volunteered for the study: age 24-41 years; mean weight 76.7 (SD 18.6) kg; height 1.79 (SD 0.05) m; and BMI 24.0 (SD 5.6) kg/m^2. Each was subjected to an accurately measured rate of intravenous infusion of sterile, pyrogen free, bicarbonate solution (110.0 mmol/kg; $NaH^{13}CO_3$, 87.5 atom %; $NaH^{14}CO_3$, 3250 dpm/μmol), delivered continuously over a 6 h period within a 'tent' calorimeter (1400 litres capacity). Before the infusion was started, and at hourly intervals throughout the study, a 12 litre sample of breath was collected in a Douglas bag. Known amounts of CO_2 from the Douglas bag sample were trapped using hyamine in methanol (nominally, 3 ml of 560-580 mmol/l) for assessment of specific activity. Further samples of breath from this same Douglas bag collection were transferred to 11 ml evacuated exetainers for measurement of CO_2 enrichment. Recoveries of ^{14}C and ^{13}C were calculated as the product of CO_2 production and the change in specific activity or enrichment, respectively. Preliminary work had shown that small fluctuations in basal enrichment (SD 0.3 ∂ per mil, relative to the International Standard PDB), relative to the large change in enrichment of CO_2 (plateau mean 98 (SD 12) ∂ per mil, relative to PDB), could be ignored.

Time period (h)	Recovery of label (%)					
	^{13}C		^{14}C		Difference	
	Mean	SD	Mean	SD	Mean	SD
0 - 1	24.9	1.8	24.7	1.9	0.2	0.2
1 - 2	62.0	4.7	61.4	4.8	0.6	0.4
2 - 3	78.4	5.9	77.6	5.8	0.8	0.7
3 - 4	88.9	2.3	88.3	2.3	0.6	0.8
4 - 5	92.7	3.0	92.1	3.3	0.6	0.9
5 - 6	90.0	5.8	89.3	4.9	0.7	1.2

Mean recoveries of ^{13}C in breath CO_2 appeared to be consistently slightly higher than those of ^{14}C (paired *t*- test, $P<0.001$), indicating that it is not possible to eliminate a small fractionation effect. However, in view of the close agreement between recoveries of ^{13}C and ^{14}C, it is concluded that these isotopes are essentially bioequivalent in behaviour, with respect to recovery of label in CO_2; and, that there is no compelling reason to amend values for recovery of label in CO_2 when using ^{13}C instead of ^{14}C to assess substrate oxidation rates or energy expenditure.

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Reduced glycine flux with age and non-insulin-dependent diabetes mellitus (NIDDM) is associated with increased urinary excretion of 5-L-oxoproline. By A.A. JACKSON[1], F. JAHOOR[2], J. MORLESE[2], F.I. BENNETT[3], C. PERSAUD[1] and T.E. FORRESTER[3], *[1]Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX, [2]USDA/ARS Children's Nutrition Center, Baylor College of Medicine, Houston, TX 77030, USA and [3]Tropical Metabolism Research Unit, University of the West Indies, Mona, Kingston, Jamaica*

The urinary excretion of 5-L-oxoproline increases when glycine available to metabolism is limited by a competitive demand, for example, the conjugation and excretion of benzoic acid as hippuric acid (Jackson *et al.* 1987). We have used urinary 5-L-oxoproline as an indirect marker for glycine availability and found that in NIDDM excretion is related inversely to the concentration of glutathione (GSH) in circulating erythrocytes (RBC) and directly to glycosylated haemoglobin (Forrester *et al.* 1990). In the present study glycine flux was measured directly and related to urinary 5-L-oxoproline and plasma concentrations of glutamic acid, cysteine and glycine.

Subjects were studied in the Tropical Metabolism Research Unit, Jamaica. There were seven young subjects aged 19 - 31 years, eleven older subjects aged 37 - 80 years, and ten subjects with NIDDM aged 42 - 76 years. A primed and constant infusion of [$^{13}C_2$]glycine was given for 6 h in the fasted state and blood taken for the measurement of enrichment of plasma glycine by GCMS, and the concentrations of glutamic acid , cysteine and glycine by HPLC. Urine was collected at the start and end of the infusion period for the measurement of 5-L-oxoproline.

	Young		Older		NIDDM		ANOVA
	Mean	SE	Mean	SE	Mean	SE	*P* value
Glutathione (mmol/l)	3.78	0.46	3.31	0.62	3.16	0.75	0.158
Glycine flux (μmol/kg per h)	198	46	157	46	130	27	0.0084
Glycine (μmol/l)	111	26	132	50	107	33	0.37
Cysteine (μmol/l)	67	9	166	128	221	138	0.042
Glutamic acid (μmol/l)	41	18	68	42	100	65	0.06
5-L-oxoproline/creatinine (μmol/mmol)	13	9	33	22	50	28	0.0094

Glycine flux decreased significantly and 5-L-oxoproline excretion increased significantly with age and NIDDM. On multivariate analysis RBC GSH concentration and fasting glucose contributed to explaining the variation in urinary 5-L-oxoproline, and plasma cysteine and glutamic acid were related indirectly to GSH concentration. Taken together, the highly significant reduction in plasma glycine flux in NIDDM associated with increased concentrations of cysteine and glutamic acid provide very strong support for the proposal that GSH synthesis is not constrained by the availability of cysteine or glutamic acid. Rather, urinary 5-L-oxoproline is a direct consequence of a reduced availability of glycine, which limits the formation of GSH at glutathione synthase, which backs-up the pathway resulting in increased formation and excretion of 5-L-oxoproline.

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Transfer of ^{15}N from orally administered lactose[$^{15}N^{15}N$]ureide to dispensable and indispensable amino acids isolated from human urine and faecal bacteria. By NEIL R. GIBSON[1], RAFE BUNDY[1], ANGELA HOUNSLOW[1], STEPHEN A. WOOTTON[1], D. JOE MILLWARD[2] and ALAN A. JACKSON[1]. [1]*Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX,* [2]*Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, GU2 5XH*

The salvage of urea-N is of importance in the N and amino acid economy of the body (Jackson, 1995). We have shown that salvaged-N returns to the systemic N pool as both indispensable and dispensable amino acids and have interpreted this as indicating that they had been synthesized *de novo* by colonic microflora and were able to cross the colon to the systemic circulation (Yeboah *et al.* 1996; Gibson *et al.* 1997). We present further evidence for this by demonstrating the presence of ^{15}N-enriched amino acids that were isolated from human faecal bacteria and urine 48 h after an oral administration of lactose[$^{15}N^{15}N$]ureide.

Nine normal, healthy adults (six females and three males) aged 19 - 26 years were given an oral dose of lactose[$^{15}N^{15}N$]ureide with priming and intermittent doses totalling 420 (SD 66) mg/kg body weight. Lactose[$^{15}N^{15}N$]ureide is not directly available to the human "host", however, the colonic microflora may cleave the covalent bond between lactose and [$^{15}N^{15}N$]urea and further metabolize the latter. Faecal samples were collected at baseline and for 48 h following label administration. Urine samples were collected every three hours. Bacteria were extracted from faeces using a modified centrifugation technique (Stephen & Cummings, 1980). Freeze-dried bacterial fractions were hydrolysed (6 M-HCl at 100° for 16 h). Glycine, alanine, lysine and histidine were isolated from the bacterial hydrolysate and urine by preparative ion-exchange with fraction collection of all column effluent and peak identification by fluorimetric, multiscan plate reader. The fractions containing glycine, alanine, lysine and histidine were desalted, lyophilized, taken up in 0.1 M-HCl and analysed for ^{15}N enrichment in a Europa-Roboprep 20-20 combustion isotope ratio mass spectrometer.

We observed significant amounts of ^{15}N in the amino acids from bacterial hydrolysates and urine 48 h after label administration. The degree of lysine enrichment compared with zero time varied from 0.03 to 0.21 atoms % excess in the bacterial hydrolysates and from 0.006 to 0.013 atoms % excess in the urine. Glycine and alanine were significantly enriched compared to baseline in the urine and bacterial hydrolysates with histidine also being enriched in the bacterial samples. Relative enrichment ratios to lysine in bacteria were; glycine 0.47 (range 0.13 - 0.745), alanine 1.14 (range 0.39 - 1.87) and histidine 0.98 (range 0.13 - 4.68). Relative enrichment ratios to lysine in urine were; glycine 1.51 (range 0.94 - 2.57), alanine 1.94 (range 1.17 - 3.09). The higher enrichment of glycine and alanine in the urine is consistent with part of the labelling reflecting transamination reactions from ^{15}N-ammonia derived from the colon.

The ^{15}N-lysine appearance rate across the colon may be estimated assuming urinary lysine enrichment (0.0095 atoms % excess) represents enriched lysine from the colon entering the plasma lysine pool and faecal bacterial lysine enrichment (0.1207 atoms % excess) represents the colonic lysine enrichment. With plasma lysine flux being approximately 100 μmol/kg/h, the rate of lysine appearance across the colon equates to approximately 29 mg/kg/d. Overall, these results support the evidence that [$^{15}N^{15}N$]urea made available to the colonic microflora may be utilized for the synthesis of amino acids and that these could be a nutritionally significant source to their human host.

Support by the Biotechnology and Biological Sciences Research Council is gratefully acknowledged.

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Plasma glycine flux falls as oral sodium benzoate acutely depletes the glycine pool. By GLENN P. WEAVIND and ALAN A. JACKSON. *Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX*

The endogenous formation of glycine is large, but finite. In the body glycine is used to detoxify by conjugation some natural chemicals and drugs with sodium benzoate, a food preservative, as one example. Large oral doses of sodium benzoate are given in the management of inborn errors of the urea cycle.

Using prime (6 μmol/kg) and constant intravenous infusion (5 μmol/kg per h) of [^{15}N]glycine we have measured the plasma glycine flux in three normal adult males, before and after oral ingestion of sodium benzoate at 200 μmol/kg per h over 3 h followed by 400 μmol/kg per h sodium benzoate for a further 3 h. The higher dose is similar to that used in the treatment of urea-cycle disorders. Mixed venous blood was sampled at half hourly or hourly intervals. Enrichment in plasma glycine was measured by gas-chromatography/mass spectrometry on derivatized, ion-exchange-resin extracted samples. Plateau levels of enrichment were determined from the mean of three time points and flux calculated. Urinary hippurate, formed by conjugation of glycine with benzoate was determined by u.v. detection HPLC on SAX-extracted samples.

Sodium benzoate...	Nil			200 μmol/kg per h			400 μmol/kg per h		
	Glycine flux (μmol/kg per h)		Hippurate	Glycine flux (μmol/kg per h)		Hippurate	Glycine flux (μmol/kg per h)		Hippurate
	Mean	SEM	Mean	Mean	SEM	Mean	Mean	SEM	Mean
Subject 1	367	26	5.2	281	11	119	231	13	159
Subject 2	246	8	1.4	187	3	26	167	8	29
Subject 3	578	86	4.3	356	26	207	268	32	266

There was considerable variability in the individual measurements of glycine flux at baseline, with subject 2 having a value for flux half that of subject 3. Following oral sodium benzoate, there was an increase up to sixtyfold in urinary excretion of hippuric acid, but the response at the highest level of intake was less marked than at the lower level. At the same time there was a substantial reduction in plasma glycine flux, to about 72% on 200 μmol/kg per h and 59% on 400 μmol/kg per h.

The subjects differed in age and general build, nevertheless they showed similarities in their responses. The formation of glycine and its conjugation with benzoate, to form hippuric acid, take place in the liver. These data indicate that as glycine is consumed in the detoxification of sodium benzoate, a competitive demand for the available glycine is created and the plasma glycine flux falls. This fall, however, cannot be sustained at the higher dose of sodium benzoate, shown by the reduction in the rate of hippurate excretion relative to the dose given (56% on the lower dose and 37% on the higher dose). Under this circumstance plasma glycine flux appears to be maintained at the expense of effective detoxification of the sodium benzoate. These data show that the ability of the body to increase the amount of glycine available from endogenous formation is strictly limited when the demand is changed acutely by oral ingestion of sodium benzoate. We have used the urinary excretion of 5-L-oxoproline to mark the sufficiency of glycine available to the system. There is an increase in urinary 5-oxoproline, in children treated for disorders of the urea cycle as the dose of sodium benzoate approaches toxic levels, suggesting that at this time the demand for glycine has exceeded the body's capacity for glycine formation.

Beliefs, attitudes and behaviour in relation to supplement use in the UK Women's Cohort Study (UKWCS). By SARA KIRK[1], AMANDA WOODHOUSE[1], MARK CONNER[2] and the UKWCS Steering Group*, [1] *Division of Public Health, Nuffield Institute for Health, 71-75 Clarendon Road, Leeds LS2 9PL,* [2]*Department of Psychology, University of Leeds, Woodhouse Lane, Leeds LS2 9PL*

Dietary supplements are used by 17% of the female population (Gregory *et al.* 1990) despite a lack of evidence that they are needed to meet nutrient deficiency, or provide other health benefits. Data on 6572 respondents from the UKWCS (Woodhouse *et al* 1997), have shown that 54% of this sample are taking supplements. This relatively high proportion may reflect the non-random nature of the sample and the high percentage of vegetarian subjects within this cohort.

Two sub-studies are underway to explore further the issue of dietary supplement use by cohort subjects, using a qualitative framework (Miles & Huberman, 1994). One of these studies has used twenty-seven in-depth interviews, the other nineteen semi-structured interviews conducted by telephone. Together, these have identified several themes which have emerged as important in supplement-taking behaviour. These relate to concerns about food safety and beliefs about specific health properties of particular foods/supplements, for example fish oils are believed to, "oil your joints". Different aspects of "insurance" emerged including; the use of supplements to ensure an adequate intake of nutrients, either where subjects have restricted diets, intentionally or otherwise, or have concerns about farming methods reducing the nutrient quality of their food. Taking supplements was also seen as a form of "insurance", through the promotion of optimal nutrition and prevention of future illness.

Quantitative analysis of supplement use by the cohort and comparison with dietary nutrient intake has also been carried out. Preliminary results show that supplement users had significantly higher dietary intakes of vitamins C, E, thiamine and B6, NSP, carbohydrate (sugar in particular), Fe and folate compared with non-supplement users, excluding intakes from supplements. These findings support previous work by Draper *et al.* (1993) and may reflect the "inverse supplement hypothesis" in which the people most likely to use supplements are those who least need them. The use of combined qualitative and quantitative methodologies gives a broader picture of factors influencing supplement use than would be possible if a single methodology was used in isolation.

This work is funded by World Cancer Research Fund, with additional funding from M.A.F.F.

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Beef consumption and nutrient intake: analysis of the UK Women's Cohort Study. By JANET CADE[1], CLAIRE CALVERT[1], JENNIFER BARRETT[2] and the UK WOMEN'S COHORT STUDY STEERING GROUP*, [1]*Division of Public Health, Nuffield Institute for Health, University of Leeds, 71-75 Clarendon Road, Leeds LS2 9PL and* [2]*Centre for Cancer Research, University of Leeds, 26 Clarendon Road, Leeds LS2 9NZ*

Over the last year, there have been changes in the consumption of beef and beef products due to publicity surrounding the concern over cattle affected by bovine spongiform encephalopathy (BSE). Before this concern, beef was an important contributor to the diet, and the National Diet and Nutrition Survey of adults found that 76% of subjects were eating beef or veal during the period of survey, and meat and meat products contributed 16% of the average daily energy intake. With the high profile of such food related issues in the media it is important to know what impact they may have on the nutrient intake of the population. In the present study baseline data from the UK Women's Cohort Study were analysed to compare subjects who were consuming beef with those meat eaters who were not eating beef.

The UK Women's Cohort Study is currently recruiting 30 000 women aged 35-69 years, living in the UK. The cohort is aiming to obtain subjects with a wide range of dietary intakes, with approximately one third being vegetarians, and not a representative sample of the UK population. Baseline data are being collected by postal questionnaire which includes a detailed food-frequency questionnaire (FFQ) covering 217 foods or food groups. The FFQ has been adapted from the questionnaire being used by the UK arm of the European Prospective Investigation into Cancer and Nutrition (Riboli, 1992). The present analysis involved the 7 424 subjects who ate meat from the first 13 670 subjects who returned questionnaires between October 1995 and July 1996. Of the meat eaters, 1 610 (12%) did not eat beef or beef dishes (including beef stew, casserole, mince, curry and beefburgers). Beef- and non-beef- eating meat eaters were compared.

Nutrient intake/d	Non-beef eating meat eaters		Beef eating meat eaters		95% CI for difference
	Mean	sd	Mean	sd	
Total energy (MJ)	9.6	3.1	10.0	3.3	-0.5, -0.2
Protein (g)	85	27	92	29	-8,-5
Carbohydrate (g)	318	111	310	112	2, 14
Fat (g)	79	31	87	34	-9, -5
% energy from fat	31	6	33	6	-2,-1
Zinc (mg)	10	4	12	4	-2, -1
Iron (mg)	18	5	18	3	-0.2, 0.6

The Table shows that there were differences in nutrient intake for beef eating and non-beef eating meat eaters. Non-beef eaters had lower intakes of energy, protein, fat and Zn. Percentage of energy from fat was also lower in the non-beef eaters as was BMI. Fibre and vitamin C intakes were higher in the non-beef eaters. There was no difference in Fe intake. There were some differences in food intake and lifestyle between the groups.

If the differences in nutrient intake observed in this population are reflected long-term by those who have recently stopped eating beef then there may be important implications for health, particularly in relation to fat intake. We recommend that a national strategic food policy is developed so that any future dietary changes can be monitored.

This work is supported by the World Cancer Research Fund.
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Consumer perceptions of starchy foods: Possible barriers towards increasing complex carbohydrate consumption. By KARIN STUBENITSKY and DAVID J. MELA, *Consumer Sciences Department, Institute of Food Research, Reading Laboratory, Reading, RG6 6BZ*

Despite public health guidance to increase consumption of starchy food sources of complex carbohydrates, intakes remain below recommended levels. Studies from our laboratory (e.g. data from Lloyd *et al.* 1993; Paisley, 1994) suggested that increasing intakes of starchy foods was not seen as a desirable dietary change. Moreover, these foods are reportedly perceived by consumers as fattening and "boring but filling". As an initial probe to gain an improved understanding of UK consumer attitudes and beliefs regarding starchy foods and their dietary role, and perhaps suggest strategies for increasing their acceptance and intake, a questionnaire based on the theory of planned behaviour (Ajzen & Fishbein, 1980) was sent out to a UK consumer sample.

The questionnaire focused on attitudes and beliefs towards starchy foods, perceived barriers towards increasing their intake (e.g. cost, habit, social influences), perceptions of personal starchy food intake, reasons for changing starchy food consumption in the past, and socio-demographic information. Starchy foods were defined in the questionnaire as all kinds of breads, breakfast cereals, rice and rice dishes, pasta and pasta dishes, and boiled or jacket potatoes. The list excluded versions of starchy foods which contained a high proportion of fat or sugars, such as roast or fried potatoes (chips), crisps, biscuits, cakes and pastries, and others which could not directly be recognised as starchy foods, such as vegetables and vegetable dishes. The questionnaire was sent to a sample (n 800) from the consumer database of a local commercial marketing organisation, and the response rate was 52% (n 414). Responses indicate that consumers have highly divergent attitudes and beliefs regarding starchy foods. These foods are seen as nutritious and good for one's health, but also as high in energy and not helping to control weight. Possible barriers towards increasing starchy food intake were the perceptions that personal starchy food intakes were already high (mean 0.61 ± 0.05, on a scale from -3 to +3, p 0.000 vs neutral midpoint), beliefs that starchy food intakes should be reduced to achieve a healthier diet (mean -0.19 ± 0.05 on a scale from -3 to +3; p 0.000 vs midpoint 0), and the view that personal starchy food intakes did not need to be changed any further, because (depending on attitude) individual's intakes had already been increased or reduced. There were no differences in these perceptions with regard to age and gender, although the sample had a disproportionate number of females and a high proportion of higher income and social classes. However, there were few differences identified in relation to social class.

These findings indicate that consumers hold a number of confusions and misperceptions regarding current dietary recommendations and dietary roles of starchy foods, which may act as barriers towards changing dietary behaviour. Health promotion strategies aimed at increasing complex carbohydrate intakes should take these perceptions into consideration; however, further work is required to verify these findings and their foundations, and examine how these potential barriers could best be addressed in practice.

Funded by a Competitive Strategic Grant from Biotechnology & Biological Sciences Research Council.

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Sensory variety: effect on appetite, food intake and body weight in lean and overweight men. By A. M. JOHNSTONE, N. MAZLAN, S. E. MBAIWA, R. J. STUBBS and C. A. REID, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB*

Diet composition and behavioural factors such as physical activity patterns are believed to exert important influences on the current prevalence of overweight and obesity (Department of Health, 1995). However, both laboratory and dietary survey-type studies which have examined the effect of diet composition on energy balance (EB) have tended to ignore the relative importance of non-nutritional determinants of food and energy intake (EI). For instance, short-term studies (within-day) have indicated that increasing sensory variety leads to an increase in food intake (Rolls *et al.* 1980), but these studies do not address the effects of sensory variety on EB. The present study examined the effect of varying the sensory variety *per se* of nutritionally controlled diets on appetite, food intake (FI) and body weight in lean (L) and overweight (OW) men.

Six overweight men, mean age 39.8 (SD 7.3) years; weight 89.3 (SD 10.7) kg; height 1.8 (SD 0.1) m; BMI 28.2 (SD 1.2) kg/m^2 and five lean men, mean age 28.3 (SD 6.4) years; weight 72.0 (SD 6.5) kg; height 1.7 (SD 0.1) m; BMI 24.0 (SD 2.7) kg/m^2 were each studied three times during a 9 d protocol, whilst resident in the Human Nutrition Unit. On days 1-2, subjects consumed a medium fat (MF: 40% fat, 13% protein and 47% carbohydrate by energy) maintenance diet calculated at 1.5 x resting metabolic rate (RMR). On days 3-9 subjects had *ad libitum* access to isoenergetically dense MF foods (550 kJ/100 g) with every item the same macronutrient composition and energy density. Subjects had continuous *ad libitum* access to five, ten or fifteen food items on the low-variety (LV), medium-variety (MV) and high-variety (HV) treatments respectively. Subjective hunger was tracked hourly during waking hours. ANOVA was conducted on energy and nutrient intakes, subjective hunger and body weight, using diet and run as factors and subject as blocking factor.

Group	Low variety (LV)	Medium variety (MV)	High variety (HV)	F2,10	SED	Probability P =
L-food intake (kg)	2.13	2.33	2.63	11.16	106	0.005
OW-food intake (kg)	1.89	1.99	2.05	1.28	102	0.321
L-energy intake (MJ/d)	11.04	12.17	13.53	15.43	594	<0.01
OW-energy intake (MJ/d)	9.55	10.05	10.41	1.06	590	0.382

The Table above shows there was a significant graded increase in mean food and energy intake in L men as sensory variety increased. The same non-significant underlying trend was exhibited by the OW men. However, when EI was expressed in relation to expected energy requirements (ER) (at 1.5 x RMR; L, 11.00, OW, 12.12 MJ/d) it can be inferred that the OW men consistently under-ate relative to expected ER. The OW men may therefore have cognitively restrained their FI throughout the study. The lean men were more restrained than the OW men, measured by the DEBQ. This is supported by changes in body weight with an average total weight loss of 1.72 kg in the OW men, and a weight gain of 0.27 kg in the L men. There was no significant group or diet effect on subjectively rated hunger. These data suggest that non-nutritional determinants of FI can be quantitatively significant and therefore cannot be ignored in studies concerned with the influence of diet on appetite and energy balance.

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Differences in dietary intake and food selection between hospital patients and day-centre visitors: implications for supplementation. By M. LUMBERS[1], M.C. MURPHY[2], C.A.R. PITHER[2], M. H. CREEDON[2], M.W.J. OLDER[3] & S.A. NEW [2], [1] *Centre for Food and Health Care Management (Department of Management Studies) and [2]School of Biological Sciences, University of Surrey, Guildford GU2 5XH and [3]Department of Orthopaedic Surgery, Royal Surrey County Hospital, Guildford, Surrey,GU2 5XX*

Previous research has shown that hip fracture patients admitted with malnutrition have poorer outcomes compared with their normally nourished counterparts (Bastow et al, 1983). Supplementation with milk-based sip feeds has been found to improve clinical outcome in hip fracture patients (Driver 1994), although patient compliance was poor. Supplementation using foods preferred by sick elderly people may be more acceptable. The need to understand the change in eating habits and preferences of this group has been suggested by Allison (1995). The present abstract reports the findings of a pilot study of the food choice and dietary intake of fourteen hip fracture patients compared with fifteen healthy elderly females attending a day-centre. Patients agreeing to participate were selected subject to obtaining a score of ≥7 for the abbreviated mental function test. Comparison of baseline data showed that there were no significant differences between the hospital and day-centre subjects in age, body weight or lifestyle factors (such as living alone, recent bereavement, falling history). Four 24 h dietary recalls were recorded for both groups. Completed menu cards were used as memory prompts for the hospital patients.

	Hospital		Day-centre	
Nutrient	Mean	SD	Mean	SD
energy (kJ)	3650.9***	1437.1	6764.9***	1201.7
protein (g)	35.3***	10.8	67.7***	22.2
fat (g)	35.1***	11.6	62.6***	24.9
vitamin C (mg)	64.7	23.9	53.1	33.1
calcium (mg)	504.5*	181.8	749.4*	337.1
magnesium (mg)	141.0***	36.3	240.8***	55.5
iron (mg)	5.5***	2.3	9.4***	2.7
selenium (μg)	29.5**	11.3	47.4**	19.0
zinc (mg)	4.7***	1.3	7.3***	1.5

Mean value significantly different from day centre controls, * p<0.03, **p< 0.005, ***p< 0.001.

The table shows that compared with the day centre clients, significantly lower food intakes were recorded in the hospital patients which did not achieve the reference nutrient intakes (RNI) (Department of Health, 1991). Notable differences in food choice between the two groups were apparent e.g. fruit juice at breakfast was popular with hospital patients such that vitamin C intakes met the RNI. Of particular interest was that soup was a very popular choice; 50% choosing it for lunch and 70% for supper. It was also noted that cold puddings were popular with both groups. The implication for supplementation strategies is that soups and puddings appear to offer an alternative and more acceptable means of delivery than the traditional sip feeds.

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Measurement of nutritional intake and nutritional status of free living elderly : a pilot study. By N. SAINI, A.M. MILLER, S.M. MAXWELL, L. DUGDILL and A.F. HACKETT, *Centre for Education and Consumer Research, Liverpool John Moores University, IM Marsh Campus, Barkhill Road, Liverpool L17 6BD*

There is very little information concerning the eating habits of elderly people. A pilot study was undertaken to assess the respondent burden, ease of administration and participation, and feasibility of all the methods and procedures proposed to determine the nutritional intake and status of the subjects. The pilot study group consisted of twenty two women, mean age 73.6 (SD 6.04) years and eleven men, mean age 75.6 (SD 4.56) years. Food intake was estimated using a three day dietary diary quantified by food photographs and various calibrated utensils and analysed using Microdiet (Salford University).

Nutrient	Female (n 22)			Male (n 11)			Significance
	Mean	SD	(Range)	Mean	SD	(Range)	P =
Energy (MJ)	6.2	0.75	(4.9 - 7.7)	6.9	0.95	(5.4 - 8.6)	0.03
Carbohydrate (g)	183.3	27.07	(138.6 - 224.9)	213.0	60.39	(147 - 330.4)	0.06
(% energy)	46.8	7.17	(32.2 - 63.7)	47.7	9.09	(36.9 - 63.2)	0.76
Protein (g)	57.8	10.69	(33.4 - 73.6)	64.1	8.00	(42.9 - 73.6)	0.1
(% energy)	15.7	2.74	(10.2 - 19.3)	15.7	1.66	(13.4 - 18.2)	0.99
Fat (g)	59.9	15.65	(30.2 - 93.3)	64.3	21.35	(35.8 - 113.2)	0.51
(% energy)	36.4	7.3	(22.2 - 51.6)	34.9	9.5	(17.6 - 49.4)	0.62
(% energy, saturates)	13.3	3.55	(6.36 - 18.7)	11.9	4.13	(5.2 - 17.7)	0.36
Total sugars (g)	83.4	23.61	(31.8 - 125.7)	78.0	46.85	(25.6 - 158.3)	0.66
(% energy)	21.6	7.15	(7.2 - 38.9)	18.0	9.21	(4.8 - 32.9)	0.24
NSP (g)	9.5	3.2	(5.3 - 17.1)	10.6	3.9	(6.9 - 9.1)	0.43
Calcium (mg)	653.9	157.41	(458.3 - 976.0)	653.5	213.53	(272.1 - 987.3)	0.99
Iron (mg)	9.4	3.24	(5.7 - 16.7)	14.4	10.94	(6.4 - 44.4)	0.05
Vitamin D (μg)	6.5	9.13	(0.1 - 32.1)	7.8	8.86	(00.0 - 24.0)	0.8

Nutritional status was assessed using blood pressure (BP) and anthropometric measurements which comprised duplicate measurements of height, weight, demispan, mid arm circumference, triceps and biceps skinfold thickness (in triplicate).

Measurement	Female			Male			Significance
	Mean	SD	(Range)	Mean	SD	(Range)	P =
Height (m)	1.582	0.059	(1.485 - 1.704)	1.731	0.062	(1.601 - 1.811)	0.001
Weight (kg)	73.0	14.55	(52.9 - 156.6*)	78.8	15.73	(55.7 - 121)	0.39
Demispan (m)	0.733	0.029	(0.672 - 0.792)	0.811	0.029	(0.751 - 0.842)	0.001
Triceps (mm)	24.6	6.69	(11.1 - 35.33)	15.6	8.15	(6.2 - 33.3)	0.002
Biceps(mm)	15.2	7.04	(4.9 - 28.7)	8.3	4.74	(3.2 - 21.7)	0.006
BMI	31.1	9.84	(22.4 - 64.0*)	26.1	4.53	(21.8 - 38.5)	0.06
% Body fat	29.2	4.0	(21.4 - 35.3)	14.7	6.0	(4.1 - 26.0)	0.000
Systolic BP (mmHg)	158	27.04	(113 - 231)	163	32.40	(130 - 211)	0.67
Diastolic BP (mmHg)	83	8.63	(70 - 95)	80	10.93	(67 - 95)	0.44

The results showed that the mean energy intake for both men and women was low but the prevalence of overweight and obesity was high. As a group there was no evidence of under-reporting of dietary intake (energy intake / BMR = 1.47) (Macdiarmid & Blundell, 1997). Technical error for all measurements was recorded and showed no systematic bias with a reliability of 0.95 and above, although difficulties were encountered whilst measuring height. The pilot study demonstrated feasibility of methods and was invaluable not only for its practical and instructional role but also towards highlighting areas for concern regarding the diet and nutritional status of elderly people for the main survey.

* Extreme values, obtained from one respondent.

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Changes in dietary intake associated with marriage or cohabitation. By ANNIE S. ANDERSON[1], DEBBIE KEMMER[1] D.W. MARSHALL[2] and SUSAN ELEY[1], *[1]Department of Human Nutrition, University of Glasgow, Alexandra Parade, Glasgow G31 2ER, [2]Department of Business Studies, University of Edinburgh, 50 George Square, Edinburgh EH8 9JY.*

Marriage is still a central institution in British society; over 70% of people eventually marry and many of the remainder cohabit (OPCS, 1996). Little, however, is known about alterations in dietary intake during the transition from single to married status in a period associated with lifestyle change. The aims of the current study were to investigate the effect of marriage or cohabitation on food purchases, eating patterns and nutrient intake.

Couples who had not previously lived together, were recruited from the central belt of Scotland. Each partner was seen individually approximately 3 months before and then again 3 months after the date of marriage or cohabitation. On each occasion, informants participated in a semi-structured interview, a 7 day weighed diet survey, a questionnaire study on food shopping, and anthropometric measurements.

Twenty couples completed diet diaries on both occasions which were then analysed using the Food meter barcode system (Anderson *et al*, 1993) with nutrient analysis data from Holland *et al* (1991) and supplements. Pre- and post-marriage or cohabitation comparisons of nutrient intake were made using paired *t* tests.

Like the majority of the Scottish population, this group of informants (on both occasions) had a mean, percentage of energy from carbohydrate and intake of NSP lower and percentage energy from fat higher than currently recommended (Department of Health, 1991). Intakes of vitamins and minerals were adequate in men but in women, intakes of folic acid and Fe were less than the current reference nutrient intakes. Comparing pre- and post-marriage nutrient intakes, intakes of vitamin C were significantly increased in men but no changes were detected in nutrient intake in women.

		Men					Women				
		Pre-marriage		Post-marriage			Pre-marriage		Post-marriage		
		Mean	SE	Mean	SE	*P*	Mean	SE	Mean	SE	*P*
Energy	(kJ)	8630	39.7	8910	394	NS	7375	286	7414	283	NS
Fat	(% energy)	37.6	1.0	37.3	1.1	NS	40.1	0.9	38.5	0.7	NS
Carbohydrate	(% energy)	43.5	0.9	43.3	1.6	NS	42.7	1.4	42.7	1.3	NS
NSP	(g)	11.7	1.0	12.4	0.7	NS	11.3	0.9	10.8	0.8	NS
Folic acid	(mg)	201	15.0	225	16.6	NS	180	12.9	180	12.5	NS
Vitamin C	(mg)	49.0	4.5	78.0	10.7	0.003	58.1	6.7	64.2	8.5	NS
Iron	(mg)	10.8	0.6	11.1	0.6	NS	10.1	0.7	9.8	0.6	NS

Despite the reported low intakes of energy, body weight increased slightly in the group of women studied between pre- and post-marriage periods from 58.3 (SE 1.7) kg to 59.8 (SE 1.8) kg ($P<0.001$) and in men from 76.7 (SE 2.8) kg to 78.4 (SE 2.9) kg ($P<0.02$)

These findings suggest that dietary changes do occur at around the time of marriage and these appear to be more marked in men than women.

Funded by The ESRC Nation's Diet Programme: the social science of food choice (grant L209 252043)

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Dietary changes amongst overseas students in Oxford. By S.L. REEVES and C.J.K. HENRY, *School of Biological and Molecular Sciences. Oxford Brookes University, Headington, Oxford OX3 0BP*

Communities from the developing world consume foods of low energy density. Their diets are characterized by high-carbohydrate, high-fibre, low-fat foods (Yang & Read, 1996) e.g. cooked rice and noodles which have an average energy density of 5.4 kJ/g. In contrast most Western foods are high in energy density e.g. roast potatoes and chips which have an energy density of 15 kJ/g. Our primary interest was to examine how subjects modulate their food intake and energy balance when they move from a low-energy-density food intake pattern to one of high energy density. The pattern of food selection, food intake and anthropometric changes in newly arrived overseas students formed the basis of the present study.

Fifty three females and fifty six males of average age 22 (SD 3.19) years were recruited. All subjects were from Malaysia and were studying at Oxford Brookes University. The following anthropometric measurements were made; weight, height, skinfolds, waist, hip, chest, thigh and mid-arm circumference. Additionally BMR was measured in sixteen individuals. All measurements were repeated after 3 and 6 months of residence in Oxford. Within 1 week of arrival the subjects were asked to complete a 3 d dietary recall diary and a food frequency questionnaire (FFQ) of foods consumed in Malaysia. At the end of 3- and 6-month periods the subjects were asked to complete 3 d food diaries and repeat the FFQ for foods consumed in Oxford. The Table shows the differences in body weight and BMR, and the energy density and macro-nutrient composition of the diets consumed by the overseas students in Malaysia and in Oxford after 3 and 6 months.

	Malaysia		Oxford 3 months		Oxford 6 months	
	Mean	SD	Mean	SD	Mean	SD
Body weight (kg)						
Males	64.68	11.14	63.91	10.77	59.48	6.18
Females	51.56	7.89	52.71	8.09	52.36	4.86
BMR (kJ/24 h):						
Males	6310	774	7091	948	5704	773
Females	5112	494	5268	804	4546	762
Energy Density(kJ/g)	4.31	1.21	6.19	1.84	5.73*	1.92
Energy(kJ)	5865	1883	6791	1548	6615	1578
Protein(g)	82.876	33.44	61.28	2.45	60.427*	22.00
Fat(g)	45.466	36.98	72.29	37.53	63.99	23.00
Carbohydrate(g)	185.91	59.52	211.03	63.79	204.88	62.36

* Mean values were significantly different from those for Malaysian diet, $P<0.05$

A significant difference was found between the energy density of the food consumed in Malaysia and Oxford after 3 and 6 months. There was also a significant drop in the amount of protein consumed. Results from the FFQ showed that there was a decrease in the consumption of meat, fish, rice, noodles, fruit and vegetables and an increase in the consumption of sausages, burgers, bread, cereal, tea and coffee. No significant differences were found in the BMR and anthropometric measurements. It appears that Malaysian students are able to remain in energy balance and are weight stable at least during the first 6 months of residence despite the choice of energy dense food available to them in the UK. This suggests at least for the short term subjects are able to modulate their food intake in response to changes in the energy densities of food.

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Nutritional implications of secondary school pupils' lunchtime meal choices. By CAROL NOBLE[1], ANITA EVES[2], MICHAEL CORNEY[2], MICHAEL KIPPS[2] and MARGARET LUMBERS[2], [1]*Department of Applied Technology and Computing, Roehampton Institute, London SW15 3SN*; [2]*Department of Management Studies, University of Surrey, Guildford GU2 5XH*;

Previous research has shown that school meals contribute significantly to the fat intake of children (Ministry of Agriculture, Fisheries and Food, 1996), especially meals from the cash-cafeteria style of service operated by many secondary schools (Noble & Kipps, 1994). As this meal may constitute the main meal of the day for some children its nutritional quality is of importance. Secondary-school pupils (*n* 185), aged 14-15 years, were asked to select the meal that they would choose from a range of photographs of meal items that they might typically encounter in their school canteen. They were asked to explain their choices. From the same range of photographs, they were then asked to construct the meal that they perceived to be the most healthy and to explain why. The nutritional composition of the meals was determined using a computer programme based on *McCance and Widdowson's The Composition of Foods* (Holland *et al.* 1991) using portion sizes provided by the caterer.

Meal item	% pupils selecting for...	
	Meal of choice	'Healthy' meal
Can of drink	50	1
Chips	45	2
Sandwich (salad)	28	31
Pizza	26	1
Crisps	26	1
Beefburger in bun	20	14
Milk	17	68
Mini cookie	16	1
Iced bun	15	1
Fresh fruit	14	75
Salad items	10	59
Jacket potato with tuna	10	21
Sausages	8	2
Apple crumble and custard	6	2
Chicken fricasse with rice	4	1
Mixed vegetables	3	24
Mashed potato	2	8
Sliced ham	1	6
Quiche	1	24

The Table shows that the items appearing most frequently in the meal of choice were snack items, the main reason why pupils selected these foods being liking of the items. Taste, healthiness and 'quick and easy to eat' were also important. The items appearing most frequently in the 'healthy' meal were fruit, salad and vegetables and milk, and the main reason why pupils thought these to be healthy were the presence of vitamins and the absence of fat. Nutritional analysis of the two meals showed the meal of choice to contain 41.2% energy from fat, which is close to the figure of 42.8% reported by the Ministry of Agriculture, Fisheries and Food (1996). The meal perceived to be most healthy contained significantly ($P<0.05$) more fat as a proportion of energy (47.7%), owing to the selection of salad items in mayonnaise, and a larger proportion of respondents selecting quiche. Results indicated that most 14-15-year-old pupils were aware of factors that made a meal more healthy, but that 'healthiness' is not the main criterion for meal selection for most pupils. In addition, a number of pupils were apparently not aware of the sources of fat in the diet, particularly in relation to composite foods.

The authors gratefully acknowledge funding by the Ministry of Agriculture, Fisheries and Food.

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The effect of television advertising on the food choices of children from different social backgrounds. By ELIZABETH HITCHINGS and PAULA J. MOYNIHAN, *The Dental School, University of Newcastle, Newcastle upon Tyne, NE2 4BW*

Identifying effective dietary intervention methods requires information on the factors which influence food choice. One factor which may influence the food choices of children, and subsequently foods brought into the home, is television advertising. There are, however, few data to support this from studies carried out in the UK (Donkin *et al.* 1992) and there is no information from children of different social class backgrounds. In view of this the present study aimed to investigate whether foods remembered from television advertisements were consumed by children and bought by parents on request in children aged 9-10 years from different social class backgrounds.

Forty-one children aged 9-10 years of both sexes were recruited from schools in Newcastle upon Tyne. Seventeen children were from private schools and twenty-seven were from state schools in moderately deprived areas. Each child was interviewed to identify which food advertisements they remembered seeing on television recently. No visual prompts were used in order to mimic the situation when a child asks for particular food without seeing the product first as a visual memory aid. A 3 d food diary was completed by each child to obtain information on foods eaten. The relationship between the number of advertisements remembered (unprompted) and the number of these foods which were eaten was investigated using Spearman's rank correlation. Parents were interviewed to obtain information on foods which the child had recently requested them to buy. The ten most requested foods and the ten foods most frequently recalled from advertisements were compared for foods in common. The results of the correlation analysis, between food advertisements remembered and food consumed, are presented in the table.

Food group	Private schools		State schools	
	Correlation coefficient	95% confidence interval	Correlation coefficient	95% confidence interval
Breakfast cereal	0.61	0.30, 0.80	0.38	0.13, 0.73
Confectionery	0.56	0.23, 0.78	0.59	0.15, 0.84
Puddings	0.46	0.10, 0.71	0.50	0.02, 0.79
Soft drinks	0.65	0.36, 0.83	0.61	0.12, 0.84
Crisps/savoury snacks	0.59	0.27, 0.79	0.78	0.48, 0.92
Cakes and biscuits	0.55	0.11, 0.77	0.58	0.13, 0.83
Chips	0.17	0.22, 0.52	0.42	-0.08, 0.75
All foods	0.67	0.39, 0.84	0.62	0.19, 0.85

The Table shows that relationships existed between food advertisements remembered and foods eaten. Children from schools in moderately deprived areas consumed fewer foods for which they could remember the advertisement ($P=0.034$, Mann-Whitney test) than children from private schools. Four out of ten of the most frequently recalled advertisements were amongst the ten foods that the children most frequently requested the parents to buy, and included crisps, sugared cereals and chips.

These data suggest that the foods children consume may be influenced by television advertising since not only were foods remembered from advertisements consumed, but they were also requested for purchase. The association seems to be stronger in children from private schools.

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A study of food habits and nutritional status among rural Javanese pregnant women. By KATRINA M MARTIN[1], JANE EARLAND[1] and BAMBANG SUPRAPTO[2], *[1]Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Herries Road, Sheffield S5 7AY and [2]Department of Nutrition, Sebelas Maret University, Jl.Ir. Sutami 36A, Surakarta, Indonesia*

Indonesia has one of the most rapidly developing economies in the South East Asia region and yet protein energy malnutrition and nutrient deficiency diseases are common in many areas. Pregnant women are of particular concern as poor nutritional status in this group may adversely affect pregnancy outcome. The aim of the present study was to investigate the dietary habits and food beliefs of a group of pregnant women from the sub-district of Polokarto in Central Java, Indonesia.

Staff from Polokarto health centre selected two villages which they considered were the most traditional in the area and therefore more representitive of other rural areas. From the lists of pregnant women held by the village midwives, forty were randomly chosen to take part in the study. The sample size was later increased to fifty seven using random sampling. Information was collected on dietary habits and food intakes using a semi-structured questionnaire and a 24 h dietary recall. Interviews were carried out using a translator. Nutritional status was also assessed through the measurement of height and weight and plotting the results according to gestational age on the Indonesian pregnancy chart. It should be borne in mind that the assessment of gestational age is more difficult in developing countries.

The 24 h dietary recalls of the subjects proved to be remarkably homogeneous and, according to the respondents and the translator who was from the same area, represented their usual daily dietary patterns. The results showed that the diet consumed by the sample was of good quality in terms of nutrient density but lacking in quantity. Energy intakes of the majority of the sample (86%) were well below the Indonesian recommended daily intake (RDI) of 10.19 MJ (Indonesian Institute for Sciences, 1994). However, as the women in this particular area are not involved in heavy agricultural work the RDI may be too high. The contributions of the macronutrients to total energy intakes were in line with both the Indonesian and WHO recommendations (World Health Organization, 1990) with 23% energy being supplied by fats and 62% carboydrates. A lack of regular meals was, however, identified as an area of concern, with 28% of the sample having 6 h or more between meals. According to the anthropometric data, the majority of women were between 90 and 109% of their reference weight-for-height, with only 5% of the sample being below the 90% cut-off point. The anthropometric results were similiar to those found in pregnant women in East Java by Kusin (1976)

With respect to food habits during pregnancy, 73.7% of the sample believed that food consumed during pregnancy has an effect on the baby being carried and 70.2% of those interviewed identified at least one dietary change made since becoming pregnant. The most common change was to increase food intake. The most common foods that the women had cravings for were vegetables, in particular amaranth, cassava leaf and jackfruit, fruits such as oranges and apples and meatball soup ('bakso'). Foods felt to be particularly beneficial for the health of the baby included vegetables, coconut water, milk, eggs and fruit. With the exception of milk, which is expensive, these foods were all prominent in the diets of the sample. Several food taboos were identified. The most common ones were ice (42%), pineapple (32%), sugar cane (11%) and cucumber (9%). The most common reasons for avoiding a food were that it would make the infant large, resulting in a difficult delivery (ice and cucumber) or that the infant would get a skin disease (pineapple).

In conclusion, pregnant women from this area and other similar areas of Central Java would probably benefit from increasing the quantities of the naturally healthy foods which they presently consume. One way of doing this would be to eat more frequently. There are no obvious barriers to women increasing their food intake such as working away from the house or lack of income, providing that the importance of the diet is emphasized by health staff. Although dietary beliefs are common during pregnancy, none of those identified in this study was harmful to the mother or child.

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Adequacy of dietary energy and nutrient intakes among rural elderly Malays. By SUZANA SHAHAR [1], JANE EARLAND [1] and SURIAH A. RAHMAN [2], *[1]Centre For Human Nutrition, University of Sheffield, Herries Road, Sheffield S5 7AU; [2]Department of Food Science and Nutrition, Universiti Kebangsaan Malaysia, Malaysia*

Nutrition plays an important role in the quality of life of elderly people as it has long been recognized that intakes of energy and nutrients reduce the occurrence of disease and disability. The importance of early recognition of suboptimal intake as well as the obvious lack of data on nutrient intakes in the elderly in developing countries, prompted us to investigate the adequacy of the dietary intakes of a sample of apparently healthy rural elderly Malays aged 60 y and above. This work was carried out as part of a larger nutritional and health status survey of elderly people in the district of Mersing, on the East coast of Malaysia. Usual dietary habits and nutrient intakes of 337 subjects (67% of the eligible sample) were obtained using a specific dietary questionnaires consisting of a precoded 7 d dietary history with a qualitative food-frequency check list. Local food photographs and familiar household measurements were used to estimate quantities. Before the actual survey, the dietary questionnaires were validated against a seven-day weighed dietary record among a subsample of fifty elderly people. Nutrient intakes were calculated using UK Foodbase software supplemented by 180 Malaysian food.

The diet was based on rice with a small portion of fish and vegetables. Quantitatively, the energy and nutrient intakes of the elderly men studied were more favourable than those of the women as presented in the Table. However after expressing the intakes in relation to body weight for energy and protein, and nutrient densities for other nutrients, there were no differences between sexes. It was found that the mean estimated intakes of energy and almost all investigated nutrients (vitamin A, mainly from *B*-Carotene, thiamin, riboflavin, niacin, Fe and Ca) were 55 - 90% of the Malaysian recommended daily allowances (RDA) (Tee *et al.* 1988), with exceptions for protein and vitamin C. The proportion of subjects with an estimated intake of less than two-thirds of the RDA was the highest for vitamin A, thiamin, and riboflavin. Despite the inadequacy of dietary intakes, the contribution of macronutrients to total food energy were closer to the World Health Organization (1990) guidelines, as more energy was derived from carbohydrates (61%), and less from fat (25%) compared with elderly people in developed countries (Horwath, 1989). In conclusion, intakes of foods which are rich in micronutrients need to be increased in this population. However, before changes can be recommended the factors that influence food choice and intakes among this special group of elderly people need to be investigated and appropriately addressed.

Energy and nutrient intakes (per d)	Men (*n* 164)		Women (*n* 173)		Proportion of subjects with intake below 2/3 RDA‡	
	Mean	SD	Mean	SD	*n*	%
Energy (MJ)	6.36	2.08	5.52***	1.92	126	37.4
Energy (MJ/kg BW)	0.12	0.04	0.12	0.05	-	-
Protein (g)	54.97	19.53	47.95**	19.00	42	12.5
Protein (g/kg BW)	1.02	0.43	1.02	0.37	-	-
Vitamin A (μgRE) †	490.44	359.98	514.92*	467.24	213	63.2
Thiamin (mg)	0.46	0.22	0.41*	0.18	255	75.7
Riboflavin (mg)	0.75	0.37	0.64	0.36	243	72.1
Vitamin C (mg) †	48.79	36.65	40.30	30.91	94	27.9
Niacin (mg)	9.82	4.03	8.13***	3.40	179	53.1
Iron (mg) †	9.13	4.80	7.68	3.29	111	32.9
Calcium (mg) †	334.32	228.00	317.72	212.37	188	55.8

BW, body weight; RE, retinol equivalents.
Mean values were significantly different from those for men: *$P<0.05$, **$P<0.005$, ***$P<0.0001$.
†Log normal transformation before analysis.
‡Compared to the Malaysian RDA for elderly men and women aged 60 and above (moderately active).

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Dietary assessment of patients undergoing coronary artery bypass grafting. By D. MASON[1], C.J.K. HENRY[1] and R. PILLAI[2], *[1]School of Biological & Molecular Sciences, Oxford Brookes University, Headington, Oxford OX3 0BP, [2]Department of Cardiothoracic Surgery, John Radcliffe Hospital, Headington, Oxford OX3 9DU*

Coronary artery bypass grafting (CABG) is a common surgical intervention for CHD in the later stages of progressive atherosclerosis. Although CABG is widely used as an intervention strategy, it is a paradox that a high percentage of patients show regression and have to seek further medical treatment (Corbelli *et al.* 1985). This is due to the successive build-up of atheroma in the new graft, or other coronary arteries. Given the well-known relationship between diet and CHD incidence, it is of interest to investigate whether patients undergoing heart bypass surgery change their dietary patterns in light of the dietary advice given to them either pre- or post-operatively.

In this preliminary study, nineteen CHD patients admitted to the cardiothoracic ward to undergo CABG surgery were recruited. They were asked to complete a food amount frequency questionnaire (FAQ) based on their current dietary habits. The FAQ had been previously validated against 4-day estimated food records (Mason and Henry, unpublished results). The patients also completed the FAQ 6-7 weeks post-operatively, once they had resumed normal eating practices. Furthermore, patients were asked to indicate whether they were given any dietary advice, and whether this had subsequently influenced their post-operative dietary habits. Comparison of FAQ responses pre- and post-operatively are presented in the Table below.

Nutrient	Pre-op FAQ		Post-op FAQ		Difference (% pre-op)†	
	Mean	SE	Mean	SE	Mean	SE
Energy (kJ)	7444	449.3	6729	392.5	7.6	4.97
Protein (g)	70.6	3.95	68.2	4.30	1.5	5.77
Carbohydrate (g)	219.9	14.51	199.6	11.97	7.0	4.59
Total fat (g)	65.6	5.38	55.9*	4.78	11.4	6.52
Saturated fat (g)	23.6	2.15	20.4	2.31	9.8	8.35
Polyunsaturated fat (g)	12.4	1.45	9.7*	0.93	11.5	7.59
Cholesterol (mg)	193.2	14.84	178.4	15.26	2.2	9.73

*Mean values were significantly different from pre-operative $P<0.05$ (Student's paired t test).
†As percentage of Pre-op FAQ.

The results show a clear reduction ($P< 0.05$) in dietary intake of total fat post-operatively. This reduction in total fat intake seemed to be more weighted towards reduction of polyunsaturated fatty acids ($P< 0.05$). Mean intake of saturated fat was not significantly reduced, and there were no changes in intake of any other macronutrient.

Of patients 89% reported having received dietary advice either pre- or post-operatively, either from their GP (38%) or from a dietitian (62%); 69% of subjects claimed that the dietary advice had positively influenced their eating habits post-operatively. The results of this study suggest that CABG patients either do not clearly understand the dietary advice they are given, or they do not want to change their life-long eating habits. Post-operative dietary follow-up of CABG patients is an important area that requires further investigation.

We thank Dr G. Hardy of Oxford Nutrition Ltd for his financial and academic support.

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Multiple dietary goals as set by the Scottish Office may not be achievable. By SANDRA DRUMMOND and TERRY R. KIRK, Centre for Food Research, Queen Margaret College, Clerwood Terrace, Edinburgh EH12 8TS

Two of the main dietary goals set by the Scottish Office Home and Health Department (1993) are to reduce the percentage energy from fat in the diet from its present level of 40.7% to less than 35% and to reduce the percentage energy from sugar from 16.6% to less than 10%. Lower fat intakes may help prevent CHD, and encourage a reduction in body weight while lower sugar intakes may help reduce the incidence of dental caries. Yet it is clear from epidemiological studies that sugar and fat acre reciprocally related (McColl 1988) suggesting that concurrent reductions may not be achievable in practie. The present study aimed to test this premise in an intervention trial.

Thirty-one healthy men (mean age: 46 years, mean BMI: 28.8kg/m^2) were randomly allocated to one of three groups; a control group (C) which received no nutritional advice, a low fat, low sugar group (LFLS), which received advice on how to reduce both fat and sugar, and a low fat, *ad libitum* sugar group (LF) which received advice on how to reduce dietary fat only. The dietary advice was given at 2-week intervals for 6 weeks. Food diaries (4 d) were used at 2, 4 and 6 weeks to monitor compliance. Results are given in the Table below.

	Baseline		6 Weeks			Baseline		6 Weeks	
	Mean	SE	Mean	SE		Mean	SE	Mean	SE
FAT (% energy)					SUGAR (% energy)				
Control (n 10)	41.4	0.95	40.6	1.81	Control (n 10)	15.8	1.15	18.1	3.17
LFLS (n 11)	40.1	1.66	32.9**	1.33	LFLS (n 11)	16.2	1.08	17.7	1.58
LF (n 10)	39.2	1.13	31.6*	2.07	LF (n 10)	16.4	1.45	19.9	2.46
CHO (% energy)									
Control (n 10)	42.4	1.6	43.7	2.3					
LFLS (n 11)	44.1	1.5	47.3	1.1					
LF (n 10)	44.1	1.6	49.1	2.7					
FAT (g)					SUGAR (g)				
Control (n 10)	109.6	7.7	104.5	6.3	Control (n 10)	157.5	52.5	120.5	26.3
LFLS (n 11)	111.2	8.3	70.9**	6.4	LFLS (n 11)	107.3	8.8	88.4*	8.6
LF (n 10)	106.6	10.2	69.3**	6.2	LF (n 10)	107.7	12.3	106.3	15.0

Mean values were significantly different from baseline: *$P < 0.05$, **$P < 0.01$ (2 -tail t-test, using SPSS).

It can be seen that both LFLS and LF groups were successful in reducing the percentage energy from fat. In the case of sugar there was no change in the percentage energy from total sugar in the LF group but no significant reduction in the LFLS group despite advice to the contrary. Our data suggest that in this group of middle-aged men the target for dietary fat was achieved at the expense of the target for sugar. This supports the epidemiological picture and may indicate that concurrent reduction in fat and sugar are not achievable.

Supported by The Sugar Bureau.

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Seasonal fruit consumption in relation to the development of, or death from, cardiovascular disease. By BRIAN D. COX and MARGARET J. WHICHELOW, *Department of Community Medicine, Institute of Public Health, Robinson Way, Cambridge CB2 2SR*

It is accepted that fresh fruit and vegetable consumption can be protective against cardiovascular disease (CVD). Recently Powles *et al* (1996) have postulated that the year-round consumption is important, as in countries where there is a seasonal scarcity of these foods CVD mortality rates are high.

The longitudinal Health and Lifestyle Survey (Cox *et al.* 1993) is unique in having data on the seasonality of fruit consumption in British adults. The respondents were seen in 1984-5 (HALS1) and the survivors again in 1991-2 (HALS2). Mortality data were also collected. At HALS1 the frequency of consumption of fresh fruit in summer and winter was recorded, and condensed as 'infrequent' (less than once weekly), 'moderate' (1-6 times per week) and 'frequent' (daily). Fresh fruit was eaten daily in summer by 58%, but by only 39% in winter. Of the 3052 respondents aged 35-75 years, free of CVD, hypertension and diabetes at HALS1, there were 197 live cases by HALS2 and 136 deaths from CVD. The relative risks, age adjusted, of CVD morbidity or mortality in relation to summer and winter fruit consumption are shown in the Table, with each variable adjusted for all the others.

		RR	95% CI	*P*	*n*
Fruit consumption					
Winter	Summer				
Frequent	Frequent	1	-	-	1318
Moderate	Frequent	1.30	0.74,2.28	NS	437
Infrequent	Frequent	1.35	0.73,2.50	NS	335
Infrequent	Mod/Infrequent	1.88	1.23,2.87	0.003	962
Sex					
Women		1	-	-	1718
Men		1.65	0.93,2.95	NS	1334
Smoking					
Non-smoker		1	-	-	1096
Smoker		1.64	1.09,2.46	0.018	1073
Ex-smoker		0.87	0.53,1.42	NS	883

The most significant component was age ($P<0.001$), with smoking status also important. The relative risk of developing CVD was higher in those who did not habitually eat fruit every day, and significantly so in those whose winter and summer consumption was less than frequent. The lack of an increase in CVD risk for ex-smokers reflects the high proportion who were excluded (30%) from the analyses because of pre-existing disease compared with non-smokers (22%).

These findings indicate the importance of the seasonality of fresh fruit in dietary assessments in relation to CVD morbidity and mortality.

Acknowledgement: MJW was supported by a grant from the Anglia and Oxford Regional Health Authority

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Dietary intake of a British African Caribbean population sample by age and place of birth: coronary risk changing over time? By S. SHARMA, J. CADE and K. CRUICKSHANK, *Clinical Epidemiology Unit, Manchester University Medical School, Manchester M13 9PT*

CHD rates in Britain for Caribbean-born people are up to 50% lower than British averages. Nutrient intake is a major contributor to coronary risk but has rarely been studied in this population. Here we report nutrient intake data for this group in relation to place of birth and age.

A food-frequency questionnaire developed specifically for this group was interview-administered to subjects aged 25-79 years randomly selected from population registers in Manchester (Sharma *et al.* 1996).

A total of 210 subjects (eighty-five men; 125 women; mean age 57.1 and 51.2 years respectively) completed the questionnaire (response 83%).

	Caribbean born Men (*n* 71)		England born Men (*n* 12)		Caribbean born Women (*n* 95)		England born Women (*n* 23)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	59	9.2	30	2.4	54	11.0	28	2.3
BMI	27.1	3.2	26.2	3.0	29.1	4.4	25.5	6.8
% Energy as fat	31.5	4.9	34.6	5.5	31.8	5.1	35.3	4.7
% Energy as carbohydrate	52.9	5.4	50.7	7.2	53.0	5.7	50.4	5.4
West Indian foods eaten (d/week)	4.4	2.6	2.9	2.3	3.7	2.6	2.6	2.1

The Table shows that younger subjects born in England consumed a diet higher in fat and lower in carbohydrate than those born in the Caribbean although the diet was still lower in fat than that of the white population (37.6% energy men, 39.5% energy women; Gregory *et al.* 1990). The difference in BMI is probably a reflection of physical activity due to age differences.

The oldest age group of African-Caribbeans (65-79 years, *n* 31) and those aged 55-64 years (*n* 72) had 31.2% (SD 5.3) and 32.5% (SD 5.2) of total energy from fat respectively, the youngest subjects aged 25-34 years (*n* 43) had 35% (SD 4.9) reflecting a lower frequency of traditional Caribbean food consumption in this group.

This report shows that older African Caribbeans are consuming a diet in line with dietary recommendations that no more than 35% of food energy should be provided by fat and this lower fat diet could be protective against CHD. However younger, UK-born subjects are adopting a more European type diet higher in fat and lower in carbohydrate and this could result in a change in CHD risk.

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Biochemical indicators of nutritional status in elderly patients with a fractured neck of femur: comparison with age-matched controls. By M.C. MURPHY[1], S.A. NEW[1], L.S. PATEL[1], C.A.R. PITHER[1], M.H. CREEDON[1], M.W.J. OLDER[2] and M. LUMBERS[3], [1]*Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH,* [2]*Department of Orthopaedic Surgery, Royal Surrey County Hospital, Guildford GU2 5XX,* [3]*Department of Management Studies, University of Surrey, Guildford GU2 5XH*

The incidence of a fractured neck of femur (FNF) has been estimated in the region of 50 000 cases /year in the UK and incidence is predicted to increase considerably. The link between malnutrition in the elderly as a risk factor for falls has been highlighted as an area which needs further investigation. There have been many reports of poor antioxidant status in this group including a recent report by Schmuck *et al.* (1996). In the present study, nutritional status was assessed by the measurement of plasma albumin, cholesterol, vitamin C, Se and Zn levels, and erythrocyte Se-dependent glutathione peroxidase activity. Female volunteers were recruited into three groups: (1) elderly patients with an emergency admission for a FNF (FNF patients) (*n* 12), (2) healthy elderly volunteers from a Day Centre (Controls) (*n* 9) and (3) elderly patients recovering from a FNF in a convalescent hospital (Convalescing) (*n* 8). A non-fasting blood sample was taken, 4 d post-operatively for the FNF patients, plasma was separated and stored at -20° until analysis. Plasma for vitamin C determination was treated with 10 % TCA and frozen at -70° until analysis according to Omaye *et al.* (1979). Plasma albumin and cholesterol levels were assayed spectrophotometrically using kits for the Cobas Mira clinical analyser (Roche Diagnostics). Se and Zn analyses were carried out by atomic absorption spectroscopy and haemolysate glutathione peroxidase activity according to St Clair & Chow (1996). The results (means and SD) are given in the table:

	Group 1 (*n* 12) FNF patients		Group 2 (*n* 9) Control		Group 3 (*n* 8) Convalescing	
Variable	Mean	SD	Mean	SD	Mean	SD
Age (years)	77.4	8.7	73.3	8.1	84.5*	4.0
Demiquet (kg/m^2)	121.0	20.3	132.4	20.4	-	-
Albumin (g/l)	39.3**	3.4	43.1	2.8	48.1	8.4
Cholesterol (mmol/l)	4.97	1.37	5.46	0.77	5.30	0.94
Vitamin C (mmol/l)	220****	56	113	80	95	63
Selenium (μmol/l)	0.74	0.27	0.77	0.23	0.73	0.23
Zinc (μmol/l)	16.0	3.6	14.4	0.9	13.8	2.4
Glutathione peroxidase (U /g Hb)	27.15	7.33	42.35***	10.47	25.46	8.79

Mean values were significantly different from other groups * $P < 0.05$, ** P=0.01, *** P<0.005,**** P=0.001.

There was no significant differences in the age or demiquet between groups 1 and 2 but the group who went on to a convalescent home were significantly older than both of the other groups. Plasma albumin levels were significantly lower in the FNF group compared with controls (P=0.01), but were not low in the convalescing patients despite the observation that this group were extremely thin. In contrast, plasma vitamin C levels were significantly higher in the FNF patients than both other groups (P<0.005). There were no significant differences in the Se and Zn levels, however, mean Se values of all groups were below the reference range (Verlinden *et al.* 1983) and glutathione peroxidase activity was also low in both patient groups (P=0.001). This study confirms others showing low albumin status in elderly FNF patients. The high plasma vitamin C levels in the FNF group are thought to be the due to the orange juice on the hospital breakfast menu and highlight the fact that plasma vitamin C levels reflect very recent intake. These findings highlight the need for more suitable biochemical indicators of undernutrition in elderly patients.

Acknowledgements go to Dr Taylor, Mrs Wilson, the nursing staff at RSCH & Milford Hospital and to the staff at Moorcroft.

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The effect of dietary restriction and surgical trauma on body composition and energy expenditure in the rat. By A.R. BOSAGH ZADEH and P.W. EMERY, *Department of Nutrition and Dietetics, King's College London, Campden Hill Road, London W8 7AH*

Metabolic rate increases after surgery, whereas the normal adaptive response to underfeeding is a reduction in metabolic rate. The aim of the present study was to determine whether prior undernutrition would suppress the increase in energy expenditure after surgery. Twenty-four female Sprague-Dawley rats were fed *ad libitum* for 3 weeks on a semipurified diet containing 200 g protein/Kg while a further twenty rats were fed on a diet containing 30 g protein/Kg in amounts restricted to 50% of the mean intake of the *ad libitum*-fed rats. Four rats from each group were then killed for measurement of initial carcass composition. Half the remaining rats in each dietary group then underwent hysterectomy under halothane anaesthesia. A 70 mm mid-line incision was made into the peritoneum and the uterus was removed but the ovaries were left intact. The muscle layer was sutured and the skin closed with stainless steel clips. The whole procedure lasted approximately 20 min. The same dietary treatments continued after surgery. The non-operated controls were individually pair-fed with the surgical rats. 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. Results for the surgically treated rats were compared with the corresponding control groups using paired *t* tests.

	Ad Libitum				Restricted			
	Surgical		Control		Surgical		Control	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body water (g)	137.7**	3.6	130.1	3.7	83.6	1.2	83.1	0.9
Body protein (g)	38.1	1.3	38.3	1.2	24.8*	0.4	25.7	0.3
Body fat (g)	15.3**	0.8	20.4	1.5	2.4	0.2	3.6	0.6
Energy expenditure (KJ/4d)	884**	38	663	36	355**	8	291	15

Mean values were significantly different from corresponding controls: * P<0.05, ** P<0.01.

Surgery caused significant fluid retention in the *ad libitum*-fed rats but not in the restricted rats. On the other hand significant loss of body protein was only observed in the malnourished surgical rats. Prior malnutrition appeared to prevent the loss of body fat following surgery, but this may have been because the malnourished rats had already lost virtually all their fat. Surgery caused a 50% decrease in food intake in the *ad libitum*-fed rats but a 33% increase in energy expenditure compared with the pair-fed controls. In the malnourished rats energy expenditure was also significantly greater in the surgical rats than in the pair fed controls, although the magnitude of the increase was somewhat smaller (22%). Thus prior undernutrition does not prevent hypermetabolism following surgery, so that an increased energy requirement is likely to contribute to the continued wasting of depleted patients after surgery.

Financial support from the National Iranian Oil Company is gratefully acknowledged.

Nutrient intake and food sensitivity in patients with rheumatoid arthritis. By S. A. NEW[1], A. R. TERRY[1], E. E. WILLIAMS[1], A. R. BEHN[2] and R. E. S. GRAY[2], [1]*Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH,* [2]*Department of Rheumatology, Royal Surrey County Hospital, Guildford GU2 5XX*

Rheumatoid arthritis (RA) is a chronic, progressive, inflammatory tissue disorder which is characterised by periods of remission and exacerbation. Its origin, however, remains largely unknown (Madison. *et al.* 1993). The possible influence of diet on disease activity is controversial. Although many rheumatic patients believe that diet has an influence on disease symptoms, to date, clinical trials have reached divergent conclusions (Darlington & Gamlin, 1996). The present abstract reports the findings of a pilot study examining nutrient intake and food sensitivity in a group of patients with active RA.

A total of fifty subjects (aged 35-85 years, ten male; forty female) were recruited from the Rheumatology Clinic, Royal Surrey County Hospital. Personal details (including family history and medication use) and lifestyle habits (including smoking and physical activity) were recorded using a questionnaire. Subjects were also asked to complete a food frequency section which listed the major foods which have been implicated as 'problem foods' in previous RA and food intolerance studies (Haugen *et al.* 1991; Young *et al.* 1994). Twenty of the subjects (aged 58-67 years, four male; sixteen female) also agreed to undertake a 7 d estimated food record using standard portion sizes. Diaries were analysed using the Comp-Eat dietary programme.

Although mean nutrient intakes were within the reference nutrient intakes (RNI) for the majority of nutrients as shown in the Table below, between 30 and 50% of subjects failed to meet the RNI for fibre, Se, Cu, Zn and Mg. Furthermore, twenty-five patients (54%) reported taking corticosteroids, 36% (*n* 15) were on non-steroidal anti-inflammatory drugs and of these, 25% reported gastrointestinal side effects of the medication. Results from the food sensitivity questionnaire found that 66% (*n* 33) of subjects highlighted food items as problematic. The most commonly identified food groups were citrus fruits (41.2%), dairy products (15.2%), other fruit (9.7%), meat (3.6%), fish (3.0%), alcohol (3.0%), additives (3.0%), cereals (2.4%) and miscellaneous foods (18.8%) (including coffee, malt, sugar, salads and beans).

Intake (/d)	Mean	SD	Range	Intake (/d)	Mean	SD	Range
Energy (MJ)	8.9	3.1	5.5-16.2	Iron (mg)	13.1	4.7	5.7-24.0
Protein (g)	74.3	21.5	42.8-99.6	Zinc (mg)	11.3	4.1	5.8-19.1
Fat (g)	83.0	28.7	46.5-145.5	Selenium (μg)	72.8	35.5	24.0-158.9
Carbohydrate (g)	274.5	81.5	169.3-451.1	Copper (mg)	2.5	4.6	0.7-21.7
NSP (g)	16.6	8.0	9.4-33.9	Magnesium (mg)	333.3	113.6	196.0-584.0
Calcium (mg)	1017	363	436-1683	Vitamin C (mg)	102.1	80.4	31.0-287.0

With the likely drug-nutrient interactions, the nutrient insufficiencies reported in these RA patients may be of significant clinical relevance. Although the food sensitivity data are limited by the lack of formal laboratory tests, these preliminary findings certainly warrant further investigations.

We are grateful to Nurse Bennett, Department of Rheumatology for her tremendous help with subject recruitment.

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Peer counsellors improve early postpartum breastfeeding practices in an urban community in Dhaka, Bangladesh. By RUKHSANA HAIDER[1,2], ANN ASHWORTH[2], IQBAL KABIR[1] and SHARON R.A. HUTTLY[2], [1]*International Centre for Diarrhoeal Disease Research, Bangladesh and* [2]*London School of Hygiene and Tropical Medicine, London WC1E 7HT*

In Bangladesh, although mothers commonly breastfeed, inappropriate practices have been identified; rejection of colostrum, giving prelacteal foods, late initiation of breastfeeding and very low rates of exclusive breastfeeding. These often occur because mothers are inadequately informed and supported, leading to increased risk of infant morbidity and mortality, especially in poor environments. In this intervention trial, local women who had breastfed their babies and were motivated to help other mothers, were chosen for training as peer counsellors, and a total of ten counselling visits at home were scheduled. Since the study is on-going, we are presenting preliminary results in which we examine whether the first three counselling sessions (two visits before delivery in the presence of influential family members, and one within 48 h of delivery) improve early postpartum breastfeeding practices.

Forty localities of similar size in Dhaka were randomized as intervention or control areas. In each of the twenty intervention areas, a peer counsellor was then recruited and trained to motivate and support mothers to initiate early breastfeeding and to breastfeed exclusively. Socioeconomic data and information on previous infant feeding practices were collected before delivery by trained interviewers. On day 4, they collected post-delivery feeding practices.

	Control		Intervention		
	Mean	SD	Mean	SD	Risk ratio (95% CI)
Mother's age (years)†	22.6	4.3	22.9	4.1	
Mother's education (years)†	4.2	4.2	4.8	4.1	
Household members *(n)*†	4.2	2.3	4.2	2.3	
Breastfeeding initiation (h)‡	15.9	17.1	3.3***	6.4	
Colostrum given as first food (%)‡	11		68***		6.1 (4.5 to 8.4)
Other foods given after colostrum (%)‡	48		23***		0.5 (0.4 to 0.6)
Exclusively breastfeeding on day 4 (%)‡	30		85***		2.8 (2.4 to 3.4)

Significantly different from control:***$P < 0.0001$.
†*n* 363 in Control and 362 in Intervention group.
‡*n* 338 in both groups, excluding mothers who moved out during pregnancy, stillbirths and infant deaths.

Both groups were of similar age and socioeconomic status. Significantly more mothers in the intervention group initiated early breastfeeding and gave colostrum. Of the intervention mothers whose babies received prelacteals, most reported that either the baby's grandmothers had administered the prelacteals contrary to their own wishes, or they had not wanted to oppose the advice given at their local health facility or by family members. In spite of these obstacles, significantly more mothers were breastfeeding exclusively on day 4 in the intervention group (85% *v.* 30%). We conclude that peer counsellors can improve early postpartum breastfeeding practices. Peer counsellors could be even more effective if health staff did not give advice that conflicts with accepted `good practice'.

This research project is supported by the Swiss Development Co-operation.

Effect of isoenergetically dense high-protein, high-carbohydrate and high-fat snacks on appetite and food intake in normal-weight men. By E. L. SHANNON, A. M. JOHNSTONE, R. J. STUBBS and C. A. REID, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB*

As the prevalence of obesity rises in the UK, feeding behaviour and appetite control have become areas of renewed interest in attempting to reverse current secular trends in overweight and obesity. Whether eating patterns influence appetite and energy intake (EI) is still uncertain. However, it is well established that high-fat, energy-dense (HF, ED) snacks produce poor compensation and hence favour overconsumption (Drummond *et al.* 1996). Whether macronutrient composition of isoenergetically dense snacks exerts differential effects on EI is also unclear. This issue will become increasingly important as the food industry lowers the fat and/or ED of snack foods. The present study assessed (i) whether snacking *per se* influences quantitative food and EI and appetite in healthy men, (ii) whether the macronutrient composition of snacks affects appetite and EI when ED is controlled.

Eight men, mean age 27.3 (SD 6.4) years; weight 76.5 (SD 10.2) kg; height 1.80 (SD 0.1) m; BMI 24.4 (SD 2.3) kg/m^2, were each studied four times during a 9 d protocol. On days 1-2 subjects were given a medium fat (MF) maintenance diet, calculated at 1.6 x resting metabolic rate (RMR). On days 3-9, they were required to consume three mandatory, isoenergetically dense snacks of the same energy content at fixed time intervals; 11.30, 15.30 and 19.30 hours. The treatments were: high-fat (HF), high-carbohydrate (HC), high-protein (HP), and no-snack (NS). Here "high" denotes 70% by energy, the remainder split between the other two macronutrients. Snacks comprised 30% of the subjects' estimated energy requirement, and were similar in taste, texture and appearance. During the remainder of each day, subjects had *ad libitum* access to a MF diet of fixed composition (fat:carbohydrate:protein 40:47:13 by energy) with an ED of 550 KJ/100 g. ANOVA was conducted on nutrient and energy intakes, subjective hunger and pleasantness of the food, using diet, sex and run as factors and subject as blocking factor. Mean daily *ad libitum* food and energy intakes can be seen in the Table below.

	HF	HC	HP	NS	F (3,18)	P value	SED
Weight (kg)	3.18	3.23	3.62	3.93	8.77	0.008	0.24
Energy (MJ)	11.48	10.99	11.21	13.23	14.93	0.001	0.64
Snack energy (MJ)	2.61	2.61	2.57	0.00	14.93	NS	0.01

The main treatment effect for snacks was accounted for by a contrast between the NS and other conditions. There was no effect of snack composition on food and EI. At mandatory snack times, subjects felt significantly more hungry during the NS dietary treatment, relative to the other three diets (F (3,18) 4.98; P<0.001). Subjects compensated by 57% for the NS condition when compared with the other treatments. There was no perceived difference in the pleasantness of each diet, however a meal effect increased significantly throughout the day (P<0.001). These data suggest that under the conditions of the experiment (i) snacking did not exert a large effect on EI or body weight, most of the difference between snack and NS conditions being compensated for and (ii) differences in the composition of isoenergetically dense diets did not differentially affect quantitative intake in normal weight men.

Drummond, S., Crombie, N. & Kirk, T. (1996). *European Journal of Clinical Nutrition* **50**, 779-783.

Body composition and muscular strength in an elderly Thai population. By C. VARAKAMIN, J. HENRY[1], M. GOLDEN[2] and K. TONTISIRIN[3], [1]*School of Biological & Molecular Sciences, Oxford Brookes University, Oxford OX3 0BP* [2]*Department of Medicine, University of Aberdeen, Aberdeen AB9 2ZD* [3]*Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand*

Until recently most gerontology has been confined to Western societies. Consequently, little information exists on the ageing process in developing countries. We report the results of a study conducted in the south (Chun Buri) and north (Suphun Buri) of Bangkok, Thailand. The subjects at Chun Buri were living in a residential home, because lack of family (sedentary lifestyle) by the seaside whilst those at Suphun Buri lived in their own homes in seven different villages (active lifestyle) in a sub-district of Toobun Yutaline (a rural community).

A total of 515 healthy elderly subjects (343 females and 192 males) from two study sites, a residential home (RH) and a rural community (RC), aged 60 years and over were recruited. The subjects were divided into three age groups: 60-69, 70-79, and >80 years. Various anthropometric measurements were taken including weight, height and skinfolds (Lohman *et al.* 1988). The physical ability of subjects was assessed by grip strength with an adjustable hand dynamometer (Winnick & Short, 1985). The results are presented as the mean and standard deviation in both male and female subjects.

Study sites, age range, and sex	BMI		Body fat (%)		Grip strength (kg)			
					Dominant		Non-dominant	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Female RH								
60-69 (23-69)†	23.7	4.9	36.3**	4.0	17.5***	5.6	15.8***	4.8
70-79 (47-61)	23.3*	5.6	34.3**	4.8	15.1***	5.4	14.1***	4.9
>80 (24-42)	22.2	3.7	33.2***	3.9	12.8*	3.9	12.2**	4.1
Female RC								
60-69 (88-103)	22.5	3.8	33.0	6.2	22.6	4.7	22.0	4.4
70-79 (65-76)	21.8	3.3	31.9	5.8	19.7	4.5	19.1	4.1
>80 (18-35)	20.8	3.4	26.8	4.8	15.3	4.7	15.1	4.8
Male RH								
60-69 (18-20)	21.4	3.5	24.0	5.8	25.4*	8.3	25.0**	7.5
70-79 (32-37)	21.4*	2.8	21.6**	5.7	23.2	5.3	22.7	5.3
>80 (20)	20.2	3.3	20.7	6.0	20.4	5.9	21.6	5.0
Male RC								
60-69 (63-67)	21.3	3.4	21.7	7.2	31.4	7.8	31.0	6.4
70-79 (28-34)	19.8	2.7	18.6	6.5	25.0	6.3	24.9	6.5
>80 (12-14)	21.4	3.1	23.7	6.6	22.2	4.9	22.0	5.1

Mean values were significantly different from RC: *P<0.05, **P<0.01, ***P<0.001.
†The numbers of subjects are in brackets.

The results show that females in all age groups living in RH had significantly higher percentage body fat values than those living in RC. A similar trend is shown in males aged 70-79 years. Muscular strength, as determined by grip strength, was significantly lower in RH elderly females of all age groups, as well as males aged 60-69 years, living in RH.

It is concluded from this study that lifestyle may influence both body composition and muscular strength. The elderly living in the RH had access to better food and care, and apparently led a sedentary life. In contrast, the elderly living in the RC led an active life, participating in numerous occupational endeavours.

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Winnick, J.P. & Short, F.X. (1985) *Physical Fitness Testing of The Disabled Project.* Champaign, IL: Human Kinetics Publishers.

Assessment of a portable electronic tape recording automated (PETRA) system v. laboratory-weighed intakes for use in *ad libitum* feeding studies in human volunteers. By A. M. JOHNSTONE, M. J. DIVER, L. M. RYAN, C. A. REID and R. J. STUBBS, *Rowett Research Institute, Greenburn Road, Aberdeen AB21 9SB*

Techniques for measuring food intake may increase accuracy and precision of dietary assessment if they reduce volunteer workload. The PETRA system was developed to reduce subject workload as subjects can automatically record a description of the food while it is being simultaneously weighed, thus maintaining precision and accuracy of data collection. Previously, the PETRA has been evaluated against conventional self-weighed intakes by the same subject (Black *et al.* 1991). This may lead to covariance of errors between the two techniques. The present study compared food intakes by subjects using the PETRA, with precise independent laboratory-weighing (LW) of the same foods and leftovers, before and after ingestion by the subjects. Validity and precision of the PETRA was therefore assessed using the LW as a "gold standard". In so doing the sources of errors in estimating energy and nutrient intakes could be quantified.

Ten women, mean weight 57.4 (SD 5.4) kg, mean height 1.7 (SD 0.50) m, mean age 26 (SD 9.4) years and ten men, mean weight 80.0 (SD 9.0) kg, mean height 1.8 (SD 0.54) m and mean age 30 (SD 12.2) years were studied for 2 d during which they had *ad libitum* access to a range of thirty-nine common supermarket foods. Subjects (Rowett Research Institute staff/students) were given a food bag containing all the food which was eaten in the Unit and at home. Volunteers were instructed to record time of eating and food descriptors in a food diary, while using the PETRA to describe and weigh food items and leftovers.

Regression and correlation analyses were conducted on the two methods using LW as the predictor technique and PETRA as the outcome, by sex and day (1 v. 2). There was a good correlation between the two methods (food, 88.7 (SED 0.015); energy, 93.6 (SED 0.037)). The average regression slopes were 0.90 and 0.92 respectively. The slopes of the line were significantly less than 1 for weight (0.90), energy (0.92), fat (0.90), water (0.87) and fibre (0.88) ($P<0.01$). Thus PETRA consistently underestimated the intake of these variables, relative to LW, by 10% on average. There were no sex or day effects for any of these variables. The slopes for protein (0.97) and carbohydrate (0.94) were not significantly different from 1.

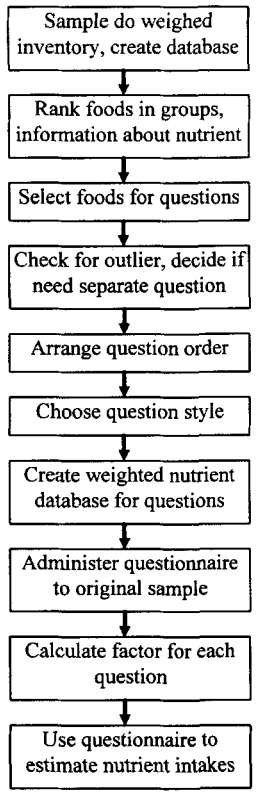
These data suggest that LW is a more accurate but not necessarily more precise method for the assessment of dietary intakes during *ad libitum* feeding studies of human subjects. PETRA consistently produced systematic methodological errors which led to underreporting of food energy and nutrient intakes by an average of about 10%. PETRA may, therefore, be more useful for comparing changes in food and energy intake using within-subject designs, than for characterizing dietary patterns that require considerable accuracy as well as precision.

Black, A.E., Jebb, S.A. & Bingham, S.A. (1991). *Proceedings of the Nutrition Society* **50**, 108A.

Computer-aided design and analysis of a food-frequency questionnaire. By A. WISE. *The Robert Gordon University, Queen's Road, Aberdeen AB15 4PH*

Food frequency questionnaires have been developed for use in many different types of study, but most researchers use their own individual method to develop them. It is very important that the questions relate directly to the group of people to be surveyed so each group of investigators designs their own questionnaire. Some use information from previous surveys, and most compare results with another method to validate the questionnaire. Most questions relate to more than one food and it is rarely clear how the nutrient composition is calculated. It is also important to establish what portion weights are culturally appropriate for each question.

A computer program has been developed to facilitate questionnaire design. The proposed protocol requires that a sample of the population undertakes a weighed inventory, the diets are entered into 'Diet5 for Windows' (Univation Ltd, The Robert Gordon University, Aberdeen) and saved as individual files. They are combined by 'Diet5 for Windows' into a single database.



The questionnaire-design program shows each food with the total weight consumed by the subjects in separate list boxes at the top of the screen, one box for each food group. Foods are simply dragged to list boxes at the bottom of the screen, one box for each question in the questionnaire. For example, several codes for different cuts of beef may be combined to one question about beef. Foods are ranked for any nutrient of particular interest to help decisions about what foods would best discriminate diets high or low in a nutrient. Then checks can be made to show which food least matches the others (outlier) that have been included in a question; this gives important information about whether a food naturally belongs to that question or deserves its own question, depending on the weight consumed by the subjects and the extent of difference to the other foods. Questions are typed in boxes and dragged around the screen to rearrange the order of the questions. They can be formatted in terms of frequency per day, week, fortnight, or month, or as amount per week.

The weighted mean analysis for each nutrient and mean portion weights are calculated for each question. The questionnaire is administered to the original subjects (or similar subjects). The program calculates a factor for each question such that when the mean portion weight is multiplied by the factor and the frequency it equals the total weight of the foods consumed by the subjects in the weighed inventory. The calculation of this factor makes allowances for misperceptions about the frequency of foods consumed (Lockie *et al.* 1988). The questionnaire can then be administered to a large number of subjects and analysed using the factor calculated from the weighed inventory.

Theoretically this approach does not need validation because the questionnaire is developed directly from a weighed inventory, which is sometimes used as the method for validation. It may be argued that the data for the weighed inventory requires validation, perhaps by doubly-labelled water or nitrogen excretion. Research is needed to apply the approach to a wide range of situations and validate the program itself.

Lockie, G.M., Wise, A. & Liddell, J.A. (1988). Journal of Human Nutrition and Dietetics 1, 29-37.

Identifying patterns of food consumption among women in the UK Women's Cohort Study: preliminary results. By JENNIFER H. BARRETT[1], ALIZON DRAPER[2], CLAIRE CALVERT[3], JANET CADE[3], and the UK WOMEN'S COHORT STUDY STEERING GROUP*, *[1]Cancer Epidemiology and Health Services Research, University of Leeds, 26 Clarendon Road, Leeds LS2 9NZ; [2]Human Nutrition Unit, London School of Hygiene and Tropical Medicine, 2 Taviton Street, London WC1H OBT; [3]Division of Public Health, Nuffield Institute for Health, 71-75 Clarendon Road, University of Leeds, Leeds LS2 9PL*

Conventional analyses of food intake data focus on the classification of subjects according to nutrient intake. Cluster analysis offers a method that will allow identification of groups of subjects with similar patterns of food consumption. It has been used successfully in biology and psychology and more recently in the nutritional sciences to identify patterns of nutrient and food intake (e.g. Hulshof *et al.* 1992).

As part of the UK Women's Cohort Study (Woodhouse *et al.* 1997) a 217-item food-frequency questionnaire (FFQ) has been completed by 6572 women aged 35-69 years, about a third of whom are self-defined vegetarians. The FFQ has been adapted from that used in the European Prospective Investigation into Cancer and Nutrition (Riboli, 1992). Food items which were similar in type and in nutrient content were combined to form seventy-four food categories to be used in the cluster analysis.

A preliminary cluster analysis was performed to classify the individuals into six groups on the basis of similar reported food intakes. The algorithm used, implemented in SPSS 6.1, assigns a case to the cluster with the nearest centroid, iteratively updating the cluster centres. The largest of the six clusters consisted of 37% of the sample, with low to average consumption of most foods compared with the rest of the cohort. The next largest group (30%) were the lowest consumers of all meats and the highest consumers of soya products and herb tea. The third group (17%) had a high consumption of snack foods, such as bread, jam, cheese, biscuits and tea or coffee. The fourth group (9%) could be characterized as "health food" consumers, with a high intake of brown pasta, low-fat dairy products and fish, and a low consumption of crisps and sugar. We also identified a small group (5%) following a traditional British diet of white bread and butter, chips and all kinds of meat, and a further group (1%) who ate extremely large quantities of fruit and vegetables.

There are many types of clustering algorithm which could be used, and our next step is to examine the robustness of this classification of cohort members, as well as its relationship to self-classification of vegetarian status. We will then compare the social and lifestyle characteristics of the groups with different dietary patterns.

This work is supported by the World Cancer Research Fund.

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* Rhys Williams, University of Leeds; Barrie Margetts, University of Southampton; and Margaret Thorogood, London School of Hygiene and Tropical Medicine.

Nutrition labelling of sugar-containing foods in 1996 compared with 1989. By JULIE McDONALD and ANDREW RUGG-GUNN, *Department of Child Dental Health, Newcastle University Dental School, Framlington Place, Newcastle upon Tyne NE2 4BW*

Department of Health reports have recommended a reduction in consumption of sugars and improvements in labelling of the sugars content of foods; legislation on nutritional labelling has been introduced, particularly in 1994, in response to an EEC directive. A survey of the nutritional labelling of 1542 food products containing sugars was undertaken in four food supermarkets (Co-op, Marks and Spencer, Netto, and Tesco) in Newcastle upon Tyne in 1996 and the results compared with those reported in a similar survey in 1989 (Tyrrell & Rugg-Gunn 1990). The methods used were similar in the two surveys: products were categorized into twelve groups and all products in each of the groups were examined for nutritional labelling. Nutrition labelling was widespread and most layouts conformed to Ministry of Agriculture Fisheries and Food recommendations: at least 64% of all varieties examined in 1996 displayed nutritional information and, if confectionery and soft drinks were excluded, at least 82% of varieties displayed nutritional information. The proportion of products labelled had increased considerably between 1989 and 1996. The exception was cakes. In 1989 25% of cakes displayed nutrition labelling and all of these stated sugar content. In 1996, 85% of cakes displayed nutrition labelling, but only 56% of these stated sugars content. Confectionery had the lowest percentage (64%) of varieties nutritionally labelled. In ten out of the twelve categories of foods, prevalence of nutrition labelling which stated sugars content, had increased between 1989 and 1996. The proportion of varieties making claims regarding sugars content which stated sugars content increased in six out of seven varieties. At least 82% of all own brand products were nutritionally labelled; Co-op and Tesco providing the most with 99%, followed by Marks and Spencer (87%), and Netto (82%). Marks and Spencer provided comprehensive labelling on most ranges of products but stocked a high proportion of luxury biscuits and chocolates in decorative tins and boxes which did not provide nutrition labelling. The effect of this was to reduce the proportion of Marks and Spencer products which provided nutrition labelling to 87%. The proportion of these products providing nutrition labelling which also labelled sugars content was highest for Marks and Spencer (100%) followed by Co-op (99%), Tesco (93%) and Netto (57%). The store which sold the cheaper range of foods had the lowest proportion of products labelled for sugars content. Of common brands of foods containing sugars, 100% of Kellogg's and Burton's products presented nutritional information which also included a statement on sugars content. In contrast, none of the varieties of Mars, Foxes and Mr. Kipling brands stated their sugars content. Food products with high sugars content, particularly confectionery, were the least likely to be labelled for sugars content. Although substantial progress in nutritional labelling for sugars content has occurred, voluntary labelling appears to allow inadequate labelling of high sugar foods.

Tyrrell, A. & Rugg-Gunn, A.J. (1990). *Community Dental Health* 7, 359-367.

Adolescent haemoglobin is predicted by maternal haemoglobin and birth weight in South Asian adolescent girls born in Southampton. By ZUHAIR S. M. AL-DALLAL[1], BARRIE M. MARGETTS[2] and ALAN A. JACKSON[1]. [1] *Institute of Human Nutrition, Bassett Crescent East, University of Southampton, SO16 7PX,* [2]*The Wessex Institute for Health Research and Development, Level B, South Academic Block, Southampton General Hospital, SO16 6YD*

Maternal anaemia, defined as maternal haemoglobin (Hb) level below 100 g/l, and low birth weight are more common in women living in the UK who are of South Asian origin than the general population. It has also been reported in a study carried out in North London (Nelson *et al.* 1994) that South Asian adolescent girls have a higher rate of anaemia than the general population. Low Hb in pregnancy has been shown to be associated with a raised placental weight: birth weight ratio, which is a predictor of high blood pressure in adult life (Barker *et al.* 1990; Godfrey *et al.* 1991). Barker *et al.* (1990) also showed that if anaemia in adolescent girls persists into their reproductive years, this has important implications for the long-term health of their offspring. To date, no study has linked maternal Hb levels, birth weight and the prevalence of anaemia in adolescent girls. Forty South Asian girls aged 11- 16 years born in Southampton, were selected at random from GP's lists, contacted, and a blood sample drawn. Birth records were searched, from which maternal Hb during pregnancy, and birth weight were obtained.

Mother's Hb (g/l)	Birth Weight (g)				Girls' Hb (g/l)			
(at pregnancy)	*n*	Mean	SD	*P* value	*n*	Mean	SD	*P* value
< 100	12	2582	524	0.0001	10	117	10	0.0001
100 - 115	16	2853	440	0.0001	8	125	8	0.0001
> 115	12	3193	179	0.0001	10	127	9	0.0001
Total	40	2873	468		28	123	10	

Birth weight and Hb levels were statistically significantly lower in girls born to mothers who had lower Hb levels during pregnancy (Table). Overall birth weights are low in this group with 20% below 2500 g. Mothers Hb and birth weight were both statistically significantly correlated to adolescent Hb level (r 0.65, $P < 0.0001$), partial correlation analyses suggested that the relationship was stronger for birth weight after adjustment for maternal Hb (r 0.43, $P < 0.03$). Current dietary intakes of Fe 12.5 (SD 4.1) mg and protein 73.5 (SD 18.8) g in the girls measured by food frequency questionnaire were not significantly related to adolescent Hb level. This study suggests that factors operating during fetal development have an important effect on Hb levels in adolescent South Asian girls.

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Physical activity among South Asian women in Southampton. By SAFIAH MOHD-YUSOF[1] and BARRIE M. MARGETTS[2],[1] *Institute of Human Nutrition, University of Southampton, Bassett Crescent East Southampton SO16 7PX,* [2]*The Wessex Institute for Health Research and Development, Southampton General Hospital, Southampton SO16 6YD*

The health benefits of physical activity are widely recognized. Physical activity has an important role in the prevention of several chronic diseases. The results of a recent health survey of the general population in England showed that more men took regular physical exercise than women (Colhoun & Prescott-Clarke, 1996). A health and lifestyles survey in the black and minority ethnic groups in England, showed that their rate of participation in sports or general physical activity was much lower than the general population. The women, particularly those from Pakistan and Bangladesh, reported the lowest rate of physical activity (Rudat, 1994). In the present study 128 women of South Asian origin (Indians, Pakistanis and Bangladeshis) were recruited either through their GP or through personal contacts. Each subject filled a self-administered questionnaire which asked about the number of times they had done four different types of moderate activity (long walks of 2 miles or more at brisk pace; heavy housework, e.g. spring cleaning, moving furniture, polishing; heavy gardening; heavy DIY e.g. mixing cement) and one type of vigorous activity (sports or exercise) during the previous week. The questions were similar to those used in the *Allied Dunbar National Fitness Survey* and the *Health Survey for England 1994*, therefore comparable with those surveys. The highest level on the scale (group 3) represents at least five times of 20 min moderate or vigorous activity during the previous week and the lowest level (group 1) represents no activity at all. Group 2 represents those who had 1-4 occasions of moderate or vigorous activity.

	Physical activity level (%)					
	Group 1		Group 2		Group 3	
Age group (years)	*Present	HSE	Present	HSE	Present	HSE
16 - 24	21	27	37	44	42	29
25 - 34	27	24	35	49	39	28
35 - 44	24	25	53	49	35	27
45 - 54	17	27	50	46	33	27
55 - 64	no data	37	50	43	50	21
16 - 64	23	31	38	45	38	24
Total no. of women	29	140	49	231	50	132

Present, the present study; HSE, Health Survey for England (Colhoun & Prescott-Clerk, 1996).

Table shows that a higher proportion of women in the youngest age groups (16-24 & 25-34 years) were in group 3. Among older women a higher percentage fell into group 2. For women of all ages (16-64) the same proportion belonged to groups 2 and 3. More than two thirds of the women in this study had done at least one type of moderate or vigorous activity during the previous week. When compared with the results of the *Health Survey for England 1994*, a higher percentage of South Asian women in the present study were in group 3 (38 *v.* 24%), which is the recommended level of activity. It is possible that activity may have been overestimated because moderate activities of at least 20 min were also included. A large number of housework activities reported in this, which were not considered in previous studies, could account for the higher level of physical activity than previously reported. The respondents in the present study were not a random sample of the South Asian women population in Southampton, so the study may not represent the true physical activity level of that population.

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Opinions about 'healthy eating' health education among South Asian family members in Scotland. By J.P. LANDMAN[1] and S. WYKE[2], *[1]Department of Dietetics and Nutrition and Centre for Food Research, Queen Margaret College, Edinburgh EH12 8TS and [2]Department of General Practice, University of Edinburgh, EH8 9DX*

In order to inform the design of appropriate and relevant health education programmes in Scotland, a three stage qualitative research study investigated opinions of health education about 'healthy eating' expressed by family members of South Asian origin. 'South Asian' describes people born in the Indian subcontinent or their descendants.

Three multilingual researchers recruited quotas of mothers (M), fathers (F) and young people (YP), to include varied religious and social backgrounds, some of whom belonged to existing community groups. Between July and October 1994, there were six focus group discussions (FGD, M, 4; YP, 2); and forty-five individual semi-structured interviews (SSI) with a total of ninety-three participants, in Edinburgh, Glasgow and Stirling. FGD and individual SSI were conducted in the participants' choice of language, translated as necessary and fully transcribed. Thirty participants were 11-19 years old and forty-eight were 20-39 years old. Forty-seven participants said they spoke English best and thirty-nine said they spoke Punjabi or Urdu best. Participants spoke about their own and perceptions of their families' experiences of existing health education. Analyses concentrated on the issues which emerged in each successive stage of the research and on comparisons of M, F and YP, in the context of participants' perceptions of their food choices.

M in three FGD, and in thirteen SSI said they wanted more 'healthy eating' education. In the company of their peers in FGD, YP said they got enough but in SSI eight YP said they wanted more 'healthy eating' information. Nine F also said they wanted more information. The perception that existing information and education under-represented South Asian foods and cuisine was a key issue for participants who said they wanted more 'healthy eating' information, as well as those who said they did not. Some participants felt that the South Asian dietary pattern was healthier than the dietary pattern they associated with 'whites'. In general, the participants said they wanted 'healthy eating' health education to be explicitly multi-cultural. This view is consistent with strongly expressed commitment to South Asian foods but eclectic and varied reported food choices (Wyke & Landman, 1997).

Our exploratory study provides evidence of support for a multi-cultural population-based strategy for 'healthy eating' education. Further research should investigate whether South Asian people of different regional origins have specific information needs.

We acknowledge funds from the Health Education Board for Scotland.

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Characteristics of fruit and vegetable eaters in north Glasgow: results from the MONICA study of 1995. By W.L.WRIEDEN[1], M.K. McCLUSKEY[2] and C. BOLTON-SMITH[2], *[1]School of Management and Consumer Studies, University of Dundee, DD1 4HT, and [2]Cardiovascular Epidemiology Unit, Ninewells Hospital and Medical School, Dundee DD1 9SY*

One of the behavioural targets set for 2005 by the Scottish diet report (Scottish Office, 1993) was that all young men and women should be eating three or more portions of vegetables and fruit/d. This is perhaps a more realistic target than the propounded '5-a-day' (Williams, 1995), given that the average consumption of fruit and vegetables in Scotland in 1989-1991 was less than 200 g/d (excluding potatoes) (Scottish Office, 1993). As part of the 1995 World Health Organisation MONICA (monitoring trends and determinants in cardiovascular disease) study in North Glasgow the dietary habits of 1500 men and women aged 25-64 years were surveyed using a standard food-frequency questionnaire (FFQ) (Bolton-Smith *et al.* 1991). The FFQ included questions on the weekly frequency of consumption of all the major types of foods. It was found that 46% of men (*n* 710) and 63% of women (*n* 790) consumed vegetables (excluding potatoes), fruit and pure fruit juice at least three times per day. The characteristics of these subjects (F&V ≥3) were compared with those who ate less than this amount (F&V<3). The percentages of each F&V group displaying a particular characteristic are given in the Table.

	Men		Women	
	F&V<3 *n* 384	F&V ≥3 *n* 326	F&V<3 *n* 295	F&V ≥3 *n* 495
Non-smokers	44.5	62.2***	41.9	67.3***
High social status[+]	7.4	25.2***	7.7	24.8***
Trying to lose weight	28.4	39.6**	50.8	62.4**
BMI <25 kg/m^2	37.8	34.5	46.3	44.1
Alcohol <24 g/d (men) or <16 g/d (women)	54.4	63.8*	86.1	85.5
Fish ≥2/week	50.6	69.2***	44.4	72.9***
Semi-skimmed or skimmed milk	55.9	69.0**	60.7	74.4***
Wholemeal or granary bread≥50% of bread eaten	10.2	37.2***	18.7	44.5***
Lard/dripping /solid vegetable fat used <1/ week	63.2	74.0**	59.2	73.8***
Chicken and other poultry≥2/week	51.2	58.2	51.4	71.2***
Processed meat or meat-filled pies<2/week	14.6	34.9***	33.3	45.4***

Significantly different proportions in F&V groups using Chi-Squared test, *P<0.05, **P<0.01, ***P<0.001.

[+] A high social status was assigned to those who fell into all three categories of non-manual occupation, educated beyond school and living in owner occupied accommodation.

There were no differences in mean BMI, blood pressure or total serum cholesterol between the two groups. However a higher proportion of the frequent fruit and vegetable eaters claimed they were trying to lose weight, were of a high social status and were non-smokers. These people tended to be more healthy eaters in general with a greater proportion attaining the other Scottish Diet behavioural targets for the year 2005. A survey in 1987 showed that West of Scotland 'healthy eaters' were more likely to be women, of a high social status, non-smokers and less frequent drinkers of alcohol (Anderson & Hunt, 1992). Eight years later it seems that the 'health divide' still remains.

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Effect of a zinc-deficient diet on the exchangeable zinc pool in human subjects. By NICOLA M. LOWE[1], LESLIE R. WOODHOUSE[2], JODY M. RANDOLPH[2], BARBARA SUTHERLAND[2], JUDITH R. TURNLUND[3], JANET C. KING[3] and MALCOLM J. JACKSON[1], *[1]Department of Medicine, University of Liverpool L69 3GA, [2]Department of Nutritional Sciences, University of California at Berkeley, CA 94720, USA, [3]USDA Western Human Nutrition Research Center, San Francisco, CA 94129, USA.*

Intravenously administered stable isotopes of Zn can be used to study the size and kinetics of rapidly exchanging Zn metabolic pools (Lowe *et al.* 1997). The sizes of Zn metabolic pools are sensitive to restrictions in dietary Zn in experimental animals (Lowe *et al.* 1991), but this has not been demonstrated in human subjects. The size of the combined pools that exchange with the plasma within a period of 2 d (EZP), can be estimated from the tracer enrichment of the plasma measured two or more days after isotope administration (Miller *et al.* 1994).

Eight healthy men participated in an 86 d Zn depletion/repletion study. The study was divided into three metabolic periods: a 16 d baseline, a 40 d depletion and a 30 d repletion period. Dietary Zn intake was 12 mg/d during baseline, <0.3 mg/d during depletion, and for three subjects (group B) 12 mg/d during repletion; the other five subjects (group A) received two intravenous infusions of 66 mg Zn 10-12 d apart then were given a diet providing 12 mg Zn/d for 17 d. EZP was estimated after intravenous administration of ^{70}Zn or ^{67}Zn at baseline (BSL), early depletion (ED), late depletion (LD), early repletion (ER), and late repletion (LR). Plasma Zn concentration (PZn) was determined by atomic absorption spectrophotometry.

	BSL (d 7)		ED (d 6)		LD (d 35)		ER (d 2)				LR (d 20)			
							A		B		A		B	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
EZP (mg)	184	28	160	19	116*	34	153	11	178	10	135*	14	151	37
PZn (μmol/l)	11.02	1.60	8.69*	1.26	3.65*	2.18	10.20	2.19	6.68*	1.65	10.00	1.73	9.43	0.93

Mean values were significantly different from baseline, * $p<0.05$ (paired t-test, 2-tail)

The size of the EZP and the PZn fell significantly during Zn depletion compared with baseline values.

This study demonstrates that the size of the combined pools that exchange with the plasma within a period of 2 d are sensitive to changes in dietary Zn levels and correlate well with total plasma Zn (r 0.58, $p<0.001$). Measurement of the EZP may provide a valid measure of body status in situations where plasma Zn concentrations are unreliable, however further studies are required to evaluate the effect of stress on the EZP.

This work was supported by USDA competitive grant 9301197.

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Zn absorption in the healthy elderly. By SIMON A. ALI[1], NICOLA M. LOWE[1], CATHERINE I.A. JACK[1], MARTIN D. REID[2], JOHN H. BEATTIE[2], JANET C. KING[3] and MALCOLM J. JACKSON[1], *[1]Department of Medicine, University of Liverpool, L69 3GA, [2]Rowett Research Institute, Bucksburn, Aberdeen, AB21 9FB and [3]USDA Western Human Nutrition Research Centre, San Francisco USA.*

A number of inconclusive studies have examined the Zn status of elderly subjects. Sandstead *et al* (1982) reviewed the available literature and concluded that elderly subjects have a reduced dietary Zn intake and that a proportion of elderly subjects are Zn deficient, based on both dietary and laboratory data. Previous studies have suggested that the healthy elderly have a reduced absorption of Zn on a standard diet (Turnlund *et al*, 1986) although this has not been observed by all authors (Couzy *et al*, 1993). In order to determine the likelihood that elderly subjects have aberrant Zn metabolism we have examined the habitual dietary Zn intake and fractional Zn absorption (FZA) using enriched stable isotopes in groups of healthy young and elderly subjects. Five elderly subjects (four male, one female aged 69-75 years) and five young subjects (four male, one female aged 21-23 years) participated in the study. Each completed a 9 day weighed food intake diary. The FZA was analysed using the dual isotope technique of Friel *et al* (1992). Each subject was given 0.4 mg 85.03% enriched ^{70}Zn intravenously and 2 mg 90.59% enriched ^{67}Zn orally with an orange drink 15 min after a standard breakfast meal. A 24 h urine collection was undertaken between days 2 and 3 of the study and the proportion of Zn absorbed calculated. All subjects then took a supplement of 10 mg Zn/d (as 44 mg $ZnSO_4.7H_2O$) for one month and the above procedure repeated. The table shows the data for both groups.

Subject	FZA (%) (SEM)	Dietary Zn intake (mg/d) (SEM)	Mean daily Zn absorbed (mg/d) (SEM)
Young Pre-supplementation	22.8 (4.9)	10.6 (1.3)	2.2 (0.3)
Elderly Pre-supplementation	12.9 (3.8)	9.2 (0.6)	1.1 (0.6) *
Young Post-supplementation	14.8 (1.3)	20.6 (1.6)	2.9 (0.1)
Elderly Post-supplementation	11.7 (3.1)	19.2 (0.7)	2.2 (0.6)

Table 1. *Zinc absorption data for both study groups before and after one month of zinc supplementation. *Values significantly differ to young group pre-supplementation ($P<0.05$)*

It is concluded that the healthy elderly subjects have a reduced absorption of Zn compared to healthy young subjects on a standard diet. Young subjects also appeared to decrease FZA following Zn supplements but elderly subjects did not.

Support from the Wellcome Trust is gratefully acknowledged.

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Micronutrient intestinal balance in growing rats with lactose-induced diarrhoea. By PATRICIO HEVIA, JUAN P. LIUZZI and ANNA M. CIOCCIA, *Laboratorio de Nutrición, Universidad Simón Bolívar, Caracas, Venezuela 89000.*

Childhood diarrhoea is a common observation in all countries of the world but its incidence is particularly high in developing countries. In Venezuela diarrhoeal diseases is considered the second cause of death among children under 1 year. Diarrhoea is also seen in gastrointestinal disorders such us caeliac and Crohn's disease and inflammatory colitis, is a frequent observation during enteral feeding, shows a high incidence in acquired immune deficiency syndrome and is also frequent among travellers. The nutritional management of diarrhoea is a matter of controversy and there is little information with regard to the availability of nutrients during diarrhoea. Accordingly, we produced diarrhoea in rats by feeding a balanced diet with 350 g lactose/Kg, and compared the intestinal balance values [IB = 100 (intake - faecal loss) / intake] of vitamins A, E, thiamin and riboflavin and of Ca, Fe, and Zn with those measured in a similar group of rats (Sprague Dawley, initial weight 81.4 ± 3.3 g) fed on a similar diet without lactose (controls). The experiment lasted 22 d and the IB of these nutrients was determined after 4 - 6, 10 - 12, 14 - 16 and 20 - 22 d of feeding these diets (Table; means values with their standard errors for eight rats).

Time (d)	4 - 6		10 - 12		14 - 16		20 - 22	
	Means	SE	Mean	SE	Mean	SE	Mean	SE
Faecal mass (g/2 d)								
Control	0.40	0.06	0.29	0.05	0.31	0.06	0.41	0.08
Lactose	7.37*	3.08	5.29*	0.86	3.53*	1.06	3.23*	0.55
Vitamin A (%) IB								
Control	95.3	0.97	95.8	0.60	94.7	0.62	93.1	1.93
Lactose	47.4*	5.76	61.9*	5.42	80.5*	6.84	65.2*	8.37
Vitamin E (%) IB								
Control	77.4	3.73	84.5	4.86	85.5	3.04	89.5	1.65
Lactose	40.9*	3.71	53.9*	4.00	74.5	5.32	88.7	2.25
Thiamine (%) IB								
Control	96.1	0.90	96.9	0.81	95.4	0.85	95.2	1.54
Lactose	94.9	1.19	93.6*	0.95	88.5*	1.94	78.7*	1.56
Riboflavin (%) IB								
Control	98.3	0.59	98.8	0.26	98.5	0.29	97.1	1.37
Lactose	97.2	0.97	96.4*	0.60	95.7*	0.64	82.5*	2.50
Calcium (%) IB								
Control	79.3	3.49	84.1	3.22	85.2	2.33	83.7	3.11
Lactose	86.4	0.85	82.5	1.81	84.5	2.00	69.6*	6.14
Iron (%) IB								
Control	45.1	5.92	58.5	7.82	59.7	5.20	69.6	5.26
Lactose	29.8*	3.74	39.3*	5.83	51.1*	6.65	28.7*	6.61
Zinc (%) IB								
Control	75.8	2.78	80.2	2.92	78.3	3.11	74.1	5.40
Lactose	54.8*	3.88	49.3*	3.40	45.5*	6.52	43.0*	5.69

* Mean values were significantly different from control, $P < 0.05$ (one - way ANOVA and Duncan's multiple range test). Pair fed controls (not shown) had IB values similar to controls.

The results showed that the inclusion of lactose in the diet resulted in an abundant diarrhoea whose severity decreased with time. This diarrhoea caused a reduction in the intestinal balance of all the studied nutrients. This reduction was substantial in the cases of vitamins A, and E as well as Fe and Zn and was less severe in the cases of the B vitamins and Ca. A correlation analysis of the data showed that faecal mass (r -0.95) and the IB of thiamin (r -0.94), riboflavin (r -0.84), Ca (r -0.85) and Zn (r -0.98) decreased with time whereas the IB of Vit A (r +0.65) and Vit E (r +0.99) increased. In contrast, the IB of Fe (r 0.07) did not change with time indicating that the effect of lactose feeding and its associated diarrhoea, affected differently the absorptive mechanism of the studied micronutrients. In summary these data show that lactose is useful for producing diarrhoea in rats and that this diarrhoea causes a reduction in the availability of the micronutrients.

Funded by CONICIT Grant S1-2265.

Elevated urinary malondialdehyde but not the presence of non-transferrin-bound iron is associated with an increased risk of poor outcome in preterm babies. By H.J POWERS[1] R.A. KIME,[1] A.T. GIBSON,[1] S. C. YONG[2] and A.S. RIGBY,[1] [1]*University Department of Paediatrics, Sheffield Children's Hospital, Sheffield S10 2TH, and* [2]*The Neonatal Intensive Care Unit, The Jessop Hospital for Women, Sheffield, S3 7RE.*

There is increasing evidence that tissue damage caused by reactive oxygen species (ROS) is part of the pathophysiology of diseases associated with prematurity, including chronic lung disease (CLD). Non-transferrin-bound Fe (NTBI) has been detected in the plasma of some babies born preterm, and this may play a role in the generation of ROS. A longitudinal study was performed to explore the association between plasma NTBI, a urinary index of lipid peroxidation (malondialdehyde, MDA), and clinical outcome in preterm babies. Ninety five babies born between 24 and 36 weeks gestation, and requiring mechanical ventilation on the day of birth, were enrolled into the study. Urine samples and blood samples were collected on the day of birth and, when possible, on postnatal days 1, 3 , 5 and 7. Urinary MDA was measured by HPLC (Knight et al. 1988) in a total of 232 samples, and expressed relative to creatinine. Plasma NTBI was measured in a total of 288 samples, also by HPLC (Kime et al. 1996).

NTBI was detected in 62% of samples from babies who were discharged well, and 67% of babies who either died or developed CLD (together defined as a poor outcome). Values ranged from zero to 51.96 µmol/l; the median was zero. There was no association between the presence of NTBI and poor outcome. On the other hand, babies with urinary MDA greater than 100 µmol/mmol creatinine were 3.8 times more likely to have a poor outcome than babies with lower MDA levels (95% CI 1.64-8.92). Correcting for postnatal day had little effect on this odds ratio. Values ranged from zero to 84 481 with an overall median of 34.0 µmol/mmol creatinine, and there was a dose- response effect; the higher the level of MDA the greater the likelihood of poor outcome. Results did not simply reflect differences in urinary creatinine between the groups.

Results support the view that ROS-related tissue damage is a factor determining outcome in preterm babies. Urinary MDA could provide a useful, relatively non-invasive predictor of outcome, whereas plasma NTBI appears to be less discriminatory.

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Micronutrient and related correlates of visual acuity in people aged 65 years and over, representative of the British population. By J.C. VAN DER POLS[1], C.J. BATES[1], A. PRENTICE[1] and P. SMITH[2], [1]*MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ and* [2]*Social Community Planning and Research, 35 Northampton Square, London EC1V 0ED*

Recent studies suggest that some micronutrients, e.g. those which prevent free radical damage, may help protect ocular tissues such as lens and retina against age-related degeneration which can result in reduced visual acuity (VA). Apart from models, the evidence is derived from epidemiological studies where prevalence of eye diseases is related to nutritional indices. An opportunity to test this in a nationally-representative elderly British population arose from the National Diet and Nutrition Survey of People Aged 65 Years and Over (Finch *et al.* 1997). Fieldwork occurred during 1995-6, the main elements being: a diet and lifestyle questionnaire, a 4 d weighed dietary record, a fasting blood sample for haematology and biochemistry; anthropometric, dental and eye examinations. VA was measured by a 3 m test, including correction with glasses and/or pin-hole, based on Glasgow acuity cards (VA range 0-1.4). This allowed parametric analysis (McGraw & Winn, 1993). A mean value from the best scores from each eye was compared with other relevant indices. Of 2624 people for whom participation was possible, 2059 completed the questionnaire; 1276 gave a blood sample, and 1487 had VA measured. The Table lists those nutritional indices (of 105 tested) which contributed to the variance of VA by both univariate and multiple regression.

	Univariate regression			Multiple regression (955 d.f.)	
Index	d.f.	Coefficient	*t*	Coefficient	*t*
Age (years)	1427	-0.021	-22.0	-0.013	-11.2
Domicile*	1427	-0.350	-18.6	-0.186	-8.2
Sex†	1427	-0.075	-4.3	-0.046	-2.6
Plasma vitamin C (μM)	1107	+0.0036	+9.6	+0.0014	+3.8
Plasma zinc (μM)	975	+0.043	+10.1	+0.017	+4.4
Dietary fibre intake (g/d)	1427	+0.012	+9.4	+0.0035	+2.7

*Living at home = 1; living in an institution = 2.

†Male = 1; female = 2.

A direct relationship implies that VA improves in conjunction with an increase in the independent variable being considered; an inverse relationship implies that it deteriorates. The contribution of each variable in the multiple regression model was significant ($P<0.05$), but the relationships with VA were not as strong as in univariate regression.

These six independent variables accounted for 36.3% of the variance; age and domicile alone accounted for about 33%. Clearly two plasma indices (vitamin C and Zn) plus dietary fibre were significant independent nutritional factors. A follow-up study of cataract and macular degeneration is being piloted, to explore these relationships further.

The authors acknowledge financial support from the Department of Health and the European Commission.

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The effect of cheese consumption as part of a meal on the calcium concentration of dental plaque. By PAULA J. MOYNIHAN, NEIL JENKINS, STEPHEN FERRIER and GARY WOODS. *The Denial School, University of Newcastle, Newcastle upon Tyne NE2 4BW*

Some foods have anticariogenic properties, conveying protection to the teeth. Promoting the consumption of such foods may aid the prevention of dental caries. Consumption of a cube of cheese has been shown to stimulate salivary flow which neutralizes plaque acids, and to increase the Ca concentration of plaque (Rugg-Gunn *et al.* 1975). This helps to saturate the environment of the enamel with $Ca_3(PO_4)_2$ thereby preventing loss of tooth mineral. Animal studies (Edgar *et al.* 1982) and experiments in which human subjects wore oral appliances with slabs of enamel attached (Silva *et al.* 1986) have also shown cheese on its own to be cariostatic and epidemiological studies of adolescents have shown that those with fewest caries consume significantly more cheese (Rugg-Gunn *et al.* 1984). It is, however, unknown whether cheese consumed as a component of a mixed meal is able to increase plaque Ca concentration. If cheese within meals is able to protect against dental caries this would have widespread practical applications for caries prevention. The aim of the present study was to investigate if cheese, consumed as part of a cooked meal, was effective in increasing plaque Ca concentration.

Sixteen adult volunteers abstained from oral hygiene for 48 hours. Plaque samples were obtained before and 5 mins after consumption of one of the following: 15 g cube cheese; pasta in cheese sauce (test meal - containing 15 g Red Leicester); pasta in mushroom sauce (control meal); chicken filled with ham and cheese (test meal - containing 15 g Red Leicester); chicken filled with mushroom and ham (control meal). Each subject tested each meal on a separate occasion. All meals were served with a green salad, garlic bread and unsweetened tea or coffee. Plaque Ca concentration was measured using atomic absorption spectrophotometry (Wright *et al.* unpublished) and results were expressed as μg/mg dry weight. The results are presented in the table.

	Baseline Ca (μg/mg)		Post-meal Ca (μg/mg)		Pre-/ post-meal difference (μg/mg)		
Meal	Mean	SE	Mean	SE	Mean	SE	*P* value
Cheese cube	4.40	0.43	8.64	0.89	4.25	0.78	
Pasta test	5.27	0.49	7.85	0.61	2.58	0.71	0.037
Pasta control	4.02	0.40	4.80	0.56	0.78	0.45	
Chicken test	5.05	0.39	7.21	0.68	2.16	0.74	0.036
Chicken control	3.80	0.33	4.28	0.73	0.49	0.67	

Paired *t* tests of the differences between pre- and post-meal samples were carried out. The pasta and chicken test meals increased plaque Ca to a significantly greater degree ($P=0.037$, $P=0.036$ respectively) than the control meals. No significant difference was found between the magnitude of increase in plaque Ca between the pasta test meal and the neat cheese ($P=0.19$). These result demonstrate that cheese consumed as a component of a meal is able significantly to increase plaque Ca and suggest that meals which contain cheese as a component may be protective against dental caries.

This work was financially supported by The National Dairy Council who the authors gratefully acknowledge.

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Differences in undercarboxylated osteocalcin, a marker of vitamin K status between British and Chinese Women. By SIÂN R. BEAVAN[1], ANN PRENTICE[1], DOROTHY M. STIRLING[1] and LIYA YAN[2], [1]*MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ and Shenyang Medical College,* [2]*Shenyang, People's Republic of China*

Osteoporotic fracture rates are highest in populations of North European origin and are considerably lower in Asians and Africans (Royal College of Physicians, 1989). Osteocalcin, a bone-specific protein, is produced by osteoblasts during bone formation. An elevated proportion of undercarboxylated osteocalcin (UCOC) in plasma has been associated with an increased risk of osteoporotic fracture (Szulc *et al.* 1993). Vitamin K is a cofactor for γ-carboxylase, an enzyme that converts specific protein-bound glutamic acid residues into γ-carboxyglutamic acid. Osteocalcin depends on vitamin K for the carboxylation of three glutamic acid residues. Circulating UCOC is regarded as a sensitive marker of vitamin K status (Bach *et al.* 1996).

The aim of the present study was to compare osteocalcin carboxylation in two populations with differing incidence of osteoporosis. Fasting plasma was obtained from eleven premenopausal and thirty-five postmenopausal Caucasian women living in Cambridge, UK, and eleven premenopausal and twenty-three postmenopausal Han-Chinese women living in Shenyang, North-East China. Osteocalcin was measured by radioimmunoassay using a commercial kit (INCStar) before and after incubation with hydroxyapatite (5 mg/250 µl). UCOC was calculated as the ratio unbound : total osteocalcin based on the fact that the fully carboxylated protein has a higher affinity for hydroxyapatite than undercarboxylated forms (Merle & Delmas, 1990). Chinese and Cambridge samples were assayed together and each estimate was performed in duplicate. The intra-assay precision of duplicate determinations was <4%, the inter-assay reproducibility was <15%.

	Premenopausal				Postmenopausal			
	Cambridge		Shenyang		Cambridge		Shenyang	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Age range (years)	41.6	3.2	30.9	2.7	66.9	1.2	67.7	0.6
UCOC (% total)	6.1	1.8	3.7	0.5	20.6*†	2.3	5.6	1.1
Total OC (µg/L)	2.2	0.3	1.7	0.2	3.7†	0.2	3.2†	0.2

* Significantly different from postmenopausal women in Shenyang using 2-sample *t* test, $P \leq 0.0001$.
† Significantly different from premenopausal women in same country using 2-sample *t* test, $P \leq 0.0002$

There was a low proportion of UCOC in both British and Chinese premenopausal women. British postmenopausal women had a significantly higher proportion of undercarboxylation than either their Chinese counterparts ($P \geq 0.0001$) or premenopausal women ($P \geq 0.0001$). No age-associated increase was observed in the Chinese group. Total osteocalcin was higher post- than premenopause in both British and Chinese women ($P \geq 0.0002$) and there was no significant difference between countries either premenopause ($P = 0.13$) or postmenopause ($P = 0.19$). This result demonstrates that there are considerable differences in the carboxylation of osteocalcin in postmenopausal women in Britain and China and may represent differences in extrinsic or intrinsic factors such as the supply and/or utilization of vitamin K. This is consistent with an association between undercarboxylation and osteoporotic fracture risk.

Liya Yan is supported by the Nestlé Foundation and the Sandoz Foundation for Gerontological Research.

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Assessment of risk factors for osteoporosis: comparison between hip fracture patients and healthy age-matched controls. By S. A. NEW[1], M. C. MURPHY[1], M. H. CREEDON[1], C. A. R. PITHER[1], M. W. J. OLDER[2] and M. LUMBERS[3], [1]*Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH,* [2]*Department of Orthopaedic Surgery, Royal Surrey County Hospital, Guildford GU2 5XX.* [3]*Centre for Food and Health Care, Department of Management Studies, University of Surrey, Guildford GU2 5XH*

Osteoporosis is a major public health problem currently resulting in 50 000 hip fractures per year in the UK alone (Cooper, 1993). The future health and economic impact of this condition is likely to be phenomenal since by the year 2030 it is predicted that elderly people will account for one in four of the adult UK population. Such demographic changes are likely to be paralleled with increases in the number of hip fractures from 1.66 million in 1990 to 6.26 million by 2050 (World Health Organisation, 1994).

Identification of risk factors for osteoporosis has yet to be fully quantified. The present abstract reports the results of a pilot study aimed to identify both dietary and non-dietary risk factors in a group of hip fracture patients compared with healthy age-matched controls.

Fifteen hip fracture patients from the local hospital and eighteen elderly women attending a Surrey day-centre were recruited during the months of October to January. All subjects obtained a score of ≥ 7 for the abbreviated mental function test. Age, weight and demispan were recorded. Menstrual history (including age of menarche, age of menopause & parity), lifestyle factors (including recent bereavement, food provision, recent falls, physical activity level and smoking habits) were assessed by questionnaire. Recent & distant past dietary habits (including intakes of Ca rich foods) were also recorded.

As shown in the Table below, the groups were well matched for age. A history of smoking was associated with an increased risk of hip fracture, but other lifestyle and menstrual factors were not significant. Consumption of Ca rich foods during childhood (up to 12 years) was higher in the cases.

	Cases (*n* 15)		Controls (*n* 18)			Cases (*n* 15)		Controls (*n* 18)	
	Mean	SD	Mean	SD		%	n	%	n
Age (years)	76.1	7.9	75.7	8.4	Smoking*	67	10	33	6
Weight (kg)	60.8	7.8	67.1	11.2	Fall history	33	5	33	6
Demispan (cm)	71.5	4.7	66.4	18.1	Living alone	40	6	67	12
BMI (kg/m^2)	25.2	3.6	27.2	4.4	Ca intake during childhood*				
Age menarche (years)	11.9	5.3	13.3	3.7	Low	7	1	11	2
Age menopause (years)	42.2	18.4	42.5	16.6	Med	67	10	83	15
Physical activity level	1.7	0.1	1.7	0.2	High	27	5	6	1

* Significant association χ^2 $P<0.05$.

These preliminary results would appear to indicate little difference in potential risk factors between hip fracture patients and the healthy elderly population. Results of the distant past Ca intake data are unexpected but may be due to recall bias and should therefore be treated with some caution. They are in agreement, however, with the findings of a recent study (Cumming & Klineberg, 1994) and thus certainly warrant more attention. Further identification of lifestyle differences between cases and controls that may be amenable to change are required.

We are grateful to the nursing staff of the Department of Orthopaedic Surgery for their assistance with subject recruitment.

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Calcium supplementation increases the bone mineral status of Gambian children. By BAKARY DIBBA, ANN PRENTICE, ELIZABETH M. E. POSKITT and TIM J. COLE, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ and Keneba, The Gambia*

Growth of rural Gambian children is poor, puberty is delayed and bone mineral status is low compared with British and American children (Lo *et al.* 1990; Prentice *et al.* 1990). Ca intake in rural areas of The Gambia is low (300-400 mg/d) due to limited consumption of dairy produce (Prentice *et al.* 1990). The present study examined, by means of a randomized, double-blind, placebo-controlled supplementation study, whether an increase in Ca intake improves growth and bone mineral status in Gambian children. Children (*n* 160: 80M, 80F) aged 8.3-12.0 years (mean age: 10.3 (SD 1.0) years) were randomized to receive 1000 mg Ca/d (calcium carbonate, Calcichew, Shire Pharmaceuticals) or matching placebo, 5 d/week for 12 months. Tablets were consumed under supervision and compliance was 100%, resulting in an increased Ca intake of 714 mg/d in the Ca group. Bone mineral content (BMC), bone width (BW) and bone mineral density (BMD) of mid-shaft (2/3 site) and distal radius (5 mm site) were measured at baseline and after 12 months (outcome) by single photon absorptiometry (Lunar SP2). Multiple regression analysis, with continuous variables transformed to natural logarithms, was used to evaluate the effect of supplementation and to correct BMC for bone and body size (Prentice *et al.* 1994). Boys and girls were analysed together since there were no differences between the sexes in any variable.

	Baseline				Outcome			
	Calcium		Placebo		Calcium		Placebo	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Mid-shaft radius								
BMC (g/cm)	0.429	0.011	0.414	0.010	0.475	0.010	0.448	0.011
BW (cm)	0.998	0.012	0.978	0.012	1.014	0.011	1.010	0.012
BMD (g/cm^2)	0.428	0.008	0.421	0.008	0.467	0.007	0.441	0.008
Distal radius								
BMC (g/cm)	0.419	0.011	0.388	0.012	0.487	0.015	0.423	0.012
BW (cm)	1.828	0.021	1.777	0.022	1.903	0.022	1.827	0.021
BMD (g/cm^2)	0.227	0.005	0.217	0.005	0.253	0.006	0.231	0.006

There were no significant differences between the groups at baseline. Ca supplementation for 12 months resulted in higher BMC, BMD and size-adjusted BMC compared with placebo. The percentage differences at outcome between Ca and placebo groups, after correcting for baseline value, were: mid-shaft radius, BMC = +4.0 (SE 1.8)%, $P = 0.026$, BMD = +5.1 (SE 1.3)%, $P = 0.0002$, size-adjusted BMC = +5.4 (SE 1.3)%, $P \leq 0.0001$; distal radius, BMC = +8.4 (SE 3.2)%, $P = 0.009$, BMD = +7.0 (SE 2.7)%, $P = 0.011$ and size-adjusted BMC = +5.5 (SE 2.7)%, $P = 0.042$. These differences were not affected by age, sex or pubertal stage. Ca supplementation had no significant effect on height (-0.0 (SE 0.0)%, $P = 0.88$), weight (+0.8 (SE 0.8)%, $P = 0.32$) or BW at either the mid-shaft radius (-1.0 (SE 0.8)%, $P = 0.21$) or distal radius (+2.0 (SE 1.2)%, $P = 0.086$).

This study has demonstrated that bone mineral acquisition can be enhanced by Ca supplementation in children accustomed to a low Ca intake. Further work is in progress to establish whether this effect is associated with changes in bone turnover and whether it persists after the supplement is withdrawn.

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The effect of maternal vitamin A status on the ATP/ubiquitin-dependent proteolytic pathway in fetal and neonatal development in the rat. By C. ANTIPATIS, M.G. THOMPSON, R.M. PALMER and C.J. ASHWORTH, *Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB*

Maternal vitamin A plays an important role in fetal cellular differentiation and maturation and affects fetal and neonatal viability. Effects of maternal vitamin A deficiency on fetal and neonatal viability and muscle metabolism in fetal and neonatal hind limb were examined. Female rats received, *ad libitum*, diets which were either vitamin A-free (VAF; *n* 8) or vitamin A-sufficient containing 7 mg retinol acetate/kg diet (VAS; *n* 8) from weaning and were mated at 10 weeks of age. Fetuses and neonates were examined on day 20 of pregnancy or within 6 h after birth (all *n* 4). The proportion of viable fetuses on day 20 did not differ (VAS: 0.96 *SE* 0.04) *v.* (VAF: 0.95 *SE* 0.03). However, the proportion of viable neonates was higher on VAS diet (VAS: 0.85 *SE* 0.09) *v.* (VAF: 0.48 *SE* 0.1, $P<0.05$). Parturition was delayed by 1 d by VAF diet (VAS: 22.5 d *v.* VAF: 23.5 d, $P<0.001$). Maternal plasma retinol concentrations were higher for dams fed on the VAS diet (VAS: 0.168 *SE* 0.012 ng/ml) *v.* (VAF: 0.075 *SE* 0.009 ng/ml, $P<0.001$) and were positively related to the litter size.

The ATP/ubiquitin dependent proteolytic system has been implicated in the control of muscle proteolysis and growth. Northern blots were performed on fetal and neonatal muscle from rats on the two diets, using probes for 6.8 kDa ubiquitin (UB) and the 14 kDa E_2 conjugating enzyme of the ATP/ubiquitin-dependent proteolytic (AUP) system (Dardevet *et al.*, 1995) and counted using a Packard imager. The data (^{32}P (cpm/band)) are shown in the Table.

Diet ...		VAF		VAS	
		F	N	F	N
E_2	1.2 kb	2059	7074	2342	24069
	1.8 kb	645	1405	840	8759
UB	0.9 kb	2032	3101	3507	16344
	1.2 kb	4392	8364	5866	26279
	2.4 kb	13862	18380	11475	32397

F, fetus; N, neonate.

Both E_2 mRNA transcripts (1.2 and 1.8 kb) were expressed at a low level in the fetuses and were more strongly expressed in the neonates. On the VAS diet E_2 expression was increased 10 fold between the day 20 fetus and the newborn rat, on the VAF diet the increases were smaller (2-3 fold). Three transcripts of UB mRNA were examined in a second group of animals, all were higher in neonates from VAS than from VAF-fed mothers and again, fetal tissues showed less expression than neonatal tissues.

These data suggest that, in the rat, there is a rapid stimulation of expression of mRNAs for components of the AUP system between the 20th day of pregnancy and the first few hours of postnatal life. Alternatively, fetal AUP components may be poorly recognized by probes to the adult forms of AUP components. The rapid postnatal expression of UB and E_2 appears to be dependent on maternal vitamin A intake. Collectively, these results suggest that maternal vitamin A intake affects neonatal survival and AUP proteolytic system between day 20 of pregnancy and day of parturition.

This work was supported by SOAEFD and the Greek State Foundation.

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Hungry season birth predicts excess childhood and adult mortality in rural Gambia. By SOPHIE E. MOORE, TIMOTHY J. COLE and ANDREW M. PRENTICE, *MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH and Keneba, The Gambia.*

The profound seasonality in rural Gambia results in variations in food intake and energy expenditure. During each annual hungry season (July-October) weight loss in pregnant women results in impaired fetal growth and small-for-date babies. These effects are known to be nutritionally mediated since they are reversed by maternal dietary supplementation (Ceesay *et al.* 1997). Seasonal growth faltering occurs in children (Cole, 1993). Patterns of disease also exhibit extreme seasonal changes with malaria and diarrhoeal disease prevailing in the hungry (wet) season.

This seasonality has been documented in the three rural villages of Keneba, Kantong Kunda and Manduar since 1949 alongside detailed recording of births and deaths providing a dataset of 3102 individuals aged 0-45 years with known month of birth and current fate (alive, 2025; known date of death, 1077). This population was used to test the hypothesis that hungry season birth predicts future survival by acting as a proxy indicator for early life exposure to nutritional and infectious stresses.

ANOVA revealed highly significant month-of-birth effects with highest mortality in people born July-December (2 months longer than the usual wet season divide). Kaplan-Meier survival plots (not shown) showed broadly similar mortality patterns until puberty. Thereafter there was a marked divergence with much higher mortality among hungry season births. Odds ratios and log rank test significance values are shown in the Table.

	Harvest season births (Jan-Jun)		Hungry season births (Jul-Dec)					
Age (years)	Alive	Dead	Alive	Dead	Overall total	Odds ratio	95% Confidence intervals	Log rank P value
All ages	979	496	1046	581	3102	1.10	0.94-1.28	0.1319
≥ 3	862	103	903	153	2021	1.42	1.08-1.86	0.0074
≥ 5	797	48	839	73	1757	1.44	0.98-2.12	0.0421
≥ 15	405	10	422	38	875	3.65	1.77-7.52	0.0001
≥ 25	169	2	157	17	345	9.15	2.02-41.48	0.0006

These findings indicate that key events during early life related to month-of-birth have a life-long consequence ultimately manifesting in premature death. This clearly parallels the 'infant and fetal origins of adult disease' theory proposed by Barker (1994). However the current finding is unique since the causes of mortality in this population are predominantly infectious and none are chronic diseases of affluence. It is probable that this phenomenon is mediated through impaired immune function. Studies are currently underway to investigate this further.

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Associations of skeletal proportions with metabolic disorders in adults. By THANG S. HAN,[1] JEREMY P. HOOPER,[2] CAROLINE E. MORRISON[2] and MICHAEL E.J. LEAN,[1] *[1]University Department of Human Nutrition and [2]Glasgow MONICA Project, Royal Infirmary, Glasgow G31 2ER.*

Early growth failure is suggested to result in altered organ and body morphology and function, and associated metabolic disorders in later life (Barker, 1994). The present study explored the associations between height, lower leg length (LLL) and demi-arm span (demi-AS) with major predisposing cardiovascular risk factors and with the presence of CHD (angina and/or angioplasty and/or heart attack and/or coronary artery bypass graft), in a subsample of 543 men and 646 women, aged 25-66 years from the fourth Glasgow MONICA (monitoring coronary risk factors) study. LLL was measured as the distance from top of the patella to the sole, lying supine, and demi-AS as the horizontal distance from web of hand between middle and fourth fingers to midpoint of sternal notch, in sitting position.

The Table shows the ANOVA with adjustments for age, social class and smoking. In men, short height, short LLL and short demi-AS were associated with hypercholesterolaemia, and long LLL was associated with diabetes mellitus. In women, short height and LLL were associated with CHD. High LLL:height or LLL:demi-AS ratios were associated with diabetes mellitus in men. In women, high demi-AS:height ratio or low LLL:demi-AS ratio was associated with CHD.

		Body lengths						Ratios (skeletal proportions)					
	Prevalence	Height		LLL		Demi-AS		LLL: height		Demi-AS: height		LLL: demi-AS	
	(%)	F	P	F	P	F	P	F	P	F	P	F	P
Men													
Hypertension*	22.9	1.7	0.19	0.4	0.52	0.4	0.52	0.2	0.65	0.5	0.49	0.0	0.86
Hypercholesterolaemia†	29.9	9.8	0.01	5.3	0.02	6.2	0.01	0.0	0.84	0.0	0.97	0.0	0.87
CHD	10.0	0.1	0.72	0.0	0.95	0.1	0.75	0.1	0.74	0.0	0.92	0.1	0.79
Diabetes mellitus‡	3.0	1.0	0.33	4.1	0.04	0.2	0.64	5.3	0.02	0.2	0.64	5.7	0.02
Women													
Hypertension	13.5	0.8	0.36	0.1	0.80	1.0	0.31	0.5	0.49	0.1	0.73	0.8	0.38
Hypercholesterolaemia†	29.1	0.0	0.99	1.1	0.30	0.7	0.39	3.3	0.07	1.9	0.17	0.3	0.59
CHD	8.5	6.7	0.01	6.2	0.01	1.0	0.31	1.0	0.32	4.3	0.04	5.9	0.02
Diabetes mellitus‡	1.8	0.1	0.80	1.2	0.27	0.2	0.67	2.4	0.12	0.0	0.84	1.1	0.29

*Systolic ≥160 and/or diastolic blood pressure ≥95 mm Hg and/or on medication for hypertension.
†Plasma cholesterol ≥6.5 mmol/l.
‡Adult onset diabetes mellitus diagnosed by doctor.

Short stature and limb lengths, and also altered skeletal proportions, which may reflect interrupted growth, are associated with several metabolic disorders. Skeletal disproportion associates with diabetes mellitus in men and CHD in women.

Barker, D.J.P. (1994). *Mothers, Babies, and Disease in Later Life*. London: British Medical Journal.

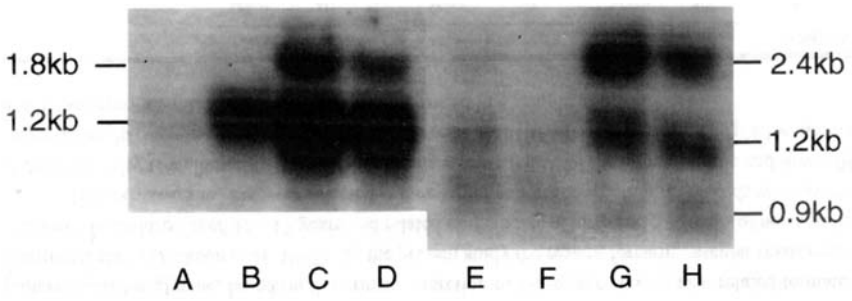
The role of the ATP/ubiquitin-dependent proteolytic pathway in maternal and fetal muscle of sheep fed moderate or high food intakes. By R.M. PALMER, M.G. THOMPSON, A. THOM, R.P. AITKEN and J.M. WALLACE, *Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB*

Rapidly growing, adolescent, pregnant ewes on high food intakes showed a reduction in the weight of both placenta and fetus at term (Wallace *et al.* 1996). Effects of this dietary regimen on fetal and maternal muscle metabolism were examined. Adolescent ewes (200 d of age) with a singleton pregnancy (*n* 4/group) consumed a mean of 5 (moderate intake, M) or 15 kg/week (high intake, H) of a diet providing 10.2 MJ metabolizable energy and 137g crude protein/kg, from days 4 - 104 of pregnancy. At 104 d ewes on diets M and H weighed 50.0 (*SE* 1.7) and 76.5 (*SE* 4.5) kg ($P<0.001$) respectively. Four maternal hindlimb muscles (gastrocnemius, plantaris, soleus and vastus) were on average 23% larger in intake H ewes, but three fetal muscles (gastrocnemius, plantaris and tibialis anterior (TA)) were smaller at this intake. For example fetal TA from H and M intake ewes contained respectively 79 (*SE* 5) *v.* 131 (*SE* 9) mg protein, 2.4 (*SE* 0.2) *v.* 3.2 (*SE* 0.2) mg RNA and 5.0 (*SE* 0.3) *v.* 7.1 (*SE* 0.6) mg DNA (all *n* 4; $P<0.05$).

Northern blots using a probe for the E_2 conjugating enzyme of the ATP/ubiquitin -dependent proteolytic system on fetal muscle from ewes on nutritional levels M and H and maternal muscles at levels M and H are shown in lanes A - D respectively. In the same order, lanes E - H show results from a probe against ubiquitin (UB).

Both UB components were more abundant in the mother than in her fetus. The 1.2 and 1.8 kb E_2 transcripts and the 2.4 and 1.2 kb mRNA encoding UB were present in greater abundance in the slower-growing diet M ewes (lanes C,G) than in diet H ewes (lanes D,H). E_2 mRNA was detected in the smaller, diet H fetus (lane B) but was scarcely detectable in the diet M fetus (lane A). The mRNA encoding UB was also expressed poorly in fetal muscle (lanes E,F).

If the UB system is involved in proteolysis in the fetus, the higher metabolic rate and remodelling of the rapidly growing fetus suggests that the UB system should be highly active in fetal life. Higher maternal levels, as indicated here, suggest that myofibrillar proteolysis in the fetus may use different UB components, with mRNA poorly recognized by the probes which recognize adult forms, or that a different, non-UB dependent proteolytic system degrades myofibrillar proteins in the fetus.



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Influence of maternal nutrition during early pregnancy on placental development and survival after birth in sheep. By LINDSAY HEASMAN, LYNNE CLARKE, KAREN FIRTH and MICHAEL E. SYMONDS, *School of Animal and Microbial Sciences, The University of Reading, Whiteknights, PO Box 228, Reading RG6 6AJ*

Maternal nutrition during the first half of pregnancy has been proposed to have a major influence on placental growth, which normally peaks at mid gestation in sheep (Kelly, 1992). Placental size is known to have a pivotal role in the regulation of fetal growth, and this has been highlighted in recent studies in which altered placental size was associated with an increased incidence of hypothermia in near-term lambs delivered by Caesarean section (Symonds & Clarke, 1996). The present study aimed to determine the effect of high and low levels of nutrition, between 30 and 80 d of gestation on placental development and outcome after birth.

Fifteen Welsh Mountain ewes were entered into the study. At 30 d of gestation (term=147 d) ewes were individually housed and fed daily on a diet of chopped hay and a barley-based concentrate to supply either 50% of the energy requirements for maintenance and pregnancy (0.5M; 85-145 g concentrate, plus 370-610 g hay, *n* 9) or 200% of energy requirements (2M; 205-260 g concentrate, plus 890-1125 g hay, *n* 6). All diets contained adequate minerals and vitamins. Ewes were scanned by ultrasound 42 d after mating in order to confirm that they were monotocous. All ewes were fed to maintenance for the remainder of pregnancy and lambs delivered by Caesarean section into a cool ambient temperature of 15° at 143-145 d of gestation. Only four lambs born to 0.5M-fed ewes survived beyond 30 min of life and so were considered as a separate group (0.5MS) from those which failed to survive (0.5MD). Placentas were separated into fetal cotyledonary and maternal caruncular tissue.

	Placental weight(g)							
	Maternal		Fetal		% Fetal		Fetal weight (g)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0.5MD	111	8	318	23	74	0.5	3760	200
0.5MS	114	4	253	19	69	1.3	3610	110
2M	117	11	262	18	69	1.7	3680	130

The level of maternal nutrition during early to mid gestation had a significant influence on outcome at birth that appeared to be linked to the weight of the fetal cotyledonary component of the placenta. This was higher (ANOVA; $P=0.08$) in lambs that failed to survive. These low-fed ewes were also characterized as possessing placenta with an increased ($P<0.05$) proportion of fetal tissue. The maternal caruncular component of the placenta was not significantly affected by maternal nutrition and there was no difference between groups with respect to lamb birth weight.

In conclusion, a reduced level of maternal nutrition between 30 and 80 d gestation can compromise adaptation after birth if this is accompanied by a compensatory increase in the fetal component of the placenta.

This work was funded by the Wellcome Trust and L.H. is supported by a Ministry of Agriculture, Fisheries and Food Studentship.

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Urinary 5-L-oxoproline excretion in teenagers in Jamaica is related to fetal growth and vascular haemodynamics. By RACHEL C. SHERMAN[1], SELBY NICHOLS[2], TERRENCE FORRESTER[2] and ALAN A. JACKSON[3], *[1]Nutritional and Biological Sciences Department, University of Newcastle upon Tyne, Newcastle upon Tyne NE2, [2]Tropical Metabolism Research Unit, University of the West Indies, Jamaica and [3]Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX*

The nutritional status of mothers during pregnancy is closely related to the blood pressure of the child at 10 - 12 years of age (Godfrey *et al.* 1994). Further, the ability of the children to satisfy their metabolic requirements for glycine, based on the urinary excretion of 5-L-oxoproline, is also related to maternal nutritional status (Jackson *et al.* 1997). In the present study the resting forearm vascular resistance was measured in children aged 15 - 17 years and related to their size at birth and 5-L-oxoproline excretion.

The sixty two subjects were selected from a larger cohort for whom birth records were available at the University Hospital of the West Indies, being representative of the upper, middle and lower fifths for birth weight category. Resting forearm vascular resistance (RFVR) was measured by plethysmography and 5-L-oxoproline relative to creatinine was measured in a sample of urine.

	Lower fifth *n* 22		Middle fifth *n* 18		Upper fifth *n* 22		ANOVA
	Mean	SD	Mean	SD	Mean	SD	*P* value
Birth weight (kg)	2.67	0.30	3.16	0.10	3.69	0.33	0.000
Placenta: birth weight	22.7	9.5	20.0	4.9	17.0*	4.2	0.022
Weight (kg)	55.8	11.6	60.1	14.4	63.5	14.2	0.17
RFVR	25.8	9.8	21.6	10.5	20.7	7.3	0.17
5-L-oxoproline (μmol/mmol creatinine)	44.5	25.0	34.8	20.3	35.9	12.7	0.29

*significantly different to lower fifth, ANOVA and post-hoc Scheffe's test.

The highest values for RVFR and 5-L-oxoproline were seen in the lowest birth-weight category, but these differences did not achieve statistical significance. With RFVR as the dependent variable in a multiple linear regression analysis, there was a highly significant relationship ($P = 0.006$) when current weight, BMI and 5-L-oxoproline were included in the model. For 5-L-oxoproline there was a highly significant relationship ($P = 0.003$) when placenta:birth weight ratio, RFVR and triceps skinfold were included in the model.

In children with lower birth weight there was a tendency towards higher forearm vascular resistance and a higher rate of 5-L-oxoproline excretion. RFVR is related to anthropometric measures and 5-L-oxoproline excretion, whereas for 5-L-oxoproline excretion, aspects of placental and fetal growth are also of importance. In experimental studies, early vascular development is perturbed by folate antagonists (Berry & Looker, 1973), which interfere with the formation of glycine. These data provide further support for the proposal that vascular competence is programmed by growth *in utero* and also indicate that the metabolism and availability of glycine might be intimately involved in this process.

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Programming of endogenous glycine formation by maternal nutritional status during pregnancy. By A.A. JACKSON[1], K. GODFREY[2], T. FORRESTER[3], D.J.P. BARKER[2], C. PERSAUD[1], J.P. LANDMAN[1,3], J. StE. HALL[3], V.COX[2] and C. OSMOND[2], *[1]Institute of Human Nutrition and [2]Medical Research Council Environmental Epidemiology Unit, University of Southampton, Southampton SO16 7PX, and [3]Tropical Metabolism Research Unit, University of the West Indies, Jamaica*

Aspects of fetal growth are closely associated with risk of chronic disease (Barker, 1994). In a prospective study, the nutritional status of mothers in Jamaica was related to the blood pressure of the child at 10 - 12 years of age (Godfrey *et al.* 1994). Fetal growth requires adequate glycine, formed in the mother, the placenta and fetus with folate as a cofactor for the glycine cleavage system. Increased urinary excretion of 5-L-oxoproline marks glycine insufficiency. Excretion is increased in pregnancy and in the newborn (Jackson, 1991). Here, we have related the excretion of 5-L-oxoproline at 10 - 12 years of age, to maternal nutritional status during pregnancy, assessed by her body composition and erythrocyte folate.

The mothers participated in a prospective study of nutrient intake and body composition during pregnancy between 1979 and 1981 (Landman & Hall, 1989). The children were contacted and a sample of urine was collected for the measurement of 5-L-oxoproline and creatinine.

Mother antenatal erythrocyte folate (μg/l)	Child 5-L-oxoproline (μmol/mmol creatinine)	Mother MUAC (mm) at 15 weeks gestation	Child 5-L-oxoproline (μmol/mmol creatinine)
<290 (*n* 11)	53.8	<24.5 (*n* 10)	52.1
291-360 (*n* 10)	38.8	24.6-26.0 (*n* 10)	41.7
361-460 (*n* 9)	37.7	26.1-28.5 (*n* 12)	37.3
>460 (*n* 11)	31.5	>28.5 (*n* 10)	31.4
All (*n* 41)	40.2	All (*n* 42)	40.2
P for trend	0.08		0.01

Urinary 5-L-oxoproline excretion increased with decreased maternal MUAC, and increased as erythrocyte cell folate decreased. With 5-L-oxoproline excretion as the dependent variable in a multiple regression analysis, there was a highly significant relationship (R^2 0.37, n 31) with an explanatory model which included maternal variables (MUAC, $P = -0.007$; weight, $P = 0.09$; biceps skinfold, $P = 0.05$; erythrocyte folate, $P = -0.3$).

The nutritional status and body composition of the mother during pregnancy was related to the excretion of 5-L-oxoproline in the child, suggesting a limited ability to satisfy the needs for glycine in children whose mothers were lighter and thinner, with a low erythrocyte folate. Glycine is used to form metabolically important compounds such as haem, glutathione, creatine, nucleotides, bile salts and collagen. Any limitation on its availability would generate competitive demands for its use and could exert widespread effects on growth and function of the individual. The present results are compatible with the hypothesis that glycine formation, or functions closely related to it, is programmed *in utero,* in relation to the nutritional status of the mother.

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Effect of a low-protein maternal diet on energy metabolism during organogenesis. By S. GAIL ISHERWOOD-PEEL, FRANCHESCA D. HOUGHTON and HENRY J. LEESE, *Department of Biology, University of York, PO Box 373, York YO1 5YW*

Epidemiological evidence has suggested a link between an adverse intra-uterine environment and the health of the offspring in later life (Barker, 1993). Langley & Jackson (1994) have shown that the offspring of rat mothers maintained on a 90g protein/kg diet throughout pregnancy have a higher systolic blood pressure than the offspring of mothers maintained on a 180g protein/kg diet. Our aim has been to investigate when during early development such defects might arise and the nature of these defects. Since we were unable to discover any impairment of development at the preimplantation stage (Leese *et al.* 1994), we have focused on organogenesis which, in the rat, occurs between day 9.5 and 12.5 of gestation. The energy metabolism of the conceptus has been measured in terms of the consumption of O_2 and glucose and the formation of lactate.

Wistar rats (8 weeks old) were fed on isoenergetic diets containing either 180 g (control) or 90 g (low) protein/kg for 2 weeks before mating and up to the appropriate day of gestation. Animals were taken at 14.00 hours since, by convention, mating is assumed to occur at the mid-point of the dark cycle (24.00 hours) so that the day following mating is designated day 0.5. On days 9.5, 10.5, 11.5 and 12.5 post-mating, pregnant rats were killed by cervical dislocation, decidua removed from the uterus and placed in M2 medium at 37°. Embryos were dissected free from the decidual mass and transferred to fresh M2 medium. For 9.5 and 10.5 d embryos, the Reichart's membrane was removed and the embryos incubated intact. For 11.5 and 12.5 d embryos, the Reichart's membrane was removed and embryos were dissected into ten and twelve component parts respectively which were incubated individually. All incubations were carried out in drops of M2 medium under oil for 1.5 h. At the end of incubation, embryos or component parts were removed, washed in phosphate-buffered saline and frozen in 5% CHAPS detergent at -20°. Spent incubation drops were frozen at -20° before analysis of glucose and lactate concentration. Embryos were dissolved by sonication and their protein content measured using Coomassie Brilliant blue. The O_2 consumption of intact 9.5 and 10.5 d embryos was assessed in separate experiments by the increase in fluorescence of pyrene according to the method of Houghton *et al.* (1996). Some rats from each diet were allowed to litter and the blood pressure of the offspring measured at 9 weeks according to the method of Langley & Jackson (1994). For ease of comparison, data for the 11.5 and 12.5 d embryos are expressed as an average of the values of the component parts. The results are expressed in terms of embryo protein content.

		Glucose consumption (pmol/μg protein per h)		Lactate formation (pmol/μg protein per h)		O_2 consumption (pl/μg protein per h)		Protein content (μg)	
g protein/kg		90	180	90	180	90	180	90	180
9.5 d	Mean	352*	535	858	992	5209	5343	25	24
	SEM	24	46	68	81	948	279	2	2
10.5 d	Mean	357*	294	938*	761	2412	2208	119	119
	SEM	15	24	57	52	260	187	6	7
11.5 d	Mean	208	199	366	348	-	-	612	683
	SEM	32	28	73	48			30	20
12.5 d	Mean	134	126	277	304	-	-	2126*	1748
	SEM	23	18	33	43			133	107

* Mean values were significantly different from 180g/kg for the same day post-mating ($P<0.05$).

There were no differences in glucose consumption and lactate production between the 90 g/kg and 180 g/kg groups on days 11.5 and 12.5, however on day 10.5 post-mating the rates of glucose consumption and lactate production in the 90 g/kg group were significantly higher than in the 180 g/kg group and on day 9.5 the glucose consumption in the 90 g/kg group was significantly lower than in the 180g/kg group. There were no differences between the O_2 consumption values in the two groups on either day. The protein content of the day 12.5 embryos in the 90 g/kg group was significantly higher than in the 180 g/kg group. The blood pressure of the offspring from the 90 g/kg diets was significantly higher than in the offspring from the 180 g/kg (150 versus 138 mmHg).

These results suggest that there is a transient rise in glucose metabolism in the day 10.5 embryo in response to feeding a low (90 g/kg) protein maternal diet. This response is typical of cells under stress. The increase in protein content at day 12.5 also supports data showing an accelerated growth of the fetus in response to a low protein diet (Langley-Evans *et al.* 1996).

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Birth weight, early feeding and body mass index at age 16-17 years by H.D. McCARTHY[1] and H.F. CRAWLEY[2], *[1]School of Life Sciences and [2]Statistics, Operational Research and Probability Methods Research Centre, University of North London, Holloway Road, London N7 8DB*

It has been suggested that intrauterine growth retardation is associated with an increased propensity to overweight in later life (Law *et al.* 1992). Furthermore, breast-feeding may offer protection against obesity in infancy, and obesity is known to track from infancy to adulthood (Braddon *et al.* 1986). Following our initial investigation into birth weight and subsequent obesity (McCarthy & Crawley, 1997) we have further utilized data from the 1970 longitudinal birth cohort study to examine the relationships between birth weight and early feeding practices, and BMI at age 16-17 years.

A sub-sample of 1183 respondents (M 479, F 704) was isolated from the 1970 longitudinal birth cohort study (Chamberlain *et al.* 1973). Selection criteria included birth weight (BW), early feeding and height and weight at 16-17 years. To investigate associations between BW, early feeding and teenage BMI, explanatory variables associated with BW (such as prematurity, parity, maternal diabetes) and other external factors such as social class, region, parental education, and smoking were included as appropriate. Models were created in GLIM4 (Francis *et al.* 1993) which best explained variations in BMI observed.

After correcting for external factors which affected BW, a significant positive ($P<0.005$) relationship between BW and BMI was observed for the sample, with heavier babies becoming heavier teenagers. Greater BMI was, however, also associated with being a non-breastfed low- birth-weight-baby (BW<2500 g) and this relationship was particularly significant among males ($P<0.001$).

It could be suggested that the mechanism by which low birth weight is associated with later obesity may be in part explained by the lower incidence of breastfeeding among low-birth-weight babies. Mechanisms by which bottle feeding has been suggested as possibly contributory to overweight include the constant concentration and flavour of formula milk which, unlike breast milk, may not allow the development of an appetite-control mechanism, and an effect on endocrine systems caused by slower gastric-emptying in bottle-fed babies (Hall, 1975).

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Nutritional regulation of GLUT4 gene expression in skeletal muscle during postnatal development. By M. KATSUMATA, K. A. BURTON and M. J. DAUNCEY, *Department of Cellular Physiology, The Babraham Institute, Cambridge CB2 4AT*

The insulin-sensitive glucose transporter, GLUT4, is the major facilitative glucose transporter in mammalian skeletal muscle. It is essential for sustained growth and normal cellular glucose metabolism (Katz *et al.* 1995). Understanding of the factors regulating the GLUT4 gene will therefore provide insight into mechanisms underlying normal growth and development. However, evidence on nutritional regulation of GLUT4 in muscle is limited, inconsistent, and focuses only on the extreme conditions of prolonged fasting and refeeding. The aim of the present investigation was, therefore, to determine the role of mild undernutrition, that nevertheless allows growth, on glucose transporter function in skeletal muscle at the molecular and cellular levels.

A 2 x 2 factorial design was used, with two ambient temperatures (35° and 26°) and two levels of food intake (high (H) and low (L), where H = 2L), thus enabling the overall effects of energy balance to be assessed. Amounts of food increased as animals grew and final intakes were 700 g and 350 g per day for H and L respectively. Six litters each of four male pigs were weaned at 3 weeks, and each littermate was assigned to one of the four treatments. At 7 weeks animals were killed humanely at 20-24 h after the last meal, for measurement of (i) GLUT4 mRNA levels in *longissimus dorsi* and *rhomboideus* muscles, by RNase protection assay using a riboprobe based on the porcine GLUT4 cDNA sequence, followed by quantitative image analysis (values in Table are optical densities = OD); and (ii) basal and insulin-stimulated 2-deoxy-glucose uptake of an isolated muscle, *flexor digitorum*.

Temperature (T)	35°		26°		Pooled	ANOVA		
Intake (I)	H	L	H	L	SE	T	I	Interaction T x I
Growth rate (g/d)	323	170	320	146	3	$P<0.01$	$P<0.01$	$P<0.01$
Efficiency of growth (g weight gain/g food)	0.95	0.97	0.92	0.79	0.02	$P<0.001$	$P<0.01$	$P<0.001$
GLUT4 mRNA (OD): *Longissimus dorsi*	6.6	10.4	5.7	12.8	1.2	NS	$P<0.001$	NS
Rhomboideus	9.2	13.2	8.8	16.0	1.3	NS	$P<0.001$	NS

A low food intake resulted in a striking increase in skeletal muscle GLUT4 mRNA levels, and the effect was greater at 26° than at 35° suggesting that energy balance also regulates GLUT4 gene expression. Results for insulin-stimulated 2-deoxy-glucose uptake showed similar trends to those for GLUT4, with the L intake resulting in higher absolute values. However, the 26°L group had the highest basal glucose uptake and consequently had the lowest increment in insulin-stimulated glucose uptake (approximately 20% compared with 70% in the three other groups). Moreover, the 26°L animals had lower growth efficiencies than the other groups ($P<0.05$). This suggests that the sub-optimal energy balance of the 26°L group may have affected the subcellular distribution of glucose transporters between the intracellular pool and the plasma membrane. It is concluded that (i) postnatal undernutrition, which is mild enough to allow growth, paradoxically upregulates GLUT4 gene expression in skeletal muscle; and (ii) the degree of upregulation of GLUT4 mRNA, and the subcellular distribution and function of glucose transporter proteins are dependent on energy balance.

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Blood pressure changes programmed by exposure to maternal protein restriction are transmitted to a second generation through the germ line. By SIMON C. LANGLEY-EVANS, REBECCA L. DUNN and ALAN A. JACKSON, *Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX*

Hypertension and CHD are programmed *in utero* by exposure of the fetus to maternal undernutrition. Low weight at birth and other indices of fetal growth retardation correlate well with adult blood pressure. These observations are reproducible in animals and it has been demonstrated that the feeding of low protein diets to rats during pregnancy, induces the development of hypertension in the offspring (Langley-Evans *et al*, 1996). Human observations suggest that maternal weight at birth is as strong a predictor of birthweight as maternal nutritional status (Godfrey *et al*, 1996). It is therefore of interest to determine whether a hypertensive phenotype acquired through parental exposure to undernutrition may be transmissible to subsequent generations.

Six adult female rats were mated and fed on either an 180g casein/kg (control, *n* 3) or 90g casein/kg (low protein, *n* 3) diet throughout pregnancy. At littering all dams were fed on the same non-purified laboratory rat diet (18.9 g protein/kg). At 7 weeks of age the blood pressures of the offspring of the low protein fed group were significantly elevated relative to the pressures of the control group (control: mean 135 (SE 5) mmHg, low protein: mean 147 (SE 9) mmHg, P=0.019). Two females and one male from each litter were selected for further study. Four series of matings (*n* 3 per group) were established when the rats were 12 weeks old. Low protein exposed males were crossed with low protein exposed or control females. Control males were crossed with low-protein-exposed or control females. The blood pressures of the offspring from these crosses were determined at 8 weeks of age (Table).

Prenatal dietary experience of parents (g/kg casein)			Body weight (g)		Systolic blood pressure* (mmHg)	
Male	Female	*n*	Mean	SE	Mean	SE
180	180	14	124	12	145^{bc}	4
180	90	22	128	11	141^{bc}	4
90	180	19	127	12	150^{c}	3
90	90	19	125	14	128^{a}	3

*Blood pressures are adjusted for inter-litter effects. ^{a,b,c} Mean values not sharing a common superscript letter were significantly different, $P<0.001$ (ANOVA).

Intergenerational effects upon blood pressure were observed. The low-protein-exposed males fathered the offspring with highest blood pressure when mated with females exposed to control diet. The cross of hypertensive males with hypertensive females produced the offspring with the lowest blood pressures. The phenotypes acquired by the parental generation *in utero* can therefore be transmitted in an epigenetic fashion through the male germ line and be modified by factors in the female germ line.

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The role of the renin-angiotensin system in the intrauterine programming of hypertension. By RACHEL C. SHERMAN, ALAN A. JACKSON and SIMON C. LANGLEY-EVANS, *Division of Human Nutrition, University of Southampton, Biomedical Sciences Building, Bassett Crescent East, Southampton, SO16 7PX*

Epidemiological studies have provided evidence that intrauterine growth retardation is associated with an increased risk of hypertension and cardiovascular disease in later life (Barker *et al.* 1993). It is suggested that the nutritional profile of the mother permanently programmes the metabolic and physiological functions of the fetus (Barker *et al.* 1993). Experiments in animals support this hypothesis (Langley-Evans *et al.* 1996). Rats exposed to a maternal low-protein diet *in utero* have significantly elevated systolic blood pressures in later life (Langley-Evans *et al.* 1996).

In the present study the role of the renin-angiotensin system in the elevation of blood pressure by maternal low-protein diets was evaluated. Rats were fed on either a 180g casein/kg (control) diet or a 90g casein/kg (low-protein) diet throughout gestation, or a 90g casein/kg diet for specific 1-week periods during gestation. Rats were culled immediately after birth, or following blood pressure measurements at 4 or 12 weeks of age, for determination of pulmonary angiotensin-converting enzyme (ACE) activity (Langley & Jackson, 1994). Plasma renin activity (PRA) (Langley-Evans *et al.* 1996) was measured at 4 weeks only.

Pulmonary ACE activity was higher in the animals exposed to a 90g casein/kg diet throughout gestation compared with the controls at the neonatal stage and at 4 weeks of age, this was significant by 12 weeks of age. The table shows data from 4 week old animals.

	SBP (mmHg)		ACE (units/mg protein)		PRA (ng/ml per h)	
Dietary group	Mean (*n*)	SE	Mean (*n*)	SE	Mean (*n*)	SE
180g/kg d 0-22 controls	93 (32)	4	0.024 (12)	0.001	6.41 (6)	1.03
90g/kg d 0-22	121* (32)	4	0.027 (5)	0.004	4.45 (6)	0.97
90g/kg d 0-7	107* (40)	5	0.029* (19)	0.002	6.07 (8)	1.04
90g/kg d 8-14	106* (40)	4	0.034** (25)	0.002	6.00 (6)	0.77
90g/kg d 15-22	112* (24)	4	0.044** (18)	0.004	10.28*(8)	2.05

Mean values were significantly different from the controls: *P<0.05 **P<0.01

Rats exposed to a 90g casein/kg diet for any discrete 7 d period during gestation had significantly elevated pulmonary ACE activity at the neonatal stage and at 4 weeks (Table) and increased systolic blood pressures at 4 weeks (Table) and 12 weeks. Rats exposed to a 90g casein/kg diet only between days 15 and 22 of gestation had significantly elevated pulmonary ACE activity and plasma renin activity at 4 weeks of age.

These data indicate that the renin-angiotensin system is altered in rats exposed to a low-protein diet *in utero* at any point during gestation and is particularly sensitive to programming during the final week of gestation. The effects of this programming are apparent from birth.

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Postnatal undernutrition markedly upregulates cardiac α1 and α2 thyroid hormone receptor gene expression. By P. WHITE and M. J. DAUNCEY, *Department of Cellular Physiology, The Babraham Institute, Cambridge CB2 4AT*

Thyroid hormones (TH) play central roles in muscle development and function: postnatally, in skeletal muscle TH stimulate conversion from slow to fast myosin heavy chain isoforms, and similarly in cardiac muscle they increase α-myosin heavy chain transcription. The actions of TH are mediated by nuclear receptors (TR) which act as transcription factors. The TH binding capacity of these receptors is profoundly influenced by energy status, and undernutrition results in a reduction in total TH binding of muscle nuclei. However, there are two distinct TR genes, α and β, which encode four major isoforms (α1, α1, β1, β2). These have the potential to alter the expression of numerous genes, either positively or negatively, and are expressed in a tissue-specific manner; in muscle the α isoforms predominate. The aim of the present investigation was therefore to determine the role of mild undernutrition, that nevertheless allows growth, on TRα1 and α2 gene expression in cardiac and skeletal muscles during postnatal development.

Our studies were undertaken in the pig because it is a particularly suitable biological model for the human infant. We first cloned the porcine TRα isoforms using the polymerase chain reaction, with oligonucleotide primers based on conserved regions of the human cDNA sequences. Differences in the 3′ region of the two α isoforms account for major differences in TH binding (α1 = positive, α2 = negative). We designed a riboprobe to the 3′ region, to allow assessment of the relative abundance of both α1 and α2 mRNA levels by RNase protection assay. Cardiac ventricular and skeletal (*longissimus dorsi*) muscles were obtained from eight pairs of 6-week-old littermate male pigs which had been kept at 26° and given a high (60 g/kg per d) or low (20 g/kg per d) food intake, as two meals/d, for the previous 3 weeks. All animals gained weight during this period but the rate of gain was lower for those on the low intake.

	Cardiac muscle				Skeletal muscle			
	High intake		Low intake		High intake		Low intake	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
TRα1	6.3	1.0	12.3*	2.0	26.5	2.1	29.9	3.2
TRα2	28.8	3.8	51.4†	6.9	3.9	0.4	5.6	0.8

Values are optical densities, obtained from autoradiographs by quantitative image analysis.
Mean values were significantly different from high intake: *P = 0.03, †P = 0.02 (paired t test, 2-tail).

There were marked muscle-specific differences in relative expression of the two TRα isoforms and in the extent to which they were affected by nutrition. Expression of TRα2 predominated over TRα1 in heart, whereas in skeletal muscle the opposite trend was observed. Especially striking was the finding that undernutrition upregulated gene expression of both TR isoforms in heart. In skeletal muscle, by contrast, there was no significant change in expression. These novel findings suggest that, in view of the role of TH in conversion of myosin isoforms, the high level of TRα2 in cardiac muscle will ensure slow sustained contractility, while the preponderance of TRα1 in *l. dorsi* will ensure the presence of a high proportion of fast-twitch fibres for rapid movement. We suggest that the elevated cardiac TRα2 expression during undernutrition may also profoundly affect myocardial function: a high level of this non-TH binding variant, combined with our previously demonstrated reduction in circulating TH levels, would reduce cardiac α-myosin transcription, leading in turn to a lower intrinsic contractile ability and operational heart rate.

Response of blood pressure and glomerular filtration rate (GFR) to salt, in offspring of female rats exposed to a 9% casein diet during pregnancy. By SIMON J.M. WELHAM, ALAN A. JACKSON & SIMON C. LANGLEY-EVANS, *Nutrition Department, Southampton University, Bassett Crescent East, Southampton. SO16 7PX.*

Exposure to a maternal 90g casein/kg diet during rat pregnancy has been shown to induce elevations of blood pressure in the offspring, relative to rats exposed to an 180g casein/kg diet (Langley-Evans *et al.* 1996). In contrast to 180g casein/kg exposed rats, hypertensive, low protein exposed animals are insensitive to the further hypertensive effect of high salt consumption (Langley-Evans & Jackson 1996). In the present study, the blood pressure and renal response of 90g and 180g casein/kg exposed offspring to salt was further examined.

Six female Wistar rats were mated and, at conception, supplied either a 90g casein/kg or an 180g casein/kg diet (three animals in each group) which was maintained throughout pregnancy. At 19 weeks of age, the female offspring had their blood pressures determined using tail cuff plethysmography and were supplied either water alone or water plus a 15g/l NaCl solution. After 7 days on the fluid regimen, the animals again had their blood pressures determined and were then anaesthetized and cannulated for the direct measurement of blood pressure and GFR.

	Tail cuff blood pressure (mmHg)						Cannulation blood pressure (mmHg)						GFR (ml/min)		
	Before fluids			After fluids			Systolic			Diastolic					
Group	MEAN	SE	n	MEAN	SE	n	MEAN	SE	n	MEAN	SE	n	MEAN	SE	n
180g/kg W	120	4.71	12	114	6.74	12	134	10	8	117	10	8	2.65	0.08	8
180g/kgW+S	126	6.34	5	139	5.71	6*	118	16	5	102	18	5	2.39	0.39	5
90g/kg W	143	8.06	6*	140	7.29	6*	148	10	5*	126	9	5	2.42	0.15	5
90g/kgW+S	133	7.56	5	123	9.05	6	146	14	5*	125	14	5	2.52	0.27	5

* Significantly different compared to 180g casein/kg exposed controls supplied with water ($P<0.05$). W - Water S - Salt.

The systolic blood pressure of the 90g casein/kg exposed animals was significantly higher than that of the 180g casein/kg exposed offspring before the salt loading (Table). Exposure to 15g/l NaCl solution caused the blood pressure of the 180g casein/kg exposed animals to rise to a level comparable with that of the 90g casein/kg exposed animals supplied with water only. The 90g casein/kg exposed offspring did not demonstrate an elevation in blood pressure in response to salt. Direct cannulation showed a clear difference between the systolic blood pressures of the 90g and 180g casein/kg exposed animals. There was also a trend for the diastolic blood pressure of the 90g casein/kg exposed animals to be elevated above those of the 180g casein/kg exposed group. GFR was not altered by salt administration in either maternal dietary group. Values obtained using the tail cuff method of blood pressure determination, showed an altered reponsiveness to salt between the groups. However, direct cannulation showed that neither 90g or 180 casein/kg exposed animals showed elevated blood pressures as a result of salt loading, possibly as a result of the action of the anaesthetic. Blood pressure was not increased in 90g casein/kg exposed animals suggesting that Na homeostasis may be altered.

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Effect of two strains of *Saccharomyces cerevisiae* on performance of preruminant calves. By JUAN M. PINOS, MARIA E. ORTEGA-CERRILLA, GERMAN MENDOZA, RICARDO BARCENA and JOSE AYALA, *Colegio de Postgraduados, Universidad Autonoma Chapingo, 56230 Montecillo, Edo. De Mexico, Mexico.*

Rumen development depends on DM intake in order to wean calves as early as possible. Feed additives such as *Saccharomyces cerevisiae* have been fed to calves to increase concentrated feed intake, and also to improve calf health (Seymour *et al.* 1995). The present study evaluated the effect of two strains of *S. cerevisiae* on daily concentrated feed intake, body weight, ruminal pH and total volatile fatty acid (VFA) concentration when fed to preruminant calves. Twenty-four 4-d-old Holstein calves (twelve male, twelve female; body weight 41.7 (SD 4.9)kg were housed in individual pens and randomly assigned to T1 (control treatment), T2 (1 g/d per calf Levucell SC2; no. 5008, 20 x 10^9 colony forming units (CFU)/g) or T3 (1 g/d per calf Levucell SB2; no. 5005, 20 x 10^9 CFU/g). Calves were fed *ad libitum* whole milk (107 g/kg body weight) and concentrated feed (g/kg: crude protein 192.4, neutral detergent fibre 329.5, acid detergent fibre 116.9). Concentrated feed intake was recorded daily, calves were weighed every week, and rumen fluid was collected by oesophageal probe every 2 weeks, until calves were weaned at 8 weeks of age. Data were analysed for a completely randomised design and means were compared by orthogonal contrasts.

	Body weight (kg)				Concentrated feed intake (g/d)				Ruminal pH				Ruminal VFA (mmol/l)			
Weeks	T1	T2	T3	SE	T1	T2	T3	SE	T1	T2	T3	SE	T1	T2	T3	SE
0	42	41	42	1.7	-	-	-	-	-	-	-	-	-	-	-	-
2	44	45	47	1.7	67	87	80	13	5.9	5.6	5.7	0.3	58	69	79	1.7
4	48	49	47	1.6	238	260	204	49	5.8	5.2	5.5	0.4	69	127	114	1.6
6	53	53	55	2.0	494	646	528	69	5.3	5.2	5.0	0.1	102	135	108	2.0
8	58	60	62	2.3	898	1051	915	97	5.5	5.4	5.3	0.1	102	117	112	2.3

The addition of *S. cerevisiae* did not affect ($P>0.05$) body weight gain, concentrated feed intake, ruminal pH or total VFA concentration. It has been observed that responses are highly variable when S. *cerevisiae* is used as a feed additive (Kumar *et al.* 1994). Many factors may affect the results obtained, such as the ability of different strains to increase production, degradation of fibre in the rumen and flow of microbial protein from the rumen. Also, composition of the diet, age of the animals, level of intake and culture media where the yeast was grown are important (Newbold *et al.* 1995). The results observed in the present study might be due to the inability of the strains fed to the calves to stimulate a production response, or that the level of addition, of *S. cerevisiae* was not sufficient to show any benefit.

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Effect of feeding barley straw upgraded by edible mushroom *Pleurotus* in sheep. By URIEL CORONEL and MARIA E. ORTEGA-CERRILLA, *Colegio de Postgraduados, 56230 Montecillo, Edo. de Mexico,, Mexico*

Improvement of the acceptability and nutritional value of straw for ruminants through physical and chemical treatments has been documented. An alternative route is the utilization of edible mushrooms such as *Pleurotus*, which can degrade the lignocellulose complexes and lignin in the straw (Karunanandaa *et al.* 1995). The purpose of the present experiment was to evaluate the digestibility and N balance of rations containing barley straw upgraded by *P. ostreatus* alone, or by *P. ostreatus, P. florida* and *P. djamour* together. Nine 4-month-old "criollo" lambs weighing about 18 kg were kept in individual metabolic cages. Animals were fed on a ration containing either 700 g/kg of untreated barley straw (US) or barley straw upgraded by *P. ostreatus* (PS) or by *P. ostreatus, P. florida* and *P. djamour* (FDS) together with 300 g/kg or a compound feed (735 g ground sorghum grain, 150 g soyabean meal, 70 g molasses, 15 g urea and 30 g mineral premix/kg). Animals had an adaptation period of 15 days, then feed intake was adjusted to 90%. All faeces and urine were collected during the following 8 days. Data were analysed for a completely randomized design and means were compared by Tukey test.

Treatment	DM intake	Digestibility (%)			N balance
	(g/d)	OM	NDF	ADF	(g/d)
US	320	77.36[a]	51.89	31.48	0.15[a]
PS	451	88.97[b]	53.77	32.29	3.57[b]
FDS	454	87.59[b]	51.89	34.72	3.22[b]
SE	26.43	1.14	1.47	2.12	0.08

OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre.
[a,b] Values with different superscript letters were significantly different, $P<0.01$.

Treatment of barley straw with *Pleurotus* significantly increased OM digestibility and N balance. However there were no differences in NDF and ADF digestibilities. The increase in fibre digestibility appears to be due to weakening of ligno-carbohydrate complexes, which largely depends on the substrate and its preferential use by the mushroom, as well as its ability to produce the enzymes to degrade these complexes (Agosin *et al.* 1985). The results obtained suggest that the strains of *P. ostreatus, P. florida* and *P. djamour* used in this study did not degrade lignin to a great extent, or that additional factors such as cellulose crystallinity may act as a digestion ceiling for further improvement in digestibility. The higher N balance was probably due to mushroom residues in the straw after mushroom harvesting, or to the ability of some micro-organisms associated with the mushroom to fix atmospheric N_2 and increase N content of upgraded straws.

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Iron status and cognitive function in UK adolescent girls. By RUTH ASH and MICHAEL NELSON, *Department of Nutrition and Dietetics, Kings College London, Campden Hill Road, W8 7AH*

Fe-deficiency anaemia (IDA) and Fe deficiency (ID) are common among UK adolescent girls (Nelson *et al.* 1993, 1994). IDA and ID affect academic performance and cognitive performance can be improved by Fe supplementation. Non-verbal IQ appears to be the cognitive area most affected and studies point to reversible effects on arousal, attention span, memory and concentration. The cognitive defects associated with ID have been linked to alterations in monoamine neurotransmitter (dopamine, serotonin and noradrenaline) levels and function.

Girls (*n* 539) aged 11.5 - 15.5 years, attending three comprehensive schools in greater London, took part in IDA/ID screening in either February 1995 or February 1996. An age/height matched sample of 139 girls, classed either: IDA (*n* 47) on the basis of capillary measurements of haemoglobin (Hb <120 g/l) and packed cell volume (PCV< 37%); ID (*n* 38) on the basis of raised Zn protoporphyrin (>700 µg/l); or Fe replete IR (*n* 53), were selected for a 10 week, double blind, Fe/placebo intervention. They completed a three day formatted food diary and measurements at baseline (March 1995 or 1996) and post intervention (June 1995 or 1996). Measurements included: venous blood counts and serum ferritin and an assessment of cognitive function using the British ability scales (BAS) (NEFR-Nelson, 1982).

BAS tests were completed by 139 girls at baseline. Five tests were used; these stressed visual and verbal reasoning, immediate recall and reasoning speed. Scores from each test were converted to age-normalized *t* scores and the mean *t* score used to derive an overall IQ. Mean IQ was 109 (*SD* 14) points, (*SEM* 1.2) and median IQ 111 points. Although IQ increased with better Fe status, there were no significant differences in IQ for girls of different Fe status (IDA:107 (*SD* 13)points; ID:109 (*SD* 13)points; IR:111 (*SD* 16)points) and no correlation between IQ and Hb (r 0.12, P=0.17).

Girls were divided into two IQ groups: low (IQ < median) and high (IQ≥ median) and three Hb groups: low (Hb <100 g/l), average (Hb 100-124 g/l) and high (Hb ≥ 125g/l). There was a weak (χ^2 5.92, r 0.19, P=0.052) but significant (P=0.028, Mantel-Haeszel) linear association between the IQ/Hb groups.

Table 1. IQ and haemoglobin level for 139 girls

Hb (g/l)	≤100 (*n* 4)		≤110 (*n* 14)		≤115 (*n* 13)		≤120 (*n* 18)		≤125 (*n* 17)		>125 (*n* 68)	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
IQ (points)	102	7	108	14	110	14	107	13	103	13	111	16

A total of 114 girls completed BAS tests post intervention. Mean IQ_2 was 116 (*SD* 14) points and median IQ 117 points. The difference in IQ score (IQ_2 - IQ_1) was 6 (*SD* 9) points. IQ scores increased significantly for all girls who received Fe, with the greatest increases in the IDA and ID girls. IDA and ID girls in the placebo group did not significantly increase their IQ scores. There was a significant effect of treatment (Fe or placebo) by Fe status on the difference in IQ scores (repeated measures MANOVA F 3.93, P=0.022).

Table 2. Pre- (IQ_1) and post-intervention (IQ_2) IQ scores for girls receiving iron supplementation or placebo

	Iron						Placebo					
	IDA (*n* 26)		ID (*n* 19)		IR (*n* 24)		IDA (*n* 19)		ID (*n* 17)		IR (*n* 29)	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
IQ_1	107	14	111	12	114	15	107	12	107	14	109	17
IQ_2	113	15	118	12	118	13	111	14	110	11	118	16
P value	< 0.001		<0.001		0.001		0.173		0.150		< 0.001	

Baseline IQs were not significantly different for girls of different Fe status, although there was a trend for a higher IQ with better iron status and a significant association between IQ and Hb. There was a significant effect of treatment by Fe status, reflected in the fact that significant increases in IQ were only found in IDA and ID girls who had received Fe supplementation. The results suggest that poor Fe status in adolescent girls has a detrimental influence on cognitive function and that Fe supplementation in these girls may improve cognitive function.

The authors would like thank the girls and staff at the schools who took part and to acknowledge funds from King's College, London and the Meat and Livestock Commission.

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The effect of meal frequency on energy intake and hunger ratings in obese subjects in a chamber calorimeter. By *Moira A. Taylor* and *John S. Garrow, Department of Human Nutrition, St. Bartholomew's Hospital Medical College, Charterhouse Sq, London EC1M 6BQ*

Less frequent meals may result in poorer compliance with a weight-reducing diet compared with a more frequent meal pattern. The patient may experience greater hunger and subsequently consume more 'snack' foods in addition to their prescribed diet. The aim of the following studies was to investigate the effect of meal frequency on ratings of hunger and consumption of additional foods in obese subjects prescribed a weight-reducing diet.

In study 1, eleven female subjects (BMI: median 40.6 kg/m^2, interquartile range 35.3-41.5 kg/m^2; age: median 39 years, interquartile range 27-45 years) were admitted to an indirect chamber calorimeter for two consecutive 48 h periods. In each period, 4.184 MJ (1000 kcal)/24 h was given as either two isoenergetic meals (2/24 h) at 11.00 hours and 19.00 hours, or as six isoenergetic meals (6/24 h), at intervals of 2 hours between 09.00 hours and 19.00 hours (randomized-crossover design). Subjects completed a visual analogue scale rating of hunger at 2 h intervals between 09.00 hours and 21.00 hours (not at all hungry to extremely hungry).

The protocol in study 2 was identical to study 1, apart from the availability of a range of additional foods, to which the subjects had *ad libitum* access throughout the study. Eight female subjects completed study 2 (BMI: median 36.6 kg/m^2, interquartile range 31.8-40.2 kg/m^2; age: median 48 years, interquartile range 40-73 years).

Total energy expenditure was measured for the final 24 h of each of the 2 d periods.

In study 1 subjects rated significantly greater hunger at 11.00 h (P=0.013) and 19.00 h (P=0.004) when they were given two meals per 24 h compared with six meals per 24 h. Total energy expenditure was not significantly different between the two meal patterns (P>0.05). All subjects were in negative energy balance on the 4.184 MJ/24 h diet.

In study 2, despite the effects in hunger seen in the first study, total energy intake was not significantly different between the two meal patterns even when subjects were given free access to additional foods (P=0.575) (total energy intake 6/24 h, median 10.71 MJ/24 h, interquartile range 8.68 - 13.02 MJ/24 h; 2/24h, median 9.91 MJ/24 h, interquartile range 7.60 - 14.85 MJ/ 24h).

All subjects given free access to additional foods exceeded their prescribed dietary intake of 4.184MJ/24 h and in the majority of cases (8/9) energy intake also exceeded total energy expenditure (total energy expenditure: 6/24 h, median 9.23 MJ/24 h, interquartile range 7.49 - 10.15 MJ/24 h; 2/24 h - median 8.93 MJ/24 h, interquartile range 7.50 - 11.23 MJ/24 h). Total energy expenditure was not significantly different between the two meal patterns (P= 0.612).

Meal pattern had no major effect on total energy intake over the short term of two days despite the increased hunger peaks associated with the less frequent meal pattern. However, the discomfort of hunger might have important implications for compliance in patients following a weight reducing diet in the long term.

Inter-relationship between circulating leptin concentrations, hunger and energy intake in healthy subjects receiving tube feeding. By R.J. STRATTON[1], G. JENNINGS[1], R.J. STUBBS[2] and M. ELIA,[1] [1]*MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH,* [2]*Rowett Research Institute, Greenburn Road, Aberdeen AB2 9SB*

Leptin is implicated as an important factor in the regulation of appetite and food intake. However, there is some uncertainty as to whether energy intake regulates leptin or if leptin regulates energy intake. The present study aimed to investigate these issues by studying temporal relationships between plasma leptin concentrations, preceding and subsequent voluntary and total energy intakes, in healthy subjects, before and during enteral tube feeding. Six healthy males, aged 36 (SD 8) years, BMI 22.1 (SD 2.27) kg/m^2 (weight stable) were resident in the metabolic suite for 9 days (limited physical activity). Following a 2 d maintenance diet (1.5 x BMR), for days 3 - 9 volunteers were allowed a*d libitum* access to covertly manipulated food (food items isoenergetically dense (550 kJ/100g) and with equal energy provision from each of the macronutrients). During days 5 - 7, volunteers were also fed diurnally for 12 h (09.00 - 21.00 hours), via a nasogastric tube (standard, commercial, 4.18 kJ/ml feed, mean feed provision 6.9 (SD 0.5) MJ). Serial leptin measurements (human leptin radioimmunoassay kit, Linco Research, USA) were made after an overnight fast on days 3, 5 and 8, followed by skinfold measurements at four sites for the calculation of percent body fat (Durnin & Womersley, 1974). Oral and total (including tubefeed) energy intakes were calculated for the 24 h preceding, and the 24 h following the leptin measurement (which also included the subsequent mornings food intake up to 12 hours, mostly breakfast intake). Hunger scores (using 100 mm visual analogue scales, 'not at all hungry'(score 0) to 'as hungry as I have ever felt'(score 100)) were recorded hourly during waking hours. Repeated measures ANOVA was used to analyse normally distributed data, which for leptin required log transformation.

	Day 3		Day 5		Day 8	
	Mean	SD	Mean	SD	Mean	SD
Leptin (ng/ml) *	2.82	-	3.43	-	4.23	-
Previous 24 h total energy intake (MJ/d)†	10.5	1.07	16.8	3.09	19.3	2.48
Subsequent days oral energy intake (MJ/d)‡	14.0	2.08	13.0	3.73	13.8	3.17
Mean daily hunger score, 0 to 100 ‡	31	11	28	11	30	13

*Leptin (geometric mean) P<0.01 ANOVA
†Includes tube feeding period, days 5 - 7, P<0.01 ANOVA.
‡No significant change ANOVA.

Significant interaction was found between fasting plasma leptin and the previous day's total energy intake (P<0.01). However, there was no significant relationship between leptin and either (1) subsequent oral energy intake up to 12.00 hours, (mean intakes 2.5, 2.0 and 2.4 MJ for days 3, 5 and 8); or (2) the first rated hunger of the day (mean hunger scores 45, 42, 43 mm); or (3) the timing of morning food consumption; or (4) subsequent 24 h oral energy intake or mean daily hunger score (see Table). During the study, there was no detectable change in percent body fat.

This study suggests that, over the time frame of the study, nasogastric tube feeding (equivalent to BMR) failed to suppress voluntary food intake. In addition, circulating leptin concentrations responded to previous 24 h energy intake but elevated leptin concentrations failed to suppress subsequent hunger or voluntary energy intake.

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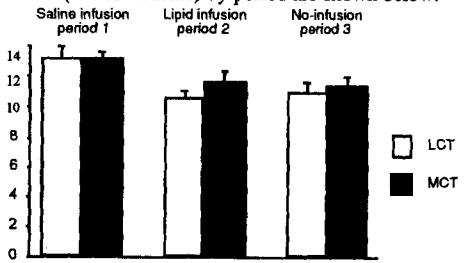
Parenteral infusion of medium-chain triacylglycerol (MCT) and long-chain triacylglycerol (LCT): effect on appetite and food intake in healthy men. By K.J. CASEY, A.M. JOHNSTONE, C.A. REID, I. DUYSBURG, M. ELIA and R. J. STUBBS, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB*

Increasing both the fat content and energy density of the diet leads to poor compensation in food intake. Blundell argues that ingested fat exerts potent suppression of appetite at the level of the gut, during the absorptive phase, but weak postingestive feedback overall (Blundell *et al.* 1995). Furthermore fat-type may affect feeding since isoenergetic substitution of MCT for LCT, at high doses exerts significant but modest attenuation of the excess energy intake (EI) and weight gain that typically occurs on high-fat, energy dense diets (Stubbs & Harbron, 1996). Since nutrient oxidation correlates with satiety and MCT are preferentially oxidized compared with LCT, MCT may exert more potent suppression of feeding at the postabsorptive level. The present study examined the effects of peripheral parenteral infusion of LCT (Intralipid) *v.* MCT on quantitative food and EI in order to assess (i) the influence of peripheral parenteral lipid infusion on energy compensation and (ii) whether the fatty acid chain length of infused triacylglycerols differentially influences subsequent EI.

Four men, mean age 32.7 (SD 12.3) years, height 1.72 (SD 0.06) m, weight 65.9 (SD 20.1) kg were each studied while resident in the Human Nutrition Unit for two 9 d periods. On days minus 1-2 they were given a medium fat (MF: 40% fat, 13% protein and 47% carbohydrate by energy) maintenance diet at 1.6 x resting metabolic rate (RMR). On days 1-3 (period 1) subjects received 500 ml/d physiological saline. On days 4-6 (period 2) the subjects received either pure MCT or LCT emulsion (4.2 MJ/d in 500 ml infusate) (Braun Medical Ltd). On days 7-9 (period 3) subjects received no infusion. Throughout days 1-9 subjects had access *ad libitum* to MF (550 kJ/100 g) foods offered on a 3 d rotating menu. Subjects recorded self-rated hunger every waking hour during the study. ANOVA was conducted on intakes of food, energy and subjective hunger with period and subject as blocking factors. Mean energy intakes (minus infusate) by period are shown below.



Energy intakes were significantly reduced during period 2, (F (1, 63) 15.35, $P < 0.001$).This effect apparently carried over into period 3 after lipid infusion had stopped. There was no differential effect of lipid type on food or energy intakes during this period. However, one of the subjects developed a phlebitis during the LCT treatment, which may have decreased the ability of this study to discriminate between the two lipid forms. There were no period or treatment effects on subjects' hunger. We conclude that intravenously infused fat exerts significant feedback on energy intake which, however only leads to partial energy compensation when subjects have access *ad libitum* to a diet of fixed composition.

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Mood effects of reduced-fat dietary treatment. By PAULAN POLET[1], PETER J. ROGERS[1], JANE WARDLE[2], ELIZABETH A. ATKINSON[1], LLOYD VALLIS[1], LORNA RAPOPORT[2], MOIRA TAYLOR[3] and PAT JUDD[3], *[1]Institute of Food Research, Reading RG6 6BZ, [2]Health Behaviour Unit, Department of Epidemiology and Public Health, University College London, London WC1E 6BT, [3]Department of Nutrition and Dietetics, King's College, University of London, London W8 7AH*

Despite the acknowledged importance of nutritional influences on behaviour (e.g. Rogers, 1995), only relatively recently has attention been paid to the psychological effects of the widely recommended low-fat, cholesterol-lowering diets (Wardle, 1995). This issue has become prominent because of the apparent association between cholesterol-lowering interventions and an increase in non-illness mortality (Muldoon *et al.* 1990).

The present study evaluated the effects on mood of reduced-fat diets in patients with moderately raised cholesterol levels. A low-fat diet and a modified-fat 'Mediterranean' diet were compared with a 'waiting list' control group. This was part of a larger study of 176 patients who were randomized to the three groups, with the active treatment groups receiving combined dietary advice and psychological support regularly over a 12-week period. The data shown in the Table are for thirty-eight patients who additionally completed sets of mood booklets before (T_0) and 2, 6 and 12 (T_{12}) weeks after the start of dietary treatment. Each set contained three booklets covering 3 d during these weeks. Patients rated their mood throughout the day at 2 h intervals, each time using a separate double page of the booklet on which was printed twenty-four adjectives describing moods and hunger. Ratings were made according to a 5 point scale (0 = 'not at all' to 4 = 'extremely').

	Low fat (*n* 13)				Mediterranean (*n* 15)				Waiting list (*n* 10)			
	T_0		T_{12}		T_0		T_{12}		T_0		T_{12}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight (kg)	74.7	14.0	71.7**	12.9	73.3	14.6	71.0*	13.1	71.0	13.5	70.8	13.3
Fat (% energy)	36.7	3.3	17.9**	6.2	34.2	6.6	19.7*	4.5	31.2	6.3	29.8†	9.9
SFA (% energy)	13.3	2.4	5.3**	2.9	11.2	2.8	5.2**	1.4	10.3	3.2	10.4†	4.9
Total cholesterol (mmol/l)	6.82	0.72	6.20**	0.68	7.09	0.88	6.28**	1.23	7.09	0.67	6.82	0.42
LDL (mmol/l)	4.61	0.79	4.15*	0.73	4.75	0.97	3.85**	1.02	4.90	0.70	4.64‡	0.53
HDL (mmol/l)	1.58	0.58	1.44	0.52	1.33	0.49	1.31	0.46	1.46	0.35	1.43	0.33
Triacylglycerols (mmol/l)	1.47	0.89	1.32	0.43	1.99	1.31	2.38	1.83	1.61	0.93	1.62	0.86

Values at T_{12} were significantly different from values at T_0, *P<0.05, **P<0.01.
†Values for waiting list significantly different from low fat and Mediterranean at T_{12} (P<0.05; Bonferroni).
‡Values for waiting list marginally significantly different from Mediterranean at T_{12} (P=0.08; Bonferroni).

The Table shows that both the low fat and Mediterranean groups were successful in reducing the percentage energy from fat in their diet and their total blood cholesterol and LDL levels. The magnitude of these changes were similar to those found for the whole group of 176 patients.

Initial principal component analysis reduced the number of mood variables to two, explaining 54% of the total variance and these were labelled as 'energetic arousal' and 'tension/dysphoria'. Since mood varies substantially across the day, analyses for diet effects were carried out on daily mood ratings at four time points: immediately after waking up in the morning and just before going to bed at night, with the time in between split into two. Residual maximum likelihood analyses, with pretreatment data as covariate, revealed no significant main or interaction effects of diet or week. However, there were significant main effects of time of day (P<0.01) and significant diet x time of day interactions (P<0.01) for both principal components and hunger. Patients in the waiting list group were less energetic on waking up compared with those in the low fat and Mediterranean groups, whereas the low fat group patients tended to be more tense and dysphoric during the middle parts of the day. Hunger was lower in the waiting list group patients than in the other groups on waking up, but not at later times in the day.

These findings show no marked adverse effects on mood of dietary treatments designed to lower blood cholesterol levels.

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Post-ingestive effects of fat and carbohydrate on physiological and psychological measures. By ANITA S. WELLS[1], N. W. READ[1] and I. A. MACDONALD[2], *[1]Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Herries Road, Sheffield S5 7AU and [2]Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH*

The fat and carbohydrate (CHO) contents of a meal influence many aspects of postprandial physiology and psychology including heart rate (HR), energy expenditure (EE), muscle nerve sympathetic activity, sleepiness, perception of pain, alertness and mood. However, no studies have measured both physiological and psychological effects of pure nutrients at the same time, in the absence of cognitive and orosensory influences. The aim of the present investigation was to compare the effects of gastric infusions of fat and CHO on physiological and psychological measures.

Nine healthy subjects (six male) were each tested on 3 days, with each test day separated by at least 2 days. Between 10.15 and 10.30 hours, subjects were given an isovolumic rapid gastric infusion of either sucrose solution (100% energy CHO), lipid emulsion (100% energy fat, 200g/l intralipid, Kabi Pharmacia Ltd, Milton Keynes, Bucks), or a non-nutrient control (9g NaCl; saline). Measures of HR, EE (using the ventilated hood system), mood and sleepiness (Wells *et al.* 1997) were collected before the infusions and every 0.5 h for 3.5 h after. The infusions of lipid and sucrose were isoenergetic, containing one third of individual subject's estimated daily energy requirements (mean 3227 kJ range 2479 - 3971 kJ). Subjects were blinded to the nature of the infusions.

	F value, ANCOVA repeated measures			Mean post infusion change					
	Infusion	Time	Infusion x Time	Saline		Sucrose		Lipid	
	df (2,16)	df (6,48)	df (12,96)	Mean	SE	Mean	SE	Mean	SE
EE (kJ/kg per 24 h)	65.54‡	<1	3.91‡	1.73	1.04	18.22*	0.16	6.29*†	0.81
RQ	94.50‡	2.86‡	1.31	-0.04	0.02	0.13*	0.02	-0.06*†	0.01
HR (beats/min)	11.77‡	<1	2.22‡	0.43	1.11	9.70*	2.18	7.18*	1.25
Sleepiness	<1	6.64‡	2.61‡	-0.54	0.29	-0.44	0.30	-0.35	0.37
Attentive-dreamy	<1	4.16‡	1.95‡	-9.05	3.77	-8.30	4.71	0.68	6.93
Energetic arousal	<1	7.65‡	2.56‡	3.13	1.30	3.14	1.38	2.16	1.82
Tension	3.79‡	1.76	<1	0.68	0.77	1.13	0.43	1.59	0.75
Hedonic tone	3.94‡	<1	1.03	0.94	0.92	0.98	0.77	-1.54*†	0.83
Hungry-satiated	14.64‡	7.92‡	1.91‡	2.56	5.70	20.06*	7.01	24.54*	5.20

Post hoc tests demonstrate; * significantly different from saline ($p<0.05$), † significantly different from sucrose ($p<0.05$)
‡ *F* value significant ($p<0.05$)

The Table shows that the nature of the infusion significantly influenced post-ingestive EE, HR, alertness and mood. In addition, post-hoc analysis indicated that 0.5 h after the gastric infusions, HR and EE were significantly higher after the sucrose than after the lipid and saline. The greatest differences in sleepiness, dreaminess and energetic arousal were apparent 3 - 3.5 h after the infusions. At 3 h after the lipid infusion, subjects felt significantly more dreamy than they did after the sucrose and saline infusions. Sleepiness was also greater and energetic arousal lower 3.5 h after the lipid infusion than after the saline infusion.

In conclusion, the presence of lipid and sucrose in the gut induces significant and differing physiological and psychological effects, which are independent of cognitive and orosensory influences.

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Dissociation of mood and appetite effects of breakfast 'meals' varying in carbohydrate and energy content. By J. E. L. DAY[1], P. J. ROGERS[1], A. M. AHERNE[1], S. STEPHENS[1], G. M. FINCH[1], S. LONG[2] and L. M. MORGAN[2], *[1]Institute of Food Research, Reading RG6 6BZ and [2]School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

We previously found that postprandial mood was significantly affected by the fat : carbohydrate ratio in the meal, and moreover that optimal effects were associated with the macronutrient composition usually selected by the subjects at those particular times of day (either breakfast or lunch) (Lloyd *et al.* 1994, 1996). These meals were equi-energetic, whereas in the present study meal size (and energy density) was varied through the addition of carbohydrate (CHO) (see Table). Each 'meal' contained 46 g double cream and 10 g sucrose, plus either 0, 40 or 90 g maltodextrin (Cerestar PUR 1915), and was made up with water to a volume of 450 ml. The subjects (nine normal weight men, aged 20-30 years) were given these meals for breakfast 1 week apart according to a counterbalanced, within-subjects design. Immediately before and then at 30 min intervals after consuming this breakfast the subjects rated their appetite and mood (100 mm line scales), and 2.5 h later at midday they received *ad libitum* a buffet lunch.

Breakfast energy content reduced appetite (ratings of hunger, desire to eat, etc.) and food intake at lunch in a dose-related fashion (see Table). However, total food intake (breakfast + lunch) was somewhat greater after the higher energy meals, showing that energy compensation was incomplete. In contrast, mood and in particular 'energetic arousal' (combination of ratings of alert, energetic, lively, drowsy, tired, sluggish) was better after the intermediate breakfast. Analysis of food diary data showed that on average the energy and carbohydrate content of the subjects' typical breakfast (1.64 (SE 0.37) MJ; 61 (SD 12) g carbohydrate) were most similar to this intermediate meal.

	Breakfast energy and CHO content					
	Low (1.03 MJ, 10 g CHO)		Intermediate (1.70 MJ, 50 g CHO)		High (2.54 MJ, 100 g CHO)	
	Mean	SE	Mean	SE	Mean	SE
Lunch intake (MJ)	6.63a	0.22	6.25	0.38	5.55b	0.35
Hunger† (mm)	64.8a	3.0	60.2b	3.5	54.1c	3.5
Energetic arousal† (arbitrary units)	43.6a	19.7	60.2b	20.5	17.7c	19.8

a,b,c Means within a row denoted by different letters were significantly different ($P<0.05$; LSD test following ANOVA).
† Mean of ratings from 30 min after breakfast to just before lunch; pre-breakfast ratings entered as a covariate.

The results show a dissociation between the mood and appetite effects of this manipulation of meal size. Unlike appetite, energetic arousal during the postprandial interval was not simply inversely related to meal size, but was highest following the meal that corresponded most closely in size and carbohydrate content to the breakfast usually eaten by this group of subjects. The physiological basis of this effect is unknown, although the rapid onset of the changes in energetic arousal (differences due to breakfast energy content evident within 30 min) indicates the involvement of pre-absorptive mechanisms, rather than for example modulation of brain serotonergic function by carbohydrate as proposed by Wurtman *et al.* (1981). The results are, however, consistent with the suggestion that the mood effects of eating play a significant role in reinforcing food choice (Rogers, 1995).

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Intriguing positive effect of Placebo (sorbitol) in Mg trial for Premenstrual Syndrome (PMS). By MIRIAM C. DE SOUZA [1], ANN F. WALKER [1], PAUL A. ROBINSON [2], ANDREW MORRIS [3] and KIM M. BOLLAND [3], *[1]Hugh Sinclair Unit of Human Nutrition, Department of Food Science and Technology, The University of Reading, Reading, RG6 6AP; [3] MPS Research Unit, Department of Applied Statistics, The University of Reading, Reading, RG6 6FN, [2]Department of Clinical Biochemistry, Royal Berkshire Hospital, Reading, RG1 5AN*

In PMS studies, it has been shown that a woman taking the placebo benefits by relief of symptoms in expectancy of the effectiveness of the remedy under test (Magos, 1990). Sorbitol, used as a food sweetener, has often been used as a placebo. Hypoglycaemia associated with some forms of PMS may be linked with low intracellular sorbitol, as hyperglycaemia has been shown to enhance intracellular, accumulation of sorbitol with subsequent inositol depletion. As Mg is thought to be an important regulator of inositol transport, giving Mg may increase affinity of the inositol transport protein for inositol (Grafton *et al.* 1992).

In the pursuit of the effect of Mg supplementation in the relief of PMS, the present double-blind crossover study was designed to investigate the dose–response of three different levels of Mg (200, 350 and 500 mg/d as MgO "Heavy" precipitate salt) supplementation against the placebo (sorbitol). Volunteers were recruited through three sources; a feature article in the local newspaper, *The University of Reading Bulletin* and the *PMS Society Bulletin*. Volunteers completed a menstrual health questionnaire and their PMS symptoms were confirmed by daily records kept throughout one menstrual cycle. About ninety-eight women were selected (average age 34 years) and randomised into one of the four treatments for two consecutive menstrual cycles. After a washout period of one cycle, each subject was crossed over to a second treatment for two menstrual cycles. To accommodate all possible combinations of treatments, volunteers were arranged into twelve different groupings. The volunteers kept a record of their daily symptoms in a menstrual diary throughout the study and completed a food frequency questionnaire (FFQ). Estimated 24 h Mg output was calculated using the urinary Mg : creatinine concentration ratio of the first urine of the day.

PMS	category		PMS-A	PMS-C	PMS-D	PMS-H	Total	Estimated 24 h urinary Mg output (mg/d)		FFQ Mg
		n	(Anxiety)	(Craving)	(Depression)	(Hydration)	PMS	Mean	SE	(mg/d)
	Baseline	85	4.41	3.53	3.00	2.91	13.82	95.50	5.34	
Means	Placebo	41	1.71 **	2.21	1.29	1.65	6.87 ***	72.58 *	6.52	321.53
	200 mg	40	3.62	2.54	2.49	2.17	10.82	96.41	6.47	309.50
	350 mg	47	2.84	2.68	2.02	2.26	9.8	89.91	5.84	308.74
	500 mg	33	3.25	2.79	2.44	2.31	10.78	100.51	7.25	305.89

* P=0.039; ** P=0.006; *** P=0.004.

Placebo significantly reduced PMS-A (anxiety) and Total PMS symptoms compared with the Mg treatments. A significant difference was found in the urinary excretion of Mg between placebo and the three levels of Mg treatment. However, no statistical significance between the three different levels of Mg supplementation was revealed in the ANOVA. The results show that 24 h estimated urinary Mg output was not enhanced by Mg supplementation. We concluded that Mg from MgO was poorly absorbed and sorbitol effected greater reduction of PMS symptoms than MgO, whose efficacy was not fully tested, since sorbitol does not appear to be a true placebo.

This research was sponsored by CAPES/Brazil, ISFE /Switzerland and the Boots Company donated the supplements.

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Taste and appetite in patients with advanced cancer. By C. MOODY[1], C. J. SEAL[1] and C. F. B. REGNARD[2], *[1]Human Nutrition Research Centre, Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne NE1 7RU and [2]St Oswald's Hospice, Regent Avenue, Gosforth, Newcastle upon Tyne NE3 1EE*

Patients with advanced cancer often present with significant weight loss and symptoms associated with the cancer cachexia syndrome, including anorexia and poor taste sensation (Fearnon, 1992). Anorexia is ranked 4th by patients in prevalence and severity after pain, fatigue and weakness (Donnelly *et al.* 1995). These symptoms persist, even after cessation of cancer therapy. Enjoyment of food is of paramount importance to most individuals, being a measure of 'quality of life' and forming the basis of many social activities. In many progressive disease states regular eating patterns and behavioural activities become secondary to the illness and consequently may be overlooked in treatment plans. Alterations in eating patterns, exclusion of specific food groups or eating too little may all lead to an imbalanced diet which adversely affects nutritional status. The objective of the present study was to investigate the extent of anorexia and loss of taste in patients with advanced cancer to provide information which can be used to improve palliative care and enhance quality of life in this group of patients.

Thirty-six patients, average age 68.5 (range 42-89) years, attending St Oswald's Hospice either as in-patients or within the day clinic, took part in the study. All patients had progressive cancers in an advanced state, 47% with metastases. The predominant cancers present were breast (22%), prostate (17%), lung (14%) and brain (14%). Patients with known upper gastrointestinal tract or oral cancers were excluded from the study. Thirteen (36%) presented with general nutritional problems including constipation, nausea/vomiting, dry mouth and difficulty swallowing. Each patient completed a simple two–page questionnaire administered by a helper to evaluate perceptions of change in taste and appetite, use of discretionary sugar or salt and changes in preferences for particular foods. Of the thirty-six patients completing the questionnaire, 75% indicated that they had experienced a change in their appetite and a similar large proportion (70%) had experienced changes in taste sensation. In both cases, 86% of those noting a change indicated that this had been for the worse. Use of discretionary sugar in drinks, or the addition of salt during cooking and at the table had not changed during development of the disease state. However, of those questioned, 60% had modified their diet as a direct consequence of loss of appetite and 40% as a result of altered taste. Patients had developed dislikes for particular foods including meat, cheeses and some spicy foods and cravings for others including ice cream and fruit juices. Approximately 50% of the patients had stopped drinking tea or coffee.

These results suggest that changes in taste and appetite are a major contributory factor to selection of particular foods for patients with advanced cancer and that this should be taken into consideration when formulating dietary advice or in the development of care plans. Micronutrient deficiencies may be a problem in this group of patients and we are investigating this further.

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