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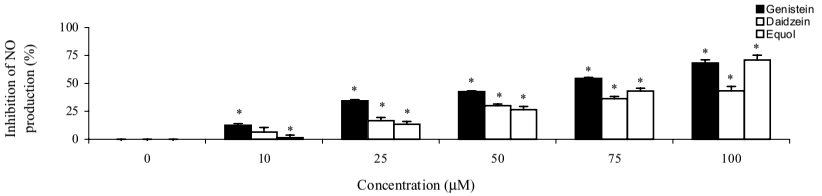
Antioxidant and nitric oxide inhibitory activity of isoflavone metabolites. By K. VAFEIADOU[1], B.A. EWINS[1], S. DE PASCUAL-TERESA[1], U. UCHIDA[3], S. MATSUGO[3], A.M. MINIHANE[1], R. TURNER[1], P.D. WEINBERG[2] and G.H. RIMBACH[1], [1]*Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences,* [2]*School of Animal and Microbial Sciences, The University of Reading, Reading RG6 6AP and* [3]*Department of Applied Chemistry and Biotechnology, Faculty of Engineering, Yamanashi University, Takeda, Kofu, Japan*

Soya isoflavones have been extensively studied because of their possible health-promoting effects. Genistein and daidzein, the major isoflavone aglycones, have received most attention; however, they undergo extensive metabolism in the gut and liver, which may affect their biological properties. This study investigated the antioxidant activity, free-radical-scavenging properties and selected cellular effects of the isoflavone metabolites equol, 8-hydroxydaidzein, *O*-desmethylangiolensin and 1,3,5-trihydroxybenzene compared with their parent aglycones; genistein and daidzein.

Electron spin resonance spectroscopy (ESR) and spin trapping in the presence of 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) were used to measure scavenging of hydroxyl anion radicals generated in the Fenton system and superoxide anion radicals generated in the xanthine/xanthine oxidase system by the test components. The FRAP (ferric reducing ability of plasma) and TEAC (trolox equivalent antioxidant capacity) assays were used to analyse the antioxidant properties of isoflavone and their metabolites. Murine macrophages RAW 264.7 were pre-treated with isoflavones (0–100 μM) for 24 h. Nitric oxide (NO) production by non-activated and macrophages RAW 264.7-activated with lipopolysaccharide (LPS) and interferon gamma (IFN-γ) was assessed by measurement of nitrite (NO_2^-) concentration in the medium using the Griess reaction. Inducible nitric oxide synthase (iNOS) protein levels were determined by Western blotting.



Inhibition of NO production (%) in macrophages RAW 264.7-activated with INF-γ and LPS after treatment with isoflavones. Values are expressed as means (SEM) of two individual experiments, $P<0.05$ (*) indicates a significant difference of the treated *v.* the control samples.

ESR spectroscopy indicated that 8-hydroxydaidzein was the most potent scavenger of hydroxyl and superoxide anion radicals. Isoflavone metabolites also exhibited higher antioxidant activity than parent compounds in standard antioxidant (FRAP and TEAC) assays. However, for the suppression of NO production by activated macrophages, genistein showed the highest potency, followed by equol and daidzein (see Figure). Neither the isoflavone metabolites nor their parent compounds had an effect on iNOS protein levels in the activated macrophages, suggesting that the inhibitory effect of genistein, daidzein and equol occurs at a post-transcriptional level. Thus the metabolism of isoflavones affects their free radical scavenging and antioxidant properties, and their cellular activity, but the effects are complex and worthy of further investigation.

Skeletal calcium accretion and calcium intake in young females during puberty: data from a 3-year longitudinal study on bone health. By J.A. NURMI-LAWTON[1], A. BAXTER-JONES[2], J.A. BISHOP[1], P. TAYLOR[3], C. COOPER[3] and S.A. NEW[1], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH,* [2]*University of Saskatchewan, Saskatoon, Canada and* [3]*Osteoporosis Centre, University of Southampton, Southampton SO16 6YD*

The importance of calcium intake for peak bone mass (PBM) development has been investigated in various calcium supplementation and calcium balance studies. Several other nutritional factors such as vitamin D and protein also influence skeletal development, although their full role remains unspecified. It has been estimated that 26% of adult skeletal calcium is laid down during 2 years around the period of peak calcium accretion (Bailey *et al*. 2000), with a large range in variation of absorption and retention rates both within and between individuals.

As part of a 3-year longitudinal study on the effects of nutrition and exercise on PBM development in young female gymnasts (G) and sedentary controls (C), this abstract presents data on influences of exercise on peak bone mineral accretion. Individuals going through their peak BMC accrual during the study period were identified (G, *n* 10; C, *n* 13) using a method based on prediction of age at peak height velocity (PHV) (Mirwald *et al*. 2002). Total body bone mineral content (TB BMC) was measured annually on three occasions by dual energy X-ray absorptiometry (DXA, Lunar DPX), and consequently two velocity measures were obtained for each subject. Information on anthropometry and pubertal development were collected at baseline, 6, 12, 24 and 36 months. Dietary intake was assessed using repeated estimated dietary records (7 d at baseline; 3 d at 6, 9, 12, 24 and 36 months) and analysed using Diet 5 for Windows (version 2000).

	Gymnasts (*n* 10)		Controls (*n* 13)	
	Mean	SD	Mean	SD
Age at PHV (years)*	12.9 [a]	0.8	11.9 [b]	0.6
Age at peak BMC velocity (years)	14.2 [a]	0.8	12.8 [b]	0.5
Peak BMC velocity (g/year)	256	54	292	116
Peak Ca accretion rate (mg/d)**	226	47	257	102
Mean Ca intake (mg/d)	664	253	819	238
Apparent retention efficiency (%)***	41	23	34	18

[a,b] Unlike superscripts indicate $P<0.001$ (*t*-test). *Estimated, based on Mirwald *et al*. (2002). **Calculated using 32.2% as the fraction of Ca in bone mineral (Ellis *et al*. 1996). ***Calculated as % of Ca accretion from Ca intake.

As shown in the Table, estimated PHV and peak BMC velocity occurred significantly later in gymnasts than in controls. Within the groups, PHV (growth spurt) occurred 0.9–1.3 years before the peak bone mineral accrual. However, there was no difference in the magnitude of bone mineral accrued at peak, or in mean dietary calcium intake between the groups. The calculated mean daily calcium accretion rates were 226 and 257 mg/d, and apparent retention efficiencies 41% and 34% in gymnasts and controls, respectively.

Although based on calculated values, these results emphasize the importance of adequate dietary calcium in both active and inactive girls by quantifying the annual skeletal mineral accretion rates. With calcium intakes around the current RNI for this age group (11–18 years; 800 mg/d), high absorption and retention efficiency are required in order to provide sufficient mineral for the growing bone. Further research on other micronutrients is required to clarify the effects of diet on peak bone mineral accrual.

Financial support from the National Osteoporosis Society is gratefully acknowledged.

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Maternal undernutrition during early to mid-pregnancy and the programming of mitochondrial cytochrome *c* and voltage-dependent anion channel (VDAC) in the ovine fetal and juvenile lung. By D.P. YAKUBU, G.S. GOPALAKRISHNAN, A. MOSTYN, M.E. SYMONDS and T. STEPHENSON, *Academic Division of Child Health, School of Human Development, University Hospital, Nottingham NG7 2UH*

Mitochondria are involved in cellular energy metabolism. Cytochrome *c* is a highly mobile electron transport protein located in the mitochondrial inter-membrane spaces and is essential in cellular energy conversion. VDAC is a general diffusion porin present in the outer mitochondrial membrane and is important in cellular energy metabolism and apoptosis. Maternal nutrition during pregnancy is known to play an important role in determining mitochondrial protein abundance in the newly born offspring (Budge *et al*. 2003). However, the extent to which such effects may be programmed *in utero* and in the subsequent juvenile remain to be established. The present study, therefore, examines the effect of maternal undernutrition over the period of placental growth on the abundance of cytochrome *c* and VDAC in the ovine fetal and juvenile lung.

Twenty-four Welsh mountain ewes of similar body weight and condition score were individually housed from day 28 of gestation and assigned to two dietary groups; nutrient-restricted (NR) and control (C). The NR group consumed 3.2–3.8 MJ d^{-1} of metabolisable energy (ME) (60% of total ME requirements) until 80 d of gestation, while the C group were fed to appetite and consumed 8.0–10.9 MJ d^{-1} of ME. From 80 d of gestation until term, both groups consumed 8.0–10.9 MJ d^{-1} of ME (150% of total ME requirements). Lung tissue was sampled from four or five singleton fetuses from NR and C ewes at mid-gestation (80 d) after humane euthanasia (barbiturate overdose, 100 mg kg^{-1} pentobarbital sodium: Euthata). Seven ewes in each group were allowed to deliver normally and their offspring grazed along with their mothers on open pasture until 6 months of age. These were then humanely slaughtered and lung tissues sampled for further analysis. Mitochondria were prepared from the lung tissues and analysed by immunoblotting.

Results (in arbitrary units (a.u.)) are presented as means with their standard errors (SEM). Statistically significant differences between nutritional groups were analysed using a Mann-Whitney *U* test.

	VDAC (a.u.)				Cytochrome *c* (a.u.)			
	NR		C		NR		C	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
80 d fetus	34	2.1	31	1.9	6	0.7*	10	1.8
6 months	8	1.5**	18	0.8	18	1.2*	25	2.4

Significant differences between nutritional groups: *P<0.05, **P<0.01.

Lung fresh and dry weights and total protein concentration were similar between nutritional groups at both sampling ages. VDAC abundance was similar in both groups at mid-gestation but significantly lower in the NR group at 6 months postnatal age (P<0.01). Cytochrome *c* was significantly lower in the NR group at both mid-gestation and 6 months postnatal age (P<0.05).

In conclusion, maternal undernutrition during early to mid-pregnancy has differential effects on the abundance of cytochrome *c* and VDAC. This has long-term effects in the juvenile lung development which are manifested in reduced VDAC and cytochrome *c* abundance. This may act to limit energy availability in the lung during periods of metabolic stress.

David Yakubu was supported by a University of Nottingham International Office Scholarship.

Budge H, Dandrea J, Mostyn A, Evens Y, Watkins R, Sullivan C, Ingleton P, Stephenson T & Symonds ME (2003) *Pediatric Research* **53**, 302–308.

Maternal supplementation during pregnancy, breast-feeding and early childhood diet as predictors of childhood cognitive function in the 1946 Birth Cohort. By K.D. BARWELL[1], M. RICHARDS[2], G. MISHRA[1], M.E.J. WADSWORTH[2] and C. BOLTON-SMITH[1], [1]*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL and* [2]*MRC National Survey of Health and Development, University College London, 1–19 Torrington Place, London WC1E 6BT*

Breast-feeding confers a modest yet consistent benefit on cognitive ability in childhood even after controlling for pertinent social confounders (Anderson *et al*. 1999). The *n*-3 polyunsaturated fatty acid (PUFA) content of breast milk is likely to be an important factor, but the additional contribution of perinatal maternal diet or diet in early childhood is unclear.

This investigation examined the effect of maternal welfare supplementation during pregnancy, duration of breast-feeding and diet at 4 years on cognitive ability at age 8 years in the 1946 Birth Cohort. The social-class-stratified cohort initially consisted of 5362 people selected from all births occurring in Britain during the period 3–9 March 1946. This analysis includes subjects with complete data on dietary, cognitive and confounder variables (n=1008).

Dietary data included the reported uptake of maternal welfare supplements (MWS) during pregnancy (milk, orange juice, and either cod liver oil or a vitamin A and D tablet), duration of breast-feeding and 24 h recall meal records at age 4 years (Prynne *et al*. 1999). Cognitive assessment was conducted at age 8 years and involved reading comprehension, word pronunciation, vocabulary, and non-verbal reasoning. Multiple linear regression was used to model the relationship between dietary factors and cognitive ability, adjusting for social class, parental education, birth order, maternal age, maternal smoking, gender and birth weight.

Nutrient factor	Adjusted multiple regression parameter estimates and 95%CI for cognitive ability at age 8 years		
	Total (n 1008)	Boys (n 487)	Girls (n 521)
MWS vitamin A/D per day			
<700 µg/<1 µg	0.7 (−0.1, 1.5)	0.2 (−1.0, 1.3)	1.1* (0.0, 2.1)
700–1500 µg/18–21 µg	1.0** (0.3, 1.6)	0.6 (−0.4, 1.6)	1.1* (0.2, 2.0)
>2000 µg/>38 µg	Reference	Reference	Reference
Diet at 4 years, energy adjusted			
Vitamin C[#] mg/d	0.1 (0.0, 0.2)	−0.1 (−0.2, 0.1)	0.2** (0.1, 0.3)
Vitamin E mg/d	0.3* (0.0, 0.5)	0.5** (0.2, 0.9)	0.02 (−0.3, 0.3)

*P<0.05, **P<0.01, [#]Square-root transformation.

Breast-feeding was not significantly associated with cognition at age 8 years. However, cognitive ability was significantly higher in children whose mothers consumed between 700 and 1500 µg/d of supplemented vitamin A (18–21 µg vitamin D) relative to mothers consuming >2000 µg vitamin A/d (>38µg vitamin D). For diet at age 4 years, vitamin C intake showed a significant association with cognitive ability among girls, and vitamin E intake was significantly and positively associated with cognition in boys.

This study suggests that nutrient supply *in utero* and in early childhood may modify childhood cognitive ability. Retinol or vitamin D toxicity could explain the decrease in cognitive ability observed in children of mothers in the highest group of daily vitamin A and D consumption. The *n*-3 PUFA in the fish oil and antioxidant function of vitamins C and E could contribute to the positive relationship between these vitamins and childhood cognitive function.

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Effect of maternal nutrient restriction in early to mid-gestation on blood pressure control at 2 years of age in the resulting offspring in sheep. By G.S. GOPALAKRISHNAN[1], S.M. RHIND[3], C.E. KYLE[3], A.N. BROOKS[4], R.M. WALKER[2], M.M. RAMSAY[1], T. STEPHENSON[1] and M.E. SYMONDS[1], [1]*School of Human Development, University Hospital,* [2]*School of Biosciences, University of Nottingham, Nottingham NG7 2UH,* [3]*Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen AB15 8QH and* [4]*AstraZeneca, Alderly Park, Cheshire, SK10 4TJ*

Suboptimal maternal nutrition at specific stages of pregnancy is associated with abnormal placental and fetal growth. Consequently, vascular structure and physiology of the resulting offspring may be altered and these individuals at increased risk of adult diseases including hypertension (Barker, 1995). Maternal nutrient restriction over the period of rapid placental growth (i.e. 30–80 d), followed by adequate nutrition up to term, results in a longer fetus with a disproportionately larger placenta (Heasman *et al.* 1998). At term, these lambs have larger kidneys which exhibit an increased abundance of glucocorticoid and type 1 angiotensin II (AII) receptor mRNA (Whorwood *et al.* 2001). This study aimed to determine the long-term consequences of maternal nutrient restriction between early to mid-gestation on subsequent blood-pressure control in offspring.

Thirteen male-singleton-bearing Scottish Blackface ewes of similar live weight and body condition were individually housed from day of mating. Eight ewes were nutrient-restricted (NR), these consumed 4.0 MJ of metabolisable energy (ME) per day (≅50% of ME requirements for maintenance and growth of the conceptus) until 95 d gestation, with five controls (C) consuming 8.0 MJ/d. After 95 d gestation, until term (147 d), all animals consumed 100% of requirements. At term, lambs were delivered spontaneously and at ~2 years of age, a carotid artery was surgically catheterised to allow blood-pressure measurement. Surgery lasted under an hour for all sheep. At least 2 d after surgery, systolic and diastolic blood pressures were measured for a resting period of 30 min, before animals were fed pellets and hay. Blood pressure was continuously recorded throughout feeding and up to 10 min following feeding.

Results are expressed as mean values and standard errors. Statistically significant differences between groups were assessed using a Mann-Whitney test.

Maternal group	Ram birth weight (kg)		Mean blood pressure before feeding (mmHg)		Mean blood pressure 10 min after feeding (mmHg)	
	Mean	SEM	Mean	SEM	Mean	SEM
NR (*n*=8)	4.0	0.3	75.2	2.6[a]	85.0	3.5
Control (*n*=5)	4.4	0.2	64.6	2.7	78.3	3.4

[a] Significantly different from control at $P<0.05$ level, as measured by Mann-Whitney *U* test.

There was no difference in ram body weight at birth or throughout the study. At 2 years of age resting blood pressure prior to feeding was significantly higher in NR offspring compared with the controls (e.g. systolic C 86.4, 1.9; NR 96.5, 2.7 mmHg (P=0.04)). There was no statistical difference between groups following feeding when C animals had similar blood pressure to NR offspring.

Nutrient restriction between early to mid-gestation, followed by adequate feeding up to term, resulted in hypertensive offspring prior to feeding. This suggests that alterations in maternal nutrition at specific stages of gestation may contribute to reprogramming of blood-pressure control, thus potentially contributing to hypertension in later life through concomitant physiological and vascular changes.

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Maternal nutrient restriction during early to mid-gestation alters insulin-like growth factor I (IGF-I) mRNA abundance independent of alterations in growth hormone receptor (GHR) mRNA abundance in the ovine fetal kidney. By K. BRENNAN[1], G.S. GOPALAKRISHNAN[1], S.M. RHIND[2], C.E. KYLE[2], A.N. BROOKS[3], M.T. RAE[2], T. STEPHENSON[1], D. OLSON[4] and M.E. SYMONDS[1], [1]*Academic Division of Child Health, School of Human Development, Queen's Medical Centre, Nottingham NG7 2UH,* [2]*Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH,* [3]*AstraZeneca, Alderly Park, Cheshire, SK10 4TJ and* [4]*Perinatal Research Centre, University of Alberta, Edmonton, Canada*

The risk of developing hypertension later in life can be influenced by maternal nutrient intake with reduced fetal nutrition adversely affecting kidney growth (Whorwood *et al.* 2001). IGF-I has an important role in kidney development and is known to be nutritionally regulated (Rogers *et al.* 1999). Previous work has shown that GHR is present at relatively high levels in the fetal kidney (Ymer & Herington, 1992) and that abundance does not necessarily parallel that of IGF-I. The aim of this study was therefore to determine the extent to which maternal nutrient restriction at specific stages of early to mid-gestation might result in altered expression of IGF-I within the fetal kidney and determine whether these changes are linked to alterations in GHR abundance.

Thirty-four singleton-bearing Scottish Blackface ewes were individually housed from the day of mating and assigned to one of four intervention groups and a control group (*n*=4–8). All ewes were fed a diet which was sufficient to meet 100% of total metabolisable energy requirements for that stage of gestation, then restricted to 50% between 0–30, 31–65, 66–110 and 0–110 d gestation. At 110 d gestation (term is 147 d) all ewes were euthanased and fetal kidneys sampled. Total RNA was extracted from the fetal tissue and reverse-transcribed. This cDNA was then used in PCR reactions using oligonucleotide primers specific for the GHR, IGF-I and an 18S control. Abundance was measured using densitometry and the results were calculated as a ratio of the GHR or IGF-I reaction to the 18S reaction, then expressed as a percentage of a single reference sample. All analyses were undertaken in triplicate and the coefficient of variance for each sample was always less than 15%. Results were analysed using a Kruskal-Wallis test, then each experimental group was compared with the control using a Mann-Whitney *U*-test.

Period of nutrient restriction (days gestation)	Kidney weight (g)		IGF-I mRNA (%)		GH Receptor mRNA (%)	
	Mean	SEM	Mean	SEM	Mean	SEM
Control	9.1	0.3	163	11	57	37
0–30	9.3	0.3	136	24	208	54
30–65	9.1	0.5	155	31	127	36
66–110	7.9	0.8	120	6*	109	37
0–110	7.9	0.4†	133	11	87	17

† Different from control at P=0.05, as measured by Mann-Whitney *U* test.
* Significantly different from control at $P<0.05$, as measured by Mann-Whitney *U* test.

Maternal nutrient restriction between 66 and 110 d and 0 to 110 d gestation led to a reduction in fetal kidney weight. The group restricted from 66 to 110 d also showed a significant decrease in the abundance of IGF-I mRNA. Mean GHR mRNA abundance was greater in all kidneys of nutrient-restricted groups compared with controls but, because of the large variation between individuals within each group, this was not statistically significant. There was no correlation between IGF-I and GHR mRNA abundance. Nutrient supply to the fetus therefore appears to regulate IGF-I in the kidney independently of the GHR, at least at this stage of development. In conclusion, maternal nutrient restriction coincident with the period of nephrogenesis results in reduced kidney growth and lower IGF-I mRNA expression in the absence of any parallel effect on GHR mRNA abundance.

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Ymer SI & Herington AC (1992) *Molecular and Cellular Endocrinology* **83**, 39–49.

Protein supplementation at specific stages of gestation can promote growth of fetal organs associated with immune competence as well as adipose tissue deposition in sheep. By G. HOPKINS[1], D. GARDNER[2], S. PEARCE[2], E. BUTT[2], J. BISPHAM[2], K.H.S. CAMPBELL[1], M.M. RAMSAY[2] and M.E. SYMONDS[2], [1]*Department of Animal Physiology, School of Biosciences, University of Nottingham, LE12 5RD and* [2]*School of Human Development, University of Nottingham, Nottingham NG7 2UH*

Maternal nutrient restriction at specific gestational stages compromises fetal growth and development (Symonds *et al*. 2001). In particular, fetal adipose tissue deposition (Symonds *et al*. 2003) and growth of organs associated with immune competence (i.e. thymus and spleen) are nutritionally regulated (Osgerby *et al*. 2002). The extent to which nutritional supplementation can promote growth and development of specific fetal organs is not known. This study aimed to determine whether protein supplementation of the maternal diet at defined stages of gestation promoted fetal growth and the development of specific fetal tissues.

Twenty-nine twin-bearing ewes of similar body weight and parity were randomly allocated to four feeding groups from 10 d gestation. Controls (n=8) were fed a standard diet of chopped hay and barley-based concentrate that was increased with gestation in order to provide sufficient metabolisable energy necessary to produce 4.5-kg lambs at term. Supplemented groups (n=7 per group) were randomly allocated to be provided with additional protein in the form of fishmeal (66% crude protein plus an equal amount of molasses to aid palatability) at either 10–40 d gestation (Group 1), 40–70 d gestation (Group 2) or from 110 d gestation (Group 3). Groups 1 and 2 therefore consumed ~ 1.0 kg of chopped hay and 200–240 g concentrate (50% barley; 25% fishmeal, 25% molasses), whilst Group 3 consumed 1–1.5 kg hay and 300–600 g concentrate. All the ewes were then humanely euthanised with an overdose of barbiturate (100 mg/kg pentobarbital sodium: Euthanal) at 140 d gestation to enable sampling of fetal and placental tissues. Only one randomly selected fetus from each ewe was used for tissue sampling and all placentomes were dissected and weighed in order to determine total placental mass. Results are given as means with their standard errors. Statistically significant differences with respect to nutritional supplementation were determined using a one-way ANOVA and Tukey's test.

Gestational period of supplementation	Fetal body weight (kg)		Thymus weight (g)		Spleen weight (g)		Pericardial adipose tissue weight (g)		Perireneal adipose tissue weight (g)		Placental weight (g)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
10–40 d	5.21	0.27	4.84[a]	0.65	5.81[a]	0.66	4.30	0.31	21.30	0.86	631.7	83.8
40–70 d	5.75	0.25	8.68[ab]	0.63	8.15[ab]	0.78	4.97[a]	0.25	22.79	1.02	753.1	39.3
110–140 d	5.45	0.29	6.53	1.07	7.28	0.52	3.49[a]	0.33	23.64	0.36	712.4	56.2
Control	5.03	0.56	6.17[b]	0.81	5.90[b]	0.63	3.99	0.21	18.69	0.60	703.5	58.8

Significant differences between groups, indicated by similar superscripts [a] or [b], P<0.05, using ANOVA and Tukey's *post hoc* test.

Protein supplementation during mid-gestation, coincident with the period of maximal placental growth resulted in the largest placentas and fetuses, although these differences were not statistically significant. The spleens and thymus from these fetuses were increased in mass, as were pericardial but not perirenal adipose tissue depots. There were no similar effects of protein supplementation on all other organs and tissues sampled.

In conclusion, protein supplementation of the maternal diet during the period of maximal placental growth specifically increases the growth of those tissues involved in immune competence. This may act to protect these offspring from infection after birth.

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Is the relationship between iron intake and body iron levels modulated by genotype? Results from the UK Women's Cohort Study. By J.E. CADE[1], J.A. MORETON[1], B.A. BRATLEY[1], J.A. RANDERSON-MOOR[2], K. KUKALIZCH[2], V.J. BURLEY[1], D.C. GREENWOOD[1], D. THOMPSON[3], M. WORWOOD[4] and D.T. BISHOP[2], [1]*Nutrition Epidemiology Group, Nuffield Institute for Health, 71–75 Clarendon Road, Leeds LS2 9PL,* [2]*Genetic Epidemiology Division, Cancer Research UK Clinical Centre, St James's University Hospital, Leeds LS9 7TF,* [3]*Department of Clinical Biochemistry and Immunology, Leeds General Infirmary, LS1 3EX and* [4]*University of Wales College of Medicine, Cardiff, CF4 4XN*

High levels of iron storage, even within the normal range, may predispose individuals to a number of chronic diseases, including heart disease and some cancers (British Nutrition Foundation, 1995). Recent genetic research has identified the mutations (in the gene HFE) commonly associated in the UK with the iron-overload disease, haemochromatosis. The UK Women's Cohort Study has recruited over 35 000 women, aged 35–69 years at recruitment, which began in 1994. A sample of 7207 women provided either a cheek cell or blood sample for measurement of two of these specific gene mutations. The C282Y and H63D mutations of HFE were assayed on 6724 (93%) and 6755 (94%) of the women, respectively. Blood iron storage levels were available on 2528 of these women. Dietary iron intake, both haem and non-haem has been estimated from a 218-item food frequency questionnaire. Genotype was strongly related to blood measures of iron storage and also to haem iron intake, intakes of red meat and body mass index.

Genotype	Total n[#]	Ferritin (μg/l)[†]	Serum iron (μmol/l)	Transferrin saturation (%)	Dietary haem iron (g/d)	BMI (kg/m^2)
Normal range...		11–306	11–29	≤50		
C282Y/C282Y	31	133.0	34.1	72.6	0.5	23.4
C282Y/H63D	171	64.5	24.6	48.8	0.7	24.9
H63D/H63D	167	44.5	22.9	41.9	0.8	25.3
C282Y/WT	717	52.9	21.6	41.4	0.6	24.3
WT/WT	5640	44.3	20.3	36.6	0.7	24.5

[#]Numbers with blood measures: C282Y homozygotes 30; compound heterozygotes 135; H63D homozygotes 40; C282Y heterozygotes 578; C282Y wild type 1705, [†] geometric mean, WT=wild type allele (non-C282Y, non-H63D).

Seventy-eight (3%) of the women had serum ferritin values which were considered to be high (>200 μg/l). Individuals who were homozygous for the C282Y mutation had higher serum ferritin (P<0.0001), transferrin saturation (P<0.0001) and serum iron (P<0.0001) levels and lower intakes of haem iron (P=0.005) and BMI (P=0.04) compared with the wild type. Women who were simple or compound C282Y heterozygotes had intermediate levels, although they were more similar to the wild type than the homozygotes. Total dietary iron, non-haem iron, vitamin C and calcium in the diet did not appear to be associated with genotype.

Despite lower red meat and haem iron intakes by the women who were homozygous for the C282Y mutation, they still had considerably higher serum ferritin levels. Significantly more of the homozygotes were vegetarian than expected (33%) compared with 23% in the wild-type group (P<0.0001). Further exploration using a multivariate approach to adjust for confounding factors such as supplementary iron intake and menopausal status is warranted.

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Identification of human cytochrome P450s and UDP-glucuronosyl transferases involved in genistein metabolism. By L.E. PRITCHETT[1], K.M. ATHERTON[1], E. MUTCH[2] and D. FORD[1], [1]*School of Cell and Molecular Biosciences, University of Newcastle, Kings Road, Newcastle upon Tyne NE1 7RU and* [2]*School of Clinical Laboratory Sciences, University of Newcastle, Framlington Place, Newcastle upon Tyne NE2 4HH*

Consumption of soya isoflavones has been linked to decreased incidence of several cancers, including cancers of the breast, prostate and colon. This effect is potentially modulated by Phase I and Phase II metabolism. The present study investigated the contribution of specific cytochromes P450 (CYPs) and UDP-glucuronosyl transferases (UGTs) to the metabolism of the isoflavone genistein.

Human liver microsomes with a known profile of activity with respect to the metabolism of several marker substrates of CYPs (CYP 1A, 2E1, 3A) or UGTs (UGT1A4, 1A9, 1A10), or microsomes prepared from insect cells expressing specific human UGTs using the baculovirus system (Supersomes, Gentest), were incubated with 100 μM genistein either for 30 min in the presence of 1 mM NADPH (CYP metabolism) or for 3 h in the presence of 1 mM UDP-glucuronic acid (UGT metabolism). NADPH-dependent metabolism was also investigated in the presence of inhibitors of specific CYPs: 50 μM furafylline (CYP1A2); 100 μM 4-methylpyrazole (CYP2E1); 1 μM ketoconazole (CYP3A4); 5 μM quinidine (CYP2D6). The resulting metabolites were analysed by HPLC. For CYP-mediated metabolism, separation was on a C18 column using a mobile phase of 0.18% trifluoroacetate in acetonitrile in a 3:1 ratio. For UGT-mediated metabolism, separation was on a C18 column using a mobile phase of 50% methanol, 50% 2 mM terabutylacetate in water.

NADPH-dependent (CYP-mediated) metabolism of genistein generated four major metabolite peaks. There was no clear correlation between relative levels of specific CYP activities in the livers tested and the relative areas of any specific peak, indicating a contribution to each metabolite of multiple CYPs. Microsomes from only three of four livers tested generated the metabolite corresponding to peak 1. Microsomes from all four livers generated the other three peaks. Inhibition of the generation of the metabolites corresponding to peaks 1, 2 and 3 was most marked in the presence of furafylline (peak 1: mean 61.9% (SEM 27.0%, n=3, P<0.01); peak 2: mean 67.6% (SEM 16.2%, n=4, P<0.01); peak 3: mean 50.1% (SEM 14.1%, n=4, P<0.05); statistical analysis by two-tailed, paired Student's t-test) and 4-methylpyrazole (peak 1: mean 49.0% (SEM 7.1%, n=3, P<0.05); peak 2: mean 64.7% (SEM 20.2%, n=4, P<0.05); peak 3: mean 56.9% (SEM 8.2%, n=4, P<0.01); statistical analysis by two-tailed, paired Student's t-test). The generation of peak 4 was most markedly inhibited by ketoconazole; however, the inhibitory effect (mean 92.9% (SEM 10.1%)) was observed in only two of the four livers tested.

The area of the peak corresponding to the major glucuronidated metabolite of genistein generated by Supersomes expressing single UGTs was UGT1A9 >1A10 >1A1. No activity was detected with UGT2B7 or 2B15. The peak area of the glucuronide showed a positive linear correlation with UGT1A1 activity in microsomes from six human livers (R^2=0.74, P<0.05) but not with UGT1A9 or UGT1A4 activity.

The data indicate a role for CYPs 1A2, 2E1 and 3A4 in the hepatic Phase I metabolism of genistein. Whilst UGT1A9 was more active with respect to glucuronidation of genistein in the absence of other UGTs, UGT1A1 appears to play the major role in a mixed, hepatic enzyme system. The involvement in genistein metabolism of CYPs and UGTs, enzymes for which polymorphisms are well-established, may lead to inter-individual variability in the response to dietary isoflavones.

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The effect of dietary zinc deficiency on expression of zinc transporter mRNAs in mouse placenta. By R.M. RUSSI, S.R. PHILLIPS, J.M. PIPER, J.C. MATHERS and D. FORD, *School of Cell and Molecular Biosciences and Human Nutrition Research Centre, University of Newcastle, Kings Road, Newcastle-upon-Tyne NE1 7RU*

Adequate fetal zinc supply is essential for development of the neonate, and zinc supply *in vitro* may influence future susceptibility to various non-communicable diseases. The placenta expresses several proteins with zinc transport activity that potentially have a role in fetal zinc nutrition. The present study examined the effect of moderate maternal zinc deficiency on expression at the mRNA level of several cloned zinc transporters in the mouse placenta.

Twelve female C57BL/6 mice were time-mated and from day 0 of gestation (presence of vaginal plug) were given access to one of two zinc-free purified mouse diets supplemented as follows: zinc-deficient (15 ppm, ZnD) or zinc-adequate (50 ppm, ZnA). The basal diet was adequate with respect to energy and all other macro- and micronutrients. All animals were housed in groups of three, in stainless-steel cages with free access to deionised water and provided with a weighed quantity of food such that all was consumed within a 24 h period. On day 16 of gestation, animals were killed by CO_2 overdose and all placentas were snap-frozen in liquid nitrogen. Levels of placental mRNA corresponding to MT and the zinc transporters ZTL1/ZnT5, ZIP1, ZnT1 and ZnT4 were compared by semi-quantitative RT-PCR in six placentas from each of twelve animals (six ZnD and six ZnA). Mean fetal weight was reduced (P<0.05 by unpaired, two-tailed Student's t-test) in ZnD animals (0.644 g, SEM=0.012, n=38) compared with ZnA animals (0.698 g, SEM=0.022, n=46).

	15 ppm Zn^{2+}			50 ppm Zn^{2+}		
	Mean	SEM	n	Mean	SEM	n
MT	0.52***	0.06	17	1.00	0.12	15
mZTL1/ZnT5	0.75*	0.06	17	1.00	0.10	16
mZIP1	0.78*	0.08	17	1.00	0.09	17
mZnT1	0.59***	0.06	16	1.00	0.09	18
mZnT4	0.59**	0.06	16	1.00	0.11	18

Levels of Zn-related mRNAs in mouse placenta following dietary zinc manipulation. Values are in arbitrary units as a ratio of 18 S rRNA levels and are normalised to data for the ZnA diet. Statistical analysis was by two-tailed, unpaired Student's t-test; *P<0.05, **P<0.01, ***P<0.001.

Expression of all transcripts was significantly reduced on the ZnD diet compared with the ZnA diet. We suggest that downregulation of MT and zinc transporters in the mouse placenta under conditions of zinc deficiency is mediated through reduced activity of the zinc-sensitive transcription factor MTF-1 (Heuchel *et al.* 1994). These responses are similar to those observed in the rat intestine (Liuzzi *et al.* 2001) and suggest that alternative regulatory mechanisms do not act in the placenta to protect the fetus from conditions of maternal dietary zinc deficiency. However, additional placental zinc transporters not examined in this study may be upregulated to maximise zinc transfer to the fetus when maternal intake of zinc is restricted.

Funded by BBSRC grant 13/D11912 (D.F. and J.C.M.) and by a BBSRC studentship (R.M.R.).

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The influence of docosahexaenoic acid on endothelial function and arterial compliance in healthy young male subjects. By H.E. THEOBALD[1], T.A.B. SANDERS[1], E.A. ASBURY[2], C.S. HAYWARD[2] and P. COLLINS[2], [1]*Nutrition Food and Health Research Centre, Franklin-Wilkins Building, King's College London, London SE1 9NN,* [2]*Cardiovascular MR Unit, Royal Brompton Hospital, Imperial College School of Medicine, London SW3 6NP*

Endothelial dysfunction is associated with increased risk of coronary heart disease (CHD) (Halcox *et al*. 2002). Long chain *n*-3 fatty acids have been shown to improve endothelial function in hypercholesterolaemic individuals (Goodfellow *et al.* 2000) and arterial compliance in subjects with type II diabetes (McVeigh *et al.* 1993). Mori *et al*. (2000) reported that 4 g DHA/d improved endothelial function in overweight hyperlipidaemic men. We report the effects of DHA on endothelial function and aortic compliance in a pilot study of 3 weeks' duration, supplementing with 2.6 g DHA as an algal triacylglycerol (DHASCO) versus placebo in thirty-two healthy men (aged 18-40 years) using a double-blind parallel design.

Endothelial-dependent (flow-mediated dilatation: FMD) and independent relaxations (glyceryl trinitrate: GTN) were determined by B-wave ultrasound and pulse wave velocity (PWV), and the augmentation index (AI) by tonometry. The results are shown in the Table.

	DHA (*n*=16)		Placebo (*n*=16)		*P*
	Mean	SEM	Mean	SEM	
Brachial artery diameter (mm)					
Pre-treatment	3.89	0.107	4.12	0.146	
End of study	3.95	0.149	4.03	0.151	0.87
FMD 1 min post-occlusion (mm)					
Pre-treatment	0.17	0.089	0.03	0.093	
End of study	0.27	0.116	0.17	0.076	0.65
FMD 5 min post-occlusion (mm)					
Pre-treatment	0.24	0.097	0.05	0.074	
End of study	0.28	0.111	0.22	0.097	0.82
GTN dilatation (mm)					
Pre-treatment	0.65	0.122	0.40	0.089	
End of study	0.59	0.125	0.44	0.082	0.19
Augmentation index					
Pre-treatment	0.75	0.046	0.72	0.028	
End of study	0.73	0.043	0.72	0.027	0.82
Aorto femoral PWV m/s					
Pre-treatment	7.75	0.429	7.16	0.190	
End of study	7.36	0.302	7.22	0.213	0.24

FMD tended to be greater and PWV decreased (implying improved compliance) following DHA supplementation, but compared with the control group did not achieve statistical significance. Further studies are needed to confirm these preliminary data using a larger sample size and by using repeat measures to decrease measurement error.

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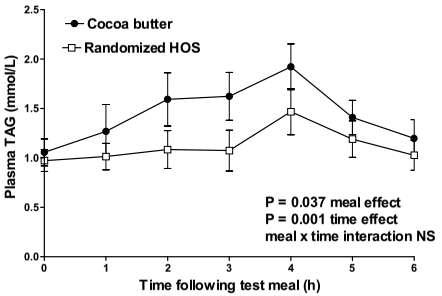
Postprandial lipaemia induced by cocoa butter compared with an inter-esterified blend of totally hydrogenated and unhydrogenated high oleic sunflower oil. By S.E.E. BERRY and T.A.B. SANDERS, *Nutrition, Food and Health Research Centre, King's College London, 150 Stamford Street, London SE1 9NN*

Cocoa butter results in similar postprandial lipaemia compared with high oleic acid sunflower (HOS) oil but randomization of cocoa butter results in reduced postprandial lipaemia (Sanders *et al*. 2003). One explanation advanced was that this was a consequence of changes in triacylglycerol (TAG) structure from symmetrical to asymmetrical TAG, which would alter the physical properties of the fat. In an earlier report, Sanders *et al.* (2001) noted that a test meal consisting of an inter-esterified blend of total hydrogenated with unhydrogenated high oleic sunflower oil (randomized HOS) resulted in decreased postprandial lipaemia compared with a meal consisting of unhydrogenated unrandomized HOS. In the present study, the postprandial lipaemic response of randomized HOS was compared with cocoa butter. Melting characteristics were determined by differential scanning calorimetry (DSC) and low resolution NMR, and TAG structure by HPLC. The postprandial changes in plasma lipids were determined in six male subjects aged 20–40 years following standardized test meals containing 50 g fat. The changes in plasma TAG are shown below (mean values with SEM).



The randomized HOS fat resulted in a 51% lower postprandial area under the curve from plasma TAG compared with cocoa butter. Analysis of the TAG molecular structure showed that 0.5% of the TAG was trisaturated in the cocoa butter whereas the proportion was 9.8% in the randomized HOS. Virtually all of the saturated fatty acids were in the *sn*-1 and *sn*-3 positions in cocoa butter but in the randomized HOS 54.4 mol% was present in the *sn*-2 position. DSC calorimetry showed two major melting peaks at 17.1 and 22.9°C for the cocoa butter, and two major peaks at 18.7 and 32.4°C and a further melting event at 43°C in the randomized HOS which was not completely melted at 50°C. The NMR analyses showed solid fat content at 37°C to be <1% for the cocoa butter but 23% for the randomized HOS and 11.9% at 52°C. The differences in physical properties of these saturated fats with similar fatty acid contents are likely to affect the rate of micelle formation and fat absorption and so may explain the observed differences in postprandial lipaemia.

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Randomized placebo controlled trial of a daily intake of 200 mg docosahexaenoic acid in vegans.
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Docosahexaenoic acid (22:6*n*-3; DHA) can be synthesised from linolenic acid (18:3*n*-3) and can also be obtained preformed from the consumption of food of animal origin, especially fish. The proportion of DHA in plasma and erythrocyte lipids of vegans is approximately one-third that of omnivores (Sanders *et al*. 1978; Lloyd-Wright *et al*. 2000). An increased intake of linolenic acid was not found to increase the proportion of DHA in blood lipids (Sanders & Younger, 1981) and it was proposed that the lower proportion of DHA in the blood lipids of vegans was attributable to the absence of DHA from their diet and their higher intake of linoleic acid, which inhibits its synthesis from linolenic acid. Although DHA is usually absent from a vegan diet; DHA is manufactured by algae via the polyketide synthase pathway that does not use linolenic acid as a precursor. We hypothesised that an intake of DHA similar to that in omnivore diets would increase plasma DHA concentrations. This hypothesis was tested in a randomised double-blind paralleled designed crossover trial in seventy-two vegan men. Subjects were recruited from the Oxford cohort of EPIC and from the London Vegan Society. Exclusion criteria were abnormal haematology, liver function tests and a serum vitamin B_{12} concentration below 200 ng/l. Subjects were randomised to receive 200 mg DHA as algal DHA (DHASCO; Martek Inc.) or placebo in identical VegeCaps for 3 months. Fasting blood samples were obtained at baseline and at the end of study for determination of serum lipids and plasma fatty acid concentrations. The results are shown below.

			Pre-treatment		Post-treatment		Treatment effect
		n	Mean	SE	Mean	SE	
Plasma DHA (mg/l)	DHA	38	21.08	1.88	31.13	1.91	$P<0.0001$
	Placebo	31	25.22	2.41	24.44	2.04	
Cholesterol (mmol/l)	DHA	38	4.13	0.16	4.27	0.18	NS
	Placebo	31	4.07	0.12	4.37	0.13	
LDL (mmol/l)	DHA	38	2.32	0.13	2.34	0.14	NS
	Placebo	31	2.34	0.10	2.45	0.12	

Plasma DHA concentrations were low compared with the reference values in our laboratory for DHA for omnivores (mean 56mg/L, 95% CI 46, 65). Plasma DHA concentrations increased by 50% on DHA treatment but still did not achieve the concentrations seen in omnivores. There were no significant changes in serum lipid concentrations between treatments but the serum cholesterol concentrations were very low compared with the general population and reference values.

Very low intakes (200mg/d) of DHA have a significant influence on DHA concentrations in plasma lipids in subjects consuming diets devoid of DHA but do not increase LDL cholesterol at this level of intake.

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Plasma conjugated linoleic acid concentrations in vegan, vegetarian and omnivore men recruited into the EPIC study: 9-*cis*, 11 *trans* octadecadienoic acid are markers of the intake of ruminant derived fat.
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The term conjugated linoleic acid (CLA), is used to describe octadecadienoic acid (18:2) isomers in which the double bonds are in conjugation. 9-*cis*, 11-*trans*-octadecadienoic acid (9,11 CLA) is a minor component of milk, dairy products and ruminant fats (<1%). Consequently, this fatty acid could be used as a biomarker for the intake of ruminant-derived fatty acids. The other major isomer is 10-*cis*, 12-*trans* octadecadienoic acid (10,12 CLA) which occurs during the alkaline isomerisation of fats. The aim of the present study was to test the hypothesis that plasma 9,11 CLA concentration was a biomarker of ruminant fat intake. In order to test this hypothesis, we measured plasma CLA concentrations in vegan, vegetarian and omnivore men recruited into the EPIC study. Plasma fatty acid concentrations were determined on citrated plasma that had been stored at −70°C nitrogen for up to 5 years. Total plasma lipids were transesterified with methanolic HCL in the presence of pentadecaenoic acid as an internal standard. Methyl esters were separated by capillary gas liquid chromatography on a 25 m × 0.25 mm capillary column (BP70 SGE) using hydrogen as carrier gas. The results expressed as mean values with 95% CI are shown below.

	Omnivores *n* 226	Vegetarians *n* 237	Vegans *n* 233
9, 11 CLA mg/l	10.1 (9.2, 11.05)	6.6 (5.7, 7.55)	3.2 (2.3, 4.1)
9,11 CLA mol%	0.25 (0.23, 0.28)	0.19 (0.17, 021)	0.11 (0.10, 0.12)
10, 12 CLA mg/l	5.0 (4.6, 5.4)	3.9 (3.6, 4.3)	2.9 (2.5, 3.3)
10,12 CLA mol%	0.14 (0.13, 0.15)	0.14 (0.13, 0.15)	0.13 (0.12, 0.14)

The absolute concentrations of both CLA isomers differed significantly between the three dietary groups ($P<0.00001$). However, when expressed as mol% of total plasma lipids the differences between plasma concentrations of 10,12 CLA no longer remained statistically significantly different ($P=0.09$) but those between 9,11 CLA remained significant ($P<0.0001$). These findings would suggest that dairy products (as consumed by vegetarians and omnivores) make a substantial contribution to 9,11 CLA intake whereas 10,11 is probably derived from other sources, possibly from intestinal fermentation or from dark-green vegetables. The higher value for 9,11 CLA in omnivores compared with vegetarians could reflect the contribution made by fats of ruminant origin. It is suggested that 9,11 CLA could be used as a biomarker of the intake of ruminant-derived fatty acids.

Influence of an alkaline mineral water on postprandial lipaemia in postmenopausal women. By S. SCHOPPEN[1], A.M. PEREZ-GRANADOS[1], B. SARRIÁ[1], S. NAVAS[1], A. CARBAJAL[2], F. SANCHEZ-MUNIZ[2] and M.P. VAQUERO[1], [1]*Department of Metabolism and Nutrition, Instituto del Frío, CSIC, C/Jose Antonio Novaís 10, 28040 Madrid, Spain and* [2]*Department of Nutrition and Bromatology I, Faculty of Pharmacy, Complutense University, Madrid, Spain*

There is evidence that some mineral waters could play a role in the prevention of cardiovascular diseases. Some authors report that bicarbonated mineral waters show effects in relation to cholesterol and lipoprotein levels (Capurso *et al*. 1999; Schoppen *et al*. 2001). This protective action could be related to gastric emptying speed, biliary secretion stimulation, and increases in gallbladder contraction. It is known that lipid postprandial metabolism plays an important role and could be a risk factor in the development of cardiovascular diseases and atherogenesis (Cruz *et al*. 2001; Anderson *et al*. 2001). After the menopause, the prevalence of these diseases increases in women. The aim of this study was to investigate the effect of an alkaline mineral water on lipid postprandial lipaemia in postmenopausal women.

The present study was carried out with nineteen postmenopausal women participating in the Menopause Program of the Area of Health and Consumption of the Madrid City Council. Women (n=19; mean age=56 years, weight=64 kg) included in the study had to have been amenorrhoeic for at least 1 year, healthy and not obese (BMI<30). None of the women were taking oestrogen replacement therapy, vitamin, mineral and phytoestrogen supplements or any other medication known to affect bone and lipid metabolism. The study was carried out in a randomised controlled cross over trial, each woman ingested a fat-rich meal (75.3g total fat) accompanied by 500 ml of control mineral water (HCO_3^- 71.1; Ca^{2+} 25.2; Mg^{2+} 2.7; Na^+ 9; K^+ 1.4 mg/l) and carbonated mineral water (HCO_3^- 2013; Ca^{2+} 52.1; Mg^{2+} 9.7; Na^+ 948; K^+ 47.7 mg/l). Fasting blood samples were taken after an overnight fast (12 h) and postprandial blood samples were obtained at 30 min, 1 h, 2 h, 4 h, 6 h and 7 h after the meal. Chylomicrons were isolated from serum by ultracentrifugation. Serum and chylomicron total cholesterol (T-Chol) and triacylglycerols (TAGs) were determined (enzymatic method; Bayer, Germany)

Areas under the curve (AUC) were compared between waters (paired Student t-test). Serum T-Chol and TAGs were not affected by the type of mineral water. The carbonated mineral water showed a significantly lower area under the curve for chylomicron T-Chol and TAGs (P=0.038, P=0.030, respectively) compared with the control mineral water. We conclude that this alkaline bicarbonated mineral water induced a low postprandial lipaemia in these women, suggesting that this water improves the triacylglycerol metabolic capacity and decreases the associated cardiovascular risk.

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Relationship between increased intake of isomers of conjugated linoleic acid and the appearance of those isomers in plasma lipids in humans. By B. LUPOLI[1], G.C. BURDGE[2], S. TRICON[3], S. KEW[3], T. BANERJEE[2], K.E. KLIEM[1], K.J. SHINGFIELD[1], D.E. BEEVER[1], R.F. GRIMBLE[2], C.M. WILLIAMS[3], P. YAQOOB[3] and P.C. CALDER[2], [1]*Department of Agriculture, The University of Reading, Earley Gate, Reading RG6 6AT,* [2]*Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX and* [3]*Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading, Whiteknights, Reading RG6 6AP*

Conjugated linoleic acid (CLA) is a term used to describe positional and geometric isomers of linoleic acid with conjugated double bonds. The principal CLA in the human diet is the *cis*-9, *trans*-11 isomer (c9,t11 CLA). Animal studies suggest a range of potential health benefits of dietary CLA, with some isomer-dependent effects being identified (Roche *et al*. 2001). There is only limited information available on the changes that increased consumption of CLA might induce in the fatty acid composition of various lipid fractions in humans. The current study investigated the effect of increased consumption of c9,t11 CLA or *trans*-10, *cis*-12 CLA (t10,c12 CLA) by healthy males on the fatty acid composition of plasma phosphatidylcholine (PC) and cholesteryl esters (CE).

CLA was provided in 750 mg capsules containing approximately 80% of either c9,t11 CLA or t10,c12 CLA in triacylglycerol form (Natural, Norway). Healthy males aged 18–46 years consumed one, two or four capsules each day for 8 weeks consecutively (n 23 per group). Blood was collected in the fasted state. Plasma PC and CE were separated by solid-phase extraction and fatty acid compositions determined by gas chromatography with flame ionisation detection.

At baseline, plasma PC and CE contained some c9,t11 CLA, but no detectable t10,c12 CLA (see Table). There was a significant increase in c9,t11 CLA or t10,c12 CLA in plasma PC and CE in individuals consuming those isomers (see Table). There was a linear dose-dependent relationship between the amount of CLA consumed from the capsules and the proportion of that isomer in both plasma PC (c9,t11 CLA, r=0.749; t10,c12 CLA, r=0.741; both P<0.001) and CE (c9,t11 CLA , r=0.774; t10,c12 CLA, r=0.854; both P<0.001). This is the first report of a dose-dependent increase in the content of specific isomers of CLA in humans.

Intake of c9,t11 CLA (mg/d)	c9,t11 CLA in subjects consuming c9,t11 CLA (g/100g total fatty acids)				Intake of t10,c12 CLA (mg/d)	t10,c12 CLA in subjects consuming t10,c12 CLA (g/100g total fatty acids)			
	Plasma PL		Plasma CE			Plasma PL		Plasma CE	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD
0	0.19	0.06	0.20	0.05	0	0	0	0	0
595	0.32	0.10	0.34*	0.08	630	0.11	0.05	0.10**	0.04
1190	0.53***	0.21	0.50***	0.17	1260	0.26***	0.18	0.20***	0.07
2380	0.78***	0.32	0.77***	0.29	2520	0.49***	0.28	0.42***	0.17

*P=0.011; **P=0.002; ***P<0.001 *v.* baseline (0 mg/d) (Dunnett's t-test).

These results indicate that both c9,11t CLA and t10,c12 CLA are readily incorporated into circulating lipid pools in healthy humans, and that this incorporation is strongly related to isomer consumption. Whether the two isomers have different effects on health-related outcomes is not yet clear.

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The *in vivo* rate of postprandial glycogen synthesis in glucocorticoid-treated rats maintained on different high protein diets. By L. BOUKARIM, N. HWALLA, N. TORBEY and O.A.OBEID, *Department of Nutrition and Food Science, American University of Beirut, Beirut, Lebanon*

Studies have shown that glucocorticoids increase the deposition of glycogen in the liver, but it is not known whether this is related to glycogen synthesis. There are many contradictory findings concerning the effect of glucocorticoids on insulin and hepatic glycogen status (Goldstein *et al*. 2002).

The present experiment was designed to study the effect of a high-protein, high-fat diet versus a high-protein, low-fat diet on *in vivo* postprandial hepatic glycogen synthesis of rats taking glucocorticoids. Twenty-eight 6-week-old male Sprague-Dawley rats were randomly divided into four groups: HP–LF, high-protein, low-fat (39% of energy from carbohydrates, 22% of energy from fat, 39% of energy from protein); HP–LF+G, high-protein, low-fat with prednisolone; HP–HF, high-protein, high-fat (22% of energy from carbohydrates, 39% of energy from fat, 39% of energy from protein); HP–HF+G, high-protein, high-fat with prednisolone. Pellets each containing 0.25 mg of prednisolone (60 d release) were implanted subcutaneously. The rats were then maintained on their respective diets for 6 weeks, and then fasted overnight. On the day of the experiment, each rat was given 1.25 g of its respective diet by gavage, immediately injected intraperitoneally with 4 mCi of ^3H_2O, and killed 1 h later. Blood and liver were taken for analysis as described by Obeid *et al*. (2000).

	HP-LF	HP-LF+G	HP-HF	HP-HF+G	*P* value*		
					Diet	G	INT
Plasma glucose (mmol/l)	8.89	8.60	8.33	7.93	0.01	NS	NS
Glycogen content (mg/g liver)	17.25	20.15	16.63	18.51	NS	NS	NS
Glycogen synthesis †	57.3	53.2	28.8	33.9	0.00	NS	NS
Glycogen via pyruvate (%)	17.4	19.8	9.31	8.59	0.02	NS	NS

*Two-way analysis of variance.
† μmol ^3H_2O incorporated into glycogen-glucose/h per g liver.

Results showed that plasma glucose concentrations of rats maintained on HP–LF diets were higher than those maintained on HP–HF diets and this was not affected by the presence of glucocorticoids. Glycogen content (mg/g liver) was similar among all groups. Hepatic glycogen synthesis was higher in rats maintained on low-fat diets compared with those on high-fat diets and this was not substantially affected by the presence of glucocorticoids. The percentage of glycogen synthesis via pyruvate (indirect pathway) was also higher in rats maintained on the low-fat diets compared with those on high-fat diets; glucocorticoids did not seem to play a role in this case either.

In conclusion, chronic administration of glucocorticoids does not seem to have an effect on either hepatic glycogen content, synthesis or the percentage of glycogen synthesis via pyruvate. In fact, increased carbohydrate content of the diet seems to be the major determinant for increased glycogen synthesis and the indirect pathway of glycogen synthesis. This may have implications for energy efficiency, because the indirect pathway of glycogen synthesis is an energy-wasteful process.

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Obeid OA, Powell-Tuck J & Emery PW (2000) *International Journal of Obesity* **24**, 508–513.

The effect of diet supplementation with glutamine, dihydroxyacetone and leucine on the *in vivo* rate of postprandial glycogen synthesis. By S.T. BITAR[1], N. HWALLA[1], N. TORBEY[1], P.W. EMERY[2] and O.A. OBEID[1], [1]*Department of Nutrition and Food Science, American University of Beirut, Beirut, Lebanon and* [2]*Department of Nutrition and Dietetics, King's College London, London SE1 9NN*

Studies conducted *in vitro* on isolated hepatocytes have shown that very little glycogen is synthesised from glucose at physiological concentrations, but the addition of three carbon compounds such as lactate, pyruvate and dihydroxyacetone (DHA) caused a significant increase in glycogen synthesis (Katz *et al.* 1976). Also, the addition of glutamine stimulated glycogen synthesis on its own and the combination of glutamine with DHA was particularly effective. Moreover, the addition of leucine to the mixture of glutamine plus DHA resulted in even greater stimulation of glycogen synthesis (Chen & Lardy, 1985)

The present experiment was designed to investigate whether the inclusion of glutamine and/or DHA and/or leucine in the diet would stimulate hepatic glycogen synthesis *in vivo*. Thirty-two male Sprague-Dawley rats were fed *ad libitum* a semi-synthetic diet supplying 21% of energy as protein, 23% as fat (maize oil) and 56% as carbohydrate (equal quantities of sucrose and starch). Rats were randomly divided into four equal groups: C, control; G, glutamine (4% of energy); GDHA, glutamine (4% of energy) plus DHA (6% of energy) and GDHAL, glutamine (4% of energy) plus DHA (6% of energy) plus leucine (1%), and maintained on the appropriate diet for 1 week, then fasted overnight. The DHA substituted for carbohydrate while glutamine and leucine substituted for casein. On the day of the experiment, each group were given by gavage 1.25 g (in 4 ml of water) of their respective diet, and immediately injected intraperitoneally with 4 mCi of ^3H_2O, killed 1 hour later and blood and liver were taken for analysis as described previously (Obeid *et al.* 2000).

	C	**G**	**GDHA**	**GDHAL**	**Pooled SD**	***P* value**
Plasma glucose (mM)	9.50^a	8.80^{ab}	8.37^b	8.50^b	0.79	0.03
Glycogen content (mg/g liver)	29.2^a	55.5^b	119^c	123^c	12.8	<0.01
Glycogen synthesis †	37.7^a	62.6^b	104^c	103^c	15.1	<0.01
Glycogen via pyruvate (%)	9.81^a	10.4^a	18.1^b	18.8^b	1.11	<0.01

* ANOVA (one way analysis of variance).
[a,b,c] Mean values in each row having the different superscript letters were significantly different, $P<0.05$.
† μmol ^3H_2O incorporated into glycogen-glucose/h per g liver.

Rats maintained on the glutamine diet showed an increase in both glycogen synthesis and content, but plasma glucose concentration and the proportion of glycogen synthesised via pyruvate were not affected. The inclusion of DHA in the diets was associated with a decrease in plasma glucose concentration, and a very pronounced increase in glycogen synthesis, content and synthesis via pyruvate (indirect pathway). The addition of leucine to the diet did not produce any effect above that found following the addition of DHA.

In conclusion, both glutamine and DHA increased *in vivo* postprandial hepatic glycogen synthesis, the later being more effective and almost doubling the proportion of glycogen synthesized via pyruvate. This may have an implication for energetic efficiency, in view of the fact that the indirect pathway of glycogen synthesis is an energy-wasting process.

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Life-long Food Skills: promoting healthy eating to looked after children. By J. PITT, O. YEATES, J. ROBINSON and L. LEVY, *Food Standards Agency, Aviation House, 125 Kingsway, London, WC2B 6NH, UK*

There are approximately 89 000 children being looked after by Social Services departments in England at any one time. More than 75% leave care with no educational qualifications and between 50 and 80% of young people leaving care are unemployed (Acheson, 1998). Children who have experienced being in care are also vulnerable to a range of other health inequalities which can include a lack of knowledge and skills in relation to purchasing, preparing and cooking food (Acheson, 1998). Looked after children are those living in care homes, with foster or adoptive carers.

Part of the Food Standard's Agency's strategic aim is to promote healthy eating amongst vulnerable groups in the population. To reflect this commitment, training seminars based on the *Eating Well for Looked After Children* report produced by the Caroline Walker Trust aimed at trainers of carers for looked after children were organised in eleven different regions in the UK.

Seventy-five per cent (194/260) of the delegates completed action plans indicating ways in which they would develop and use what they had learnt in the seminars. The majority of the delegates worked in a training capacity, others included cooks, foster carers and catering managers. Many of the suggestions to encourage eating well amongst looked after children included: limiting the availability of soft drinks and encouraging the consumption of water; promotion of healthy eating discussions amongst children and young people; introduction of theme nights; a 'ready-steady-cook' programme; establishing links with other partners and encouraging adoption of eating-well policies.

The Agency contacted the 194 delegates 12 months after the training seminars to follow up how the intended tasks stated on the action plans were progressing. A total of seventy-seven evaluation sheets were completed, of which fifty-eight (75%) were returned by post and nineteen (25%) completed by phone. Nearly half (43%) reported they had achieved all three of their aims while one-third (33%) reported achieving two of their aims. These ranged from the teaching of basic cooking skills, training days and steering groups with staff, where they had the opportunity to discuss the content of the training materials. Many respondents cited the main barriers to achieving their aims as lack of resources and time. Another fundamental barrier reported was the lack of priority given to eating a healthy, balanced diet given all the other problems that carers face when looking after these children. Staff resistance was often a deterrent to encouraging eating well. Children and young people often reported that the removal of 'treats'; although not suggested as good practice by the training seminars, did sometimes occur and could often result in uncooperative behaviour.

The majority of respondents (70%) had managed to distribute the training materials mainly through staff or foster-carer training sessions. Nine respondents (12%) had initiated training sessions for their staff, indicating further success of the seminar appraisal used by the Agency. Eleven respondents (14%) intended to organise training sessions in the near future. Training materials were also widely distributed among social workers, care homes, catering staff, child protection committees, school meal managers, school nurses, after-care teams, local authority social care departments and health centres.

Nearly two-thirds (70%) of the respondents had encouraged staff to promote eating well through a variety of initiatives such as cooking clubs, weight management schemes and kitchen gardens. The remainder had not yet managed to fulfil this aim, citing time and resource constraints as barriers.

The impact that has been achieved from this evaluation is a useful model for promoting dietary change to vulnerable groups.

Acheson D (1998) *Independent Inquiry into Inequalities in Health*. London: The Stationery Office.

Sexual dimorphism in fat patterning in a sample of 5–6-year-old children in Oxford. By J. WEBSTER-GANDY[1], J. WARREN[2] and C.J.K. HENRY[2], [1]*Research Centre for Health Studies, Buckinghamshire Chilterns University College, Chalfont St Giles, HP8 4AD and* [2]*Nutrition and Food Science Research Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane, Oxford OX3 0BP*

It is now well recognised that in addition to total body fat, fat distribution is a major risk factor for cardiovascular disease and insulin sensitivity/diabetes in both adults and children. Since fat distribution may play a major role in both morbidity and mortality, it is important to examine the nature and timing of fat patterning in children. Traditionally, sexual dimorphism in fat patterning has been regarded as occurring at puberty (Malina, 1996). However a tendency towards truncal/central fat distribution in children of pre-pubertal ages has been observed by researchers in Germany (Mast *et al*. 1998) and the USA (Arfai *et al*. 2002). The aim of this study was to investigate gender differences in fat patterning in a group of children in Oxford. Anthropometric data was collected for ninety-five girls and ninety-three boys aged 5–6 years. Body mass index (BMI), percentage body fat and fat-patterning indices (Moreno *et al*. 1997) were calculated using skinfold thickness measurements.

	Girls (*n*=95)	Boys (*n*=93)	*P*
BMI (kg/m^2)	16.3 ± 2.5	15.3 ± 1.3	<0.001
Waist circumference (cm)	54.8 ± 5.7	53.7 ± 3.4	=0.05
Hip circumference (cm)	59.2 ± 6.3	56.0 ± 4.1	<0.001
Waist:hip ratio	0.93 ± 0.05	0.96 ± 0.04	<0.0001
% fat	14.2 ± 8.0	12.4 ± 4.0	<0.05
Sum of four skinfold thicknesses*	34.78 ± 16.24	25.41 ± 6.92	<0.0001
Fat-distribution indices			
Triceps/subscapular	1.56 ± 0.41	1.60 ± 0.41	NS
Biceps+triceps/subscapular+suprailiac	1.36 ± 0.33	1.41 ± 0.29	NS
Trunk-total %	43.16 ± 6.18	42.11 ± 5.00	NS

Results expressed as mean ± SD. NS – non-significant.
* Skinfold thickness was measured at biceps, triceps, subscapular and suprailiac sites.

The three fat-patterning indices showed no difference between genders, suggesting similar levels of truncal adiposity. A significant feature of these results is the observation that whilst boys at this age had larger waist: hip ratios, the percentage body fat and skinfolds were greater in the girls. This suggests that even at this young age girls are exhibiting greater subcutaneous adiposity with no apparent central accumulation of adipose tissue. These results show that sexual dimorphism of fat patterning in children is apparent even at 5–6 years of age and that these differences can be detected using simple anthropometric measures. The children in this study were all Caucasian and therefore care should be taken if comparing these results with national samples. The challenge is to determine the association between fat patterning and risk outcome in children. It is suggested that simple anthropometric measures be used to describe fat distribution in children and these be use to identify health risk factors.

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Trends in waist:height ratios in British children aged 11–16 years over a two-decade period. By H.D. McCarthy[1] and M. Ashwell[2], [1]*Department of Health and Human Sciences, London Metropolitan University, Holloway Rd, London N7 8DB and* [2]*Ashwell Associates, Ashwell Street, Ashwell, Herts, SG7 5PZ*

The ratio of waist circumference to height (WHTR) in adults is an indicator of risk for diseases related to internal obesity (Ashwell *et al.* 1996) and a value of 0.50 or greater indicates increased risk of morbidity (Ashwell, 1998). The WHTR is now proving useful in children and we have shown in British children that both gender and linear growth between the ages of 5 and 16 years influence the WHTR (McCarthy & Ashwell, 2002). WHTR is superior in its ability to predict cardiovascular disease (CVD) risk factors in children compared with either BMI or percentage body fat (Hara *et al.* 2002; Savva *et al.* 2000). The stability of WHTR in a childhood population over a period of time has not been examined and an increase in WHTR would suggest an increase in CVD risk factors. This study compared WHTR in British children over a two-decade period and characterised further those children above the 0.50 reference point.

The data used were collected cross-sectionally in two surveys. The first was collected in 1977 for boys and 1987 for girls (Survey 1, British Standards Institute, 1990, BSI) and the second in 1997 (Survey 2, National Diet and Nutrition Survey (NDNS) of young people aged 4–18 years, Gregory *et al.* 2000). For this study, data for 3815 children aged 11.0–16.99 years from Survey 1 and 773 children aged 11.0–16.99 years from Survey 2 were selected for analysis. Waist circumference was measured midway between the 10th rib and the iliac crest. Height and weight were measured using standard procedures. WHTR and BMI were calculated and statistically analysed by age and gender.

Mean WHTR in children aged between 11.0 and 16.99 years was significantly higher in children measured in 1997 compared with those measured 10 and 20 years earlier ($P<0.0001$). The proportion of children who exceeded the boundary value in Survey 2 was 17% of boys and 12% of girls (against 5.0% and 1.5%, respectively, in Survey 1, $P<0.001$). In Survey 1, boys and girls with a WHTR above the boundary value had a significantly higher BMI and had significantly shorter legs ($P<0.001$).

These results indicate that central obesity in British children as measured by the WHTR has increased dramatically over the last two decades. Accounting for height in this measure would suggest that this increase in upper body fatness was not due to secular changes in height. That those children at the extreme of WHTR had shorter legs suggests that leg length and excess central body fat may be biologically linked. Indeed shortness in leg length (as well as excess central body fat) has been linked to morbidity in adults including insulin resistance and cardiovascular disease (Smith *et al.* 2001). This intriguing association between leg length and WHTR in children merits further investigation.

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Upper and lower body dimensions and proportions of children classified as overweight or obese by the Body Mass Index. By L. Doyle and H.D. McCarthy, *Department of Health and Human Sciences, London Metropolitan University, Holloway Rd, London N7 8DB*

Body Mass Index (BMI) is one tool for assessing overweight and obesity in children and is a reflection of both the fat and fat-free compartments of the body. Because of this characteristic, the BMI can misclassify a tall or muscular child as overweight. The length of the lower limbs and the trunk contribute largely to the stature of a child. However, the relative contribution of the lower and upper body to the BMI has not been fully explored in children. The purpose of this study was to examine the upper and lower body dimensions and proportions in children classed as either normal weight or overweight by their BMI.

The data used were collected cross-sectionally by the HUMAG Research Group, Loughborough University. Subjects were aged between 5.0 and 16.9 years (British Standards Institute, 1990). The sample (n 8079; 3502 boys, 4577 girls) was representative, as far as possible, of children in the UK at the time of data collection (1977 for boys, 1987 for girls). BMI was calculated from height and weight. For this analysis, children were divided into two groups – those with a BMI above and those with a BMI below the 91st percentile (Cole *et al.* 1995). Boys and girls were analysed separately and children were analysed at annual intervals. Leg length, trunk length, waist circumference and leg:height, waist:height and leg:trunk ratios were compared statistically.

Body dimensions and proportions varied between overweight and normal-weight children by age. Generally, children classified as overweight were significantly heavier, with larger mean waist circumferences ($P<0.001$). Children classified as overweight were generally taller although this only reached statistical significance for ages 5–9 years in girls and ages 6–12 years in boys ($P<0.01$). Trunk length was significantly greater in overweight children at all ages, except at 15 and 16 years ($P<0.001$). Leg length was similar between groups at all ages ($P>0.05$). Leg:height ratio did not show any consistent pattern between normal and overweight children, although this variable was significantly different between groups at ages 5, 7, 10, 11, 12, 14 and 16 years (with P ranging between 0.05 and 0.001) Waist:height ratio was significantly greater in the overweight children at all ages ($P<0.001$). Finally, the leg:trunk ratio was significantly lower in children classified as overweight ($P<0.001$).

These results, based upon absolute measures and ratios between the upper and lower body, indicate that much of the difference between children classed as normal or overweight by BMI was due to differences in the upper body compartment, including its length and circumference. The greater waist circumferences in the overweight children suggest that these upper body characteristics may be due to differences in body fatness (McCarthy *et al.* 2003). However the fact that trunk length was significantly greater, together with the reasonably consistent finding that overweight children were taller, would suggest that some of the higher BMI values in this group was due to a greater fat-free mass. The contribution of the lower body to BMI requires further investigation.

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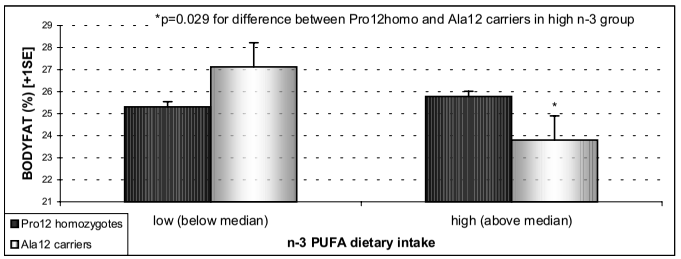
Interaction between the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma 2 gene and dietary fatty acids in relation to body fat. By C. VLASSI[1], L.F. MASSON[2], H. MCCALLUM[3], P. HAGGARTY[4] and G. MCNEILL[1], [1]*Department of Child Health,* [2]*Department of Medicine and Therapeutics,* [3]*Department of Obstetrics and Gynaecology, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD and* [4]*The Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB*

Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of the nuclear hormone receptor superfamily which is involved in the expression of fat-cell specific genes and adipocyte differentiation. *In vitro* studies have shown that *n*-3 and *n*-6 PUFAs bind and activate PPARγ (Auwerx, 1999). The Pro12Ala polymorphism has been investigated in relation to obesity and type 2 diabetes, and there is some evidence for an interaction between this polymorphism and the dietary P:S ratio in relation to BMI (Luan *et al.* 2001). The aim of this study was to evaluate whether the Pro12Ala polymorphism is associated with body fatness, either directly or by interaction with dietary fatty-acid intake.

The subjects studied were 224 healthy adults (91M, 133F) aged 18–50 years, with median BMI 23.9 [IQR 21.4–26.2] kg/m^2, who had been recruited for a study of coronary heart disease risk factors in twins. Habitual diet was assessed by the Scottish Collaborative Group 150-item semi-quantitative FFQ (version 6.3, 1999) with fatty acid intake derived from the Foodbase database (Institute of Brain Chemistry): *n*-3 and *n*-6 PUFA intakes were adjusted for total energy intake. Body fat percentage was measured by Bioelectrical Impedance. The Pro12Ala genotype was determined with a Taqman (Applied Biosystems) allelic discrimination assay using DNA extracted from blood samples.

Observed allele frequencies for the Pro12Ala mutation (Pro12Pro 70.1%, Pro12Ala 28.6%, Ala12Ala 1.4%) were in Hardy-Weinberg equilibrium. All analyses were conducted comparing Pro12Pro homozygotes to Ala12 allele carriers (Pro12Ala and Ala12Ala). After adjusting for age and gender, there was no significant difference in body fat percentage between Ala12 carriers and Pro12 homozygotes, and no significant association between energy-adjusted dietary fatty acid intake and body fat percentage. However, in the Ala12 carriers there was a decrease in percentage body fat with increasing *n*-3 PUFA intake, while this trend was not seen in the Pro12 homozygotes (see Figure). In regression analysis, the interaction between genotype and *n*-3 PUFA intake was significant (P=0.017). There were no significant interactions between genotype and the *n*-6 PUFA intake or the *n*-6/SFA, *n*-6/*n*-3 or P/S ratios.

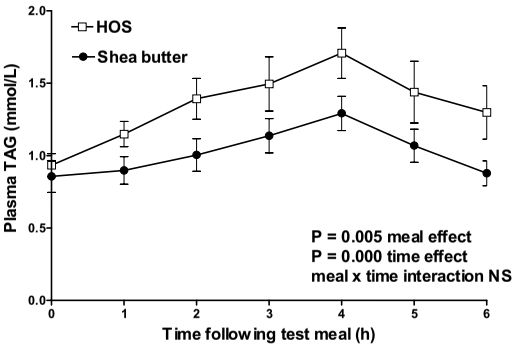


The observation that the Ala12 carriers respond differently to dietary *n*-3 PUFA deserves further investigation in future studies.

Auwerx J (1999) *Diabetologia* **42**, 1033–1049.
Luan J, Browne P, Harding A, Halsall D, Rahilly S, Chatterjee VK & Wareham N (2001) *Diabetes* **50**, 686–689.

Physical properties of stearic-acid-rich triacylglycerols modulate effects on postprandial lipaemia. By S.E.E. BERRY and T.A.B. SANDERS, *Nutrition, Food and Health Research Centre, King's College London, 150 Stamford Street, London SE1 9NN*

Cocoa butter has a unique structure with almost all of the stearic acid being present as either 1, 3 di-stearyl-oleyl glycerol (SOS) or 1 stearyl, 2 oleyl, 3 palmitoleyl glycerol (SOP). Sanders *et al.* (2001) reported that cocoa butter results in similar postprandial lipaemia as high oleate sunflower (HOS) oil. However, randomization of cocoa butter decreases postprandial lipaemia (Sanders *et al.* 2003) and it was suggested that this was a consequence of changing the fatty acid distribution on the triacylglycerol (TAG). Shea butter is rich in SOS but low in SOP and is used as a cocoa-butter substitute. In the present study, shea butter was compared with HOS. The shea butter contained 53 wt% stearic acid, 76% of the TAG was present as SOS and stearic acid accounted for 6.2 mol% in the *sn*-2 position. The melting characteristics of shea butter were determined by differential scanning calorimetry (DSC) and low-resolution NMR, and TAG structure by HPLC. DSC calorimetry showed a major melting peak at 19.5°C and minor peaks at 25.3°C and 28.8°C. NMR analyses of the shea butter showed the solid fat content to be 42.5% at 37°C and to be <1% at 52°C. A randomized cross-over design was used to compare the effects of meals containing 50 g shea butter or 50 g HOS on postprandial changes in plasma TAG 0–6 h after consumption in thirteen male subjects aged 20–43 years. The results (mean values with SEM) are shown below.



Postprandial lipaemia was significantly lower following shea butter than with the HOS. This is in contrast to our previous findings with cocoa butter. However, the shea butter, despite consisting of symmetrical TAG, had markedly different physical characteristics compared with cocoa butter, which has <1% solid fat at 37°C. These findings suggest that the tertiary physical structure of stearic-acid-rich fats may play an important role in determining their rates of digestion and absorption. Further work is necessary to test this hypothesis using model systems.

Sanders TA, Oakley FR, Cooper JA & Miller GJ (2001) *American Journal of Clinical Nutrition* **73**, 715–721.
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Effects of high and low glycaemic index foods on an atherogenic lipoproetin phenotype. By I.G. Davies, A. Wheeler, T. Mann, J.W. Wright, and B.A. Griffin. *Centre for Nutrition & Food Safety, School of Biomedical & Life Sciences, University of Surrey, Guildford, Surrey GU2 7XH.*

An atherogenic lipoprotein phenotype (ALP) is a major source of increased cardiovascular (CV) risk as part of the metabolic syndrome and type 2 diabetes. It consists of a moderate increase in plasma triacyglycerol (TAG>1.5mol/l), a low HDL cholesterol (<1mmol/l) and predominance of small, dense LDL, and can be modified by diet (Griffin & Zampelas, 1995). While conventional dietary strategies for reducing CV risk employ the use of low-fat, high-carbohydrate (LFHC) diets, there is debate over the potentially adverse effects of HC diets in raising TAG and lowering HDL (Hellerstein, 2002). In this respect, the type of carbohydrate, as defined by its capacity to raise blood glucose and insulin i.e. its Glycaemic Index (GI), may be critical. There is evidence to suggest that foods with a high GI may increase TAG and thus exacerbate an ALP, whereas foods with a low GI may decrease TAG, increase HDL and reverse an ALP. A study was designed to test the effects of high and low GI diets on an ALP. Fourteen normal, healthy men (aged 35-55years) with an ALP, were randomly assigned to one of two prescribed diets containing either a high proportion of foods with a high GI (GI >80) or a low GI (GI<60) (Foster-Powell et al, 2002). Dietary prescriptions were achieved by instructing volunteers to follow two cycles of a 7-day menu that provided a choice of foods and mixed meals (with recipes) appropriate to either their high or low GI status. The study was free-living with subjects purchasing and preparing their own food. Anthropometric measurements and blood biochemistry were determined at baseline and after 2 weeks. Dietary compliance was checked by a 5-day food diary from days 5 - 9.

	Post Low GI (n = 7)		Post High GI (n = 7)	
	% Change	P value	% Change	P value
LDL Peak density	-12	0.2	-8	0.28
Cholesterol	-10	0.03	0	0.99
TAG	-29	0.02	-5	0.32
HDL-C	-6.3	0.02	6	0.26
LDL-C	-11.6	0.07	4	0.59
Glucose	-7	0.27	-7	0.06
Insulin	-28	0.07	-12	0.14
Waist circumference	-1	0.2	-1	0.2
Body weight	-1.3	0.00	0	0.8
BMI	-1.3	0.01	0	0.9

The volunteers had all undertaken previous dietary intervention studies, were highly motivated and, with reference to their diet diaries, reliably compliant. The diets were well tolerated and achieved an overall mean difference in GI of 17 units (51 versus 68; low and high GI diets respectively). The low GI diet was accompanied by significant decreases in plasma cholesterol, triacylglycerol, HDL and LDL-cholesterol, body mass and BMI (see Table). In contrast, there were no significant differences in any of these variables after the high GI diet. There was no change in the distribution of LDL subclasses as measured by its peak density or in waist circumference for either group. In conclusion, while the low GI diet was associated with more favourable changes in plasma lipids (with the notable exception of a decrease in HDL), when compared to the high GI diet, the latter did not exert any significantly negative effects on this high-risk phenotype. Larger scale studies of extended duration will be required to substantiate the impact of GI on CV risk.

Foster-Powell K, Holt SH & Brand-Miller JC (2002) *American Journal of Clinical Nutrition* **76:1** 5-56.
Griffin BA & Zampelas A (1995). *Nutrition Research Reviews* (edited by: Gurr MI): **8**; 1-26.
Hellerstein M K (2002). *Current Opinion in Lipidology* Feb **13:1** 33-40.

Apolipoprotein E genotype and the association between habitual dietary fat intake and lipid levels. By L.F. MASSON, A. CUMMING, C. TUYA and G. MCNEILL, *Faculty of Medicine and Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD*

Apolipoprotein (apo) E is polymorphic, with three major isoforms coded for by three alleles (*ε2*, *ε3* and *ε4*), resulting in six possible genotypes: E2/2, E3/2, E4/2, E3/3, E3/4 and E4/4. In intervention studies, carriers of the *ε4* allele tended to show the greatest total and low-density lipoprotein (LDL) cholesterol responses to dietary change (Masson *et al.* 2003), but the diets were short-term and often of an extreme nature. This study aimed to investigate whether apo E genotype modifies the association between lipid levels and habitual diet, in particular saturated fatty acid (SFA) intake and the ratio of polyunsaturated fatty acids to SFA (P:S ratio).

The subjects included 239 healthy men and women (91M, 148F) aged 18–54 years who were recruited for a study of coronary heart disease (CHD) risk factors in twins. Habitual diet was assessed by a food frequency questionnaire, and a fasting blood sample was taken for lipid analyses and DNA extraction. Genotypes were determined by the polymerase chain reaction followed by HhaI digestion. Digested products were visualised under UV light following ethidium-bromide staining of an agarose gel. Subjects were classified into three genotype groups: E3/2 (n=32), E3/3 (n=145) and E4 (E3/4 and E4/4, n=55). There were no E2/2 subjects, and the seven E4/2 subjects were excluded from the analyses due to the contrasting effects of the two alleles on lipid concentrations.

After adjusting for age, gender, oral contraceptive use and percentage body fat, there were significant differences in total and LDL cholesterol levels between genotype groups, with levels in E3/2 < E3/3 < E4. Total and LDL cholesterol levels were 4.39, 4.69 and 5.00 mmol/l (P=0.007), and 2.34, 2.75 and 3.10 mmol/l (P<0.001) in E3/2, E3/3 and E4 genotype groups, respectively.

SFA intake and the P:S ratio did not differ significantly between genotype groups. Regression coefficients for the diet–lipid associations were estimated by multiple regression with adjustment for age, gender, oral contraceptive use, percentage body fat, smoking status, physical activity level and energy intake. The associations between either SFA intake or the P:S ratio and the lipid variables were not statistically significant in the whole group. However, significant coefficients and gene–diet interactions were found when the subjects were stratified by genotype group. Total and LDL cholesterol levels were significantly positively associated with SFA intake, and inversely associated with the P:S ratio in E3/3 subjects. There was also a significant inverse association between high-density lipoprotein (HDL) cholesterol and the P:S ratio in the E4 group (see Table).

Genotype group	**Saturated fatty-acid intake**			**P:S ratio**		
	Log_e cholesterol	**LDL**	**Log_e HDL**	**Log_e cholesterol**	**LDL**	**Log_e HDL**
E3/2 (n=32)	−0.001	−0.012	0.007	0.070	0.591	−0.065
E3/3 (n=145)	0.006**	0.021**	0.002	−0.265*	−1.017*	0.037
E4 (E3/4 & E4/4) (n=55)	0.001	−0.001	0.003	−0.261	−0.559	−0.720**
Gene–diet interaction	*P=0.526*	*P=0.706*	*P=0.736*	*P=0.039*	*P=0.028*	*P=0.007*

*P<0.05, **$P\leq$0.01.

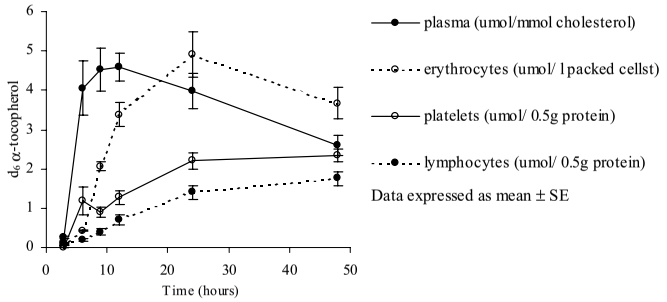
The data suggest that in this population, apo E genotype and dietary fat intake, in particular the P:S ratio, interact in determining lipid levels, and individuals with the apo *ε4* allele may be at higher risk of developing CHD, as a result of their higher lipid levels and less favourable response to diet.

Masson LF, McNeill G & Avenell A (2003) *American Journal of Clinical Nutrition* **77**, 1098–1111.

Inter-individual variation and distribution of labelled vitamin E in plasma, erythrocytes, platelets and lymphocytes. By Y.M. JEANES, W.L. HALL and J.K. LODGE, *School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey, GU2 7XH*

Vitamin E is an important lipophilic antioxidant, which appears to be beneficial in CVD. Vitamin E is absorbed with dietary fat and incorporated into chylomicrons. Following hepatic selection, α-tocopherol is distributed systemically via lipoprotein transport. The transfer of α-tocopherol between lipoproteins has been documented; however, little is known regarding the transport and distribution of α-tocopherol. This is important to ascertain, as α-tocopherol appears to influence the functional properties of platelets and lymphocytes.

The aim of the study was to determine the inter-individual variation in uptake and distribution of newly absorbed α-tocopherol into plasma, erythrocytes, platelets and lymphocytes. Twelve healthy volunteers (age 47±9.7 years) fasted for 12 h then consumed a capsule containing 150 mg deuterium labelled (d_6) RRR-α-tocopheryl acetate with a standard breakfast. Blood was collected at 3, 6, 9, 12, 24 and 48 h. Plasma, erythrocytes, platelets and lymphocytes were isolated and stored at −80°C until analysis. Vitamin E was extracted and analysed by LC/MS.



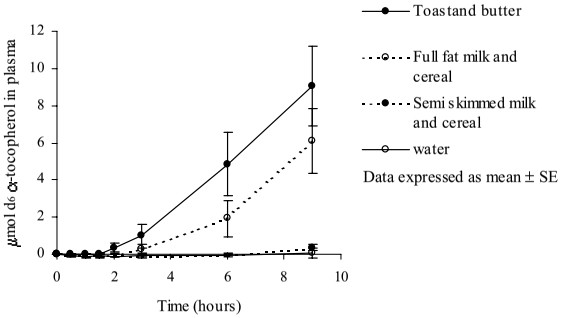
The α-tocopherol biokinetic profile is different for each blood component. α-Tocopherol rapidly entered the plasma from 3 h, whereas a slower increase was observed in erythrocytes and a gradual incorporation into lymphocytes. Interestingly α-tocopherol incorporation into platelets, in some individuals, demonstrated a biphasic response, rapid between 3 and 6 h then slower from 9 h onwards. There was large inter-individual variation in the maximum concentration and time attained for each blood component (values expressed as mean (range)), plasma; 5.1 (3.1–9.2) μmol/mmol cholesterol, erythrocytes; 5.0 (2.6–7.9) μmol/l packed cells, platelets; 2.6 (1.5–4.7) μmol/0.5 g protein, lymphocytes; 1.8 (1.1–2.9) μmol/0.5 g protein. There were also large inter-individual variations in the distribution profile of each blood component.

These data illustrate the extent of inter-individual variations in the uptake and distribution of α-tocopherol within plasma, erythrocytes, platelets and lymphocytes. These results are essential to the understanding of vitamin E biokinetics and have important implications for the design of human studies investigating functional effects of vitamin E in disease states.

Absorption of labelled vitamin E after ingestion of different breakfasts. By Y.M. JEANES, S. ELLARD, E. LEE, W.L. HALL and J.K. LODGE, *School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey, GU2 7XH*

Vitamin E is absorbed with dietary fat in the upper intestinal tract and subsequently incorporated into chylomicrons. Limited information exists on the amount and type of fat required for optimal absorption. In this study we aim to determine if the absorption of encapsulated α-tocopherol (α-tocopherol being the predominant form of vitamin E within the body) is affected by the physical properties and amount of fat within the meal it is consumed with.

Eight healthy volunteers (28±6 years) fasted for 12 h then consumed a capsule containing 150 mg deuterium-labelled (d_6) RRR-α-tocopheryl acetate with each breakfast on four separate occasions (1 week washout between each breakfast). The four breakfasts investigated were as follows; toast with butter (fat 17 g); whole-milk and cream with cornflakes (fat 17 g); semi-skimmed milk and cornflakes (fat 2 g), and water alone. 10-ml blood samples were taken at baseline and 0.5, 1, 1.5, 2, 3, 6 and 9 h after ingestion of the capsule. Plasma was separated and chylomicrons were isolated. Vitamin E was extracted and analysed by LC/MS.

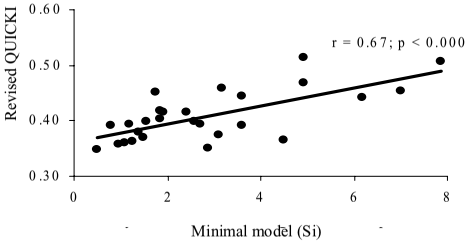


The plasma and chylomicron d_6-α-tocopherol increased significantly with time ($P<0.001$) except when vitamin E capsule was consumed with water. The increase in plasma d_6-α-tocopherol was significantly greater with toast and butter compared with the other breakfasts ($P<0.05$). A greater rate of absorption was observed between 3 and 6 h when the capsule was ingested with toast and butter compared with whole-milk with cereal ($P<0.05$).

These results indicate that the physical properties of toast and butter compared with milk and cereal significantly affect the rate and amount of α-tocopherol absorption over 9 h. A breakfast consisting of cereal and semi-skimmed milk does not provide sufficient fat for maximal absorption of α-tocopherol over 9 h. These results have important implications in the design of future vitamin E supplementation studies.

Comparison of surrogate measures of insulin sensitivity and resistance with the minimal model in Indian Asians and Caucasians. By L.M. BRADY[1], S. LOVEGROVE[1], B.A. GOWER[2], C.M. WILLIAMS[1] and J.A. LOVEGROVE[1], [1]*Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading, Reading RG6 6AP and* [2]*School of Health-Related Professions, The University of Alabama at Birmingham, Alabama, USA*

Insulin resistance is associated with features of the metabolic syndrome which is prevalent among Indian Asians living in the UK. The traditional gold standard, glucose clamp and minimal model procedures for assessing insulin sensitivity are not easily implemented in large studies. Therefore a range of less demanding surrogate techniques, which can be calculated using fasting insulin and glucose concentrations, have been developed. In the present study, insulin sensitivity (Si) determined using a frequently sampled intravenous glucose tolerance test (FSIGT) with minimal model analyses was compared with a number of surrogate measures of insulin sensitivity (the quantitative insulin sensitivity check index (QUICKI) and revised QUICKI and insulin resistance (the homeostasis model for insulin resistance (HOMA IR), the fasting insulin resistance index (FIRI), Bennett's index, fasting insulin and the insulin-to-glucose ratio) in a group of twenty-seven healthy male volunteers (fourteen British Caucasians, thirteen British Sikhs).



	r value	*P* value
Revised QUICKI	0.67	**0.000**
QUICKI	0.51	**0.007**
HOMA IR	−0.50	**0.009**
FIRI	−0.50	**0.008**
Fasting insulin	−0.44	**0.02**
Insulin/glucose ratio	−0.36	0.06
Bennett's index	−0.37	0.06

Correlation analysis identified that the strongest relationship was between Si and the revised QUICKI (r=0.67; P=0.000, see Figure). The later incorporates fasting non-esterified fatty acid (NEFA) concentrations into an equation with fasting insulin and glucose concentrations to derive a measure of insulin sensitivity. Significant positive correlations were also observed between Si and QUICKI (r=0.51; P=0.007) and significant negative associations were identified between Si and several measures of insulin resistance including HOMA IR (r=−0.50; P=0.009) (see Table). The Indian Asian group had significantly lower HDL-C concentrations (P=0.001), a significantly higher waist:hip ratio (P=0.01) and were significantly less insulin sensitive (Si) than the Caucasian group (P=0.02), as determined by the minimal model. In conclusion, the minimal model provides a sensitive measure of insulin sensitivity, which can successfully discriminate between insulin-sensitive and resistant groups even when only small numbers are studied. However, the revised QUICKI offers a strong surrogate measure of insulin sensitivity compared with the minimal model and could potentially be applied in larger studies where it is not practical to conduct more comprehensive tests.

The authors acknowledge funding from the Food Standards Agency

Food variety and its impact on food consumption in the elderly. By J.H. HOLLIS[1], C.J.K. HENRY[1] and M.S. JOSHI[2], [1]*Nutrition and Food Science Group, School of Biological and Molecular Sciences and* [2]*Psychology Department, School of Social Sciences and Law, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford OX3 0BP*

The continual consumption of a particular food leads to a progressive reduction in its pleasantness, while the pleasantness of other foods presented remains relatively unchanged. This phenomenon has been termed 'sensory-specific satiety' (Rolls *et al*. 1981*a*). As this satiety is specific to a consumed food, humans eat a greater quantity of food when they are presented with a varied meal compared to a monotonous meal (Rolls *et al*. 1981*b*). Sensory-specific satiety is reported to decrease with age (Rolls & McDermott, 1991), leading to the suggestion that food variety may be reduced in the elderly. This study aims to investigate the role that diminished sensory-specific satiety has on the eating behaviour of healthy elderly people.

Seventeen young subjects (mean age 26 (SD 5) years) and seventeen elderly subjects (mean age 70 (SD 4) years) were presented with four successive courses of sandwiches that were either of one filling (cheese) or a variety (cheese, ham, turkey, cream cheese and cucumber) and asked to eat as many as they wished. Their consumption during each meal event was then calculated.

Both young and elderly adults ate significantly more when offered a variety of sandwiches than when offered the same type of sandwich (cheese) in successive courses (P<0.05) (Fig. 1, Fig. 2). Whilst mean food intake diminished to almost zero in young adults over four successive courses of the same sandwiches, the elderly group continued to eat and consumed significantly more than the young adults in the third and fourth courses (P<0.05; Fig. 1).

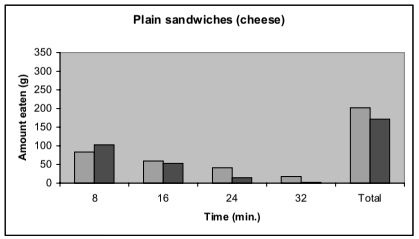


Fig. 1. Plain sandwich consumption (cheese).
Light bars = older adults; dark bars = young adults.

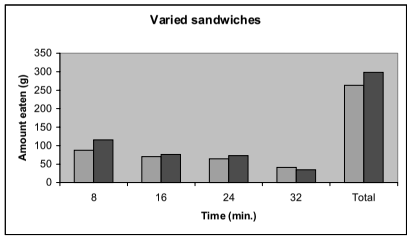


Fig. 2. Varied sandwich consumption.

This study illustrates that elderly people, similar to young people, eat more food when presented with a variety of foods rather than the same food in consecutive courses. These findings suggest that age-related reductions in sensory-specific satiety do not act to modulate food intake when older people are presented with a varied meal. In view of age-related changes in sensory-specific satiety, it is possible that external factors, such as the sight or smell of food, may be important in controlling eating behaviour in elderly people.

Rolls BJ & McDermott TM (1991) *American Journal of Clinical Nutrition* **54**, 988–996.
Rolls BJ, Rolls ET, Rowe EA & Sweeney K (1981*a*) *Physiology and Behavior* **27**, 137–142.
Rolls BJ, Rowe EA, Rolls ET, Kingston B, Megson A & Gunary R (1981*b*) *Physiology and Behavior* **26**, 215–221.

Prevalence of chronic diseases in relation to socio-demographic, lifestyle and dietary factors in Scottish adults. By M. EBRAHIMI-MAMEGHANI[1], J.A. SCOTT[1] and G. DER[2], [1]*Department of Human Nutrition, University of Glasgow, Glasgow Royal Infirmary, Glasgow G31 2ER and* [2]*Social and Public Health Sciences Unit, University of Glasgow, 4 Lilybank Gardens, Glasgow G12 8RZ*

Non-communicable diseases contributed more than half of the total reported deaths in the world in 2001 (WHO/FAO, 2003). Lifestyle factors such as diet play a key role as risk factors for chronic diseases. Defining the attributable socio-demographic and lifestyle components through longitudinal studies could be critical for the preventive reduction of chronic-disease risk factors (Mann, 2002).

Data from the West of Scotland 'Twenty-07 Study', a longitudinal cohort study, was used to investigate chronic disease prevalence in 2000 and its relation to lifestyle and dietary factors in adults from 1991 to 2000 (Ford *et al*. 1994). Two cohorts of subjects born around 1932 and 1952 were included in this study. Socio-demographic and lifestyle characteristics, dietary data and self-reported morbidity of chronic diseases were collected, and height and weight were measured in 1991 (n=1437) and 2000 (n=1128). An overall food variety score (FVS) was calculated based on the weekly consumption of food from twenty-eight biologically different food groups by food frequency questionnaire in 1991.

Significant increases were found in the prevalence of different types of chronic diseases over the 9-year period. Results showed that 16% of subjects had two or more of the conditions in 2000. Subjects with chronic diseases consumed fat-enriched meat products more frequently and alcohol drinks less frequently in 1991. More frequent consumption of savoury snacks, fat-enriched meat products, white bread; less frequent alcohol and wholegrain cereals consumption and less varied diet were associated with overweight and obesity. BMI status was observed to be a predictor of metabolic syndrome. Multivariate logistic regression analysis revealed that age, gender, FVS and change in alcohol consumption were the common socio-demographic and lifestyle predictors of chronic diseases even after controlling for metabolic syndrome at 2000.

Lifestyle factors	CVD OR (CI 95%)*	*P*	Metabolic syndrome OR (CI 95%)*	*P*	Obesity OR (CI 95%)*	*P*
Age cohort						
35	1.00	0.0000	1.00	0.0000		
55	3.31 (1.98,5.54)		2.24 (1.04,4.8)			
Gender						
Male					1.00	0.0183
Female					0.70 (0.52,0.94)	
FVS					0.95 (0.91,0.99)	0.0442
Drinking habits change						
Drinker	1.00	0.0357				
Non-drinker	0.98 (0.49,1.96)					
Give up#	2.60 (1.27,5.31)					
Start drinking#	2.19 (0.75,6.43)					
BMI status (kg/m^2)						
<25			1.00	0.0136		
25–29.9			1.51 (0.96,2.39)			
≥30			2.48 (1.35,4.55)			

*Adjusted odds ratios for significant variables for each type of disease in 2000.
#Changes in drinking status between 1991 and 2000.

Our findings, which are in consistent with other studies (WHO/FAO, 2003), revealed a number of factors predictive of the development of chronic diseases.

Ford G, Ecob R, Hunt K, Macintyre S & West P (1994) *Social Science and Medicine* **39**, 1073–1050.
Mann JL (2002) *Lancet* **360**, 783–789.
WHO/FAO (2003) *WHO Technical Report Series* **916**, Geneva: WHO.

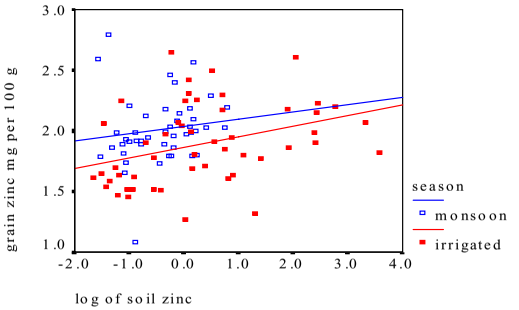
Variability in the zinc content of rice is important for human nutrition and is related to the soil environment in Bangladesh. By A.B. MAYER[1], M.C. LATHAM[1], J.M. DUXBURY[2], N. HASSAN[3] and E.A. FRONGILLO[1], [1]*Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853, USA,* [2]*Department of Crop and Soil Sciences, Cornell University, Ithaca, NY 14853, USA and* [3]*Institute of Nutrition and Food Science, Dhaka University, Bangladesh*

The aim was to explore connections between soil, crop and human micronutrient deficiencies. Zinc was used as an example of a nutrient that is often deficient in soils and humans. We explored agricultural and processing factors that could influence rice zinc. We also assessed the impact of the variability in the rice zinc content on dietary zinc intake.

Samples of soil, unpolished and polished rice were collected from farmers in Bangladesh in monsoon and irrigated rice seasons. The rice samples were digested using nitric acid and the soil nutrients extracted using diethylenetriaminepentaacetic acid (DTPA). Inductively coupled plasma-atomic emission spectrometry (ICP-AES) was used for mineral analysis of both soil and rice samples. Forty survey households were selected at random from four villages in different districts. We used 24 h recall for the dietary survey. The zinc analysis from polished rice samples collected in each household was used to individualize the nutrient intake.

During both seasons, log of soil zinc was significantly and positively related to unpolished rice zinc after controlling for soil pH and rice variety. In addition to these factors, in the monsoon season the land elevation was also a significant predictor (Full model R2 = 0.52 p<0.001 N=44). During the irrigated season soil texture was also a significant predictor, (Full model R2=0.70 p<0.001 N=51). The figure shows the relationship between rice and soil zinc. Milling removed from 10 to 50% of the unpolished rice zinc and was different in the different villages. For example, the average percentage lost was 24% in Simulia and 39% in Batabaria.

On average, polished rice contained 1.32 mg/100 g zinc (SD 0.38). We defined 'high-zinc rice' as mean + 1 SD and 'low-zinc rice' as mean − 1 SD. The children aged 5–10 years ate, on average, 378 g rice per day, which supplied 67% of their dietary zinc intake. The total dietary zinc intake would change from 6.4 mg/d if they were consuming the 'low-zinc rice' to 9.0 for the 'high-zinc rice'. Requirement was 12 mg/d on average for this group of children based on FAO guidelines and assuming low bioavailability (FAO, 1998). The total dietary zinc intake of children was therefore highly influenced by variability in the rice zinc content. Optimizing the zinc content of rice through changes in agriculture and processing would be useful for tackling human zinc deficiency. This cross-disciplinary research design will be useful for exploring zinc and other micronutrient deficiencies in many different countries and crops. The research also has implications for design and interpretation of dietary surveys that use standard food tables.



Grain zinc and log of soil available zinc. (Available soil zinc measured in ppm)

FAO/WHO (1998) *Recommended Nutrient Intakes, Report of Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements*. Rome: FAO.

Effect of moderate weight loss on the serum oestrogen, testosterone and leptin levels in obese men. By A. DJAZAYERY[1], M. MADDAH[2], M.R. ESHRAGHIAN[1], M. JALALI[1] and R. MIRDAMADY[3], [1]*School of Public Health, Tehran University of Medical Sciences, PO Box 14155–6446, Tehran,* [2]*School of Public Health, Guilan University of Medical Sciences, Rasht, Iran and* [3]*School of Medicine, Tarbyat Modarres University, Chamran Avenue, Tehran, Iran*

There is no conclusive data on the effects of weight loss on the blood levels of sex hormones, insulin and leptin in obese individuals. The objective of this study was to determine the effects of moderate weight loss on the serum levels of leptin oestrogen and testosterone and their inter-relationships in obese men. Forty-two obese men, 22–45 years old, with a body mass index (BMI)=33.6 (30–36), randomly selected from among those consulting a fertility clinic in Tehran, Iran, were put on a 5 MJ (1200 kcal) () diet (15%, 25%, and 60% of the energy coming from protein, fat, and carbohydrate, respectively) for 8–16 weeks. Fasting serum levels of the hormones, and weights and heights were determined before and after weight loss. The initial and final values of the variables are compared in the Table.

Variable	Body weight (kg)		Body mass Index (kg/m²)		Waist:hip Ratio (WHR)		Leptin (ng/dl)		Insulin (µg/IU)		Oestradiol-17 beta (pmol/l)		Testoste-rone (nmol/l)	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Initial	88.7	14.0	33.6	3.5	0.96	0.04	11.8	7.3	18.1	12.5	64.4	2.22	11.7	0.4
Final	82.6†	11.7	28.5†	2.8	0.94*	0.04	7.6*	3.1	12.3*	7.3	85.9*	22.4	13.5	5.8
Difference	-6.1		-2.1		-0.02		-4.2		-5.8		+21.5		+1.8	
Change (%)	6.9		6.9		2.1		35.6		32.0		33.4		15.4	

M=Mean, SD=Standard deviation; *P=0.01, †P=0.001 (paired t-test)

Weight loss brought about statistically significant decreases in the serum leptin and insulin levels, BMI and WHR, and a significant increase in the oestradiol level; the magnitude of leptin reduction was related only to percent weight loss (R^2=0.46, β=0.167, P=0.04). Previous investigators had reported reductions in leptin and insulin (Rissanen *et al*. 1999) following weight loss. Some authors (Leenen *et al*. 1994) had also observed statistically significant increases in testosterone; the increase of 15.4% (from 11.7 to 13.5 nmol/l) observed in our study was not significant.

Further statistical analysis of the data revealed a statistically significant negative correlation between leptin and testosterone and between leptin adjusted for BMI and testosterone, which confirms findings reported by previous workers (Blum *et al*. 1997). The association between oestradiol and testosterone was also significant. The only determinant of serum testosterone was the serum leptin level (R^2=0.38); the BMI could not explain the rest of the testosterone variance. Also, a considerable part of the leptin variance could be explained by the serum testosterone level and BMI (R^2=0.51). The causal relationships among these variables remain to be elucidated. Another finding was that, whereas there was no association between leptin and insulin initially, a significant positive correlation was found between them after weight loss. Leenen *et al*. (1994) reported such an association both before and after weight loss.

Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Muller J, Sakkebak ML, Heiman ML, Birkett M, Attanasio AM, Kiess W & Rascher W (1997) *Journal of Clinical Endocrinology and Metabolism* **82**, 2904–2910.

Leenen R, Kooy KV, Seidell JC, Deurengerg P & Koppeschaar HPE (1994) *Journal of Clinical Endocrinology and Metabolism* **78**, 1551–1520.

Rissanen P, Makimattila S, Vehmas T, Taavitsainnen M & Rissanen A (1999) *International Journal of Obesity* **23**, 645–664.

Are overweight shoppers a risk factor for the prevalence of obesity in families? By J.K. RANSLEY[2], J.K. DONNELLY[1], H. BOTHAM[1], T.N. KHARA[1], H. ARNOT[1], J.E. CADE[2] and D.C. GREENWOOD[2] , [1]*The Public Health Nutrition Unit, Trinity and All Saints, University of Leeds, Brownberrie Lane, Horsforth, Leeds LS18 5HD and* [2]*Nutrition Epidemiology Group, Nuffield Institute for Health, The University of Leeds, 71–5 Clarendon Road, Leeds LS2 9PL*

In the UK most of the food consumed at home is purchased from supermarkets and for most people this constitutes a significant proportion of their diet. An estimated 85% of 'primary' food shoppers are women and most carry the responsibility for food preparation in their homes (Ransley *et al*. 2001). The food the primary shopper chooses to buy and serve at home may therefore be an important determinant of familial dietary patterns. In a previous study we showed that more fat and energy was purchased per person in overweight families than those that were lean, regardless of age, family composition and social class (Ransley *et al*. 2003). In this study we investigated whether overweight and obese primary shoppers purchased more fat and energy per family member than lean shoppers and whether the BMI of a primary shopper was predictive of a family being classified as overweight (mean z-score for BMI ≤1.3).

A total of 214 households were recruited from a random sample of Tesco Clubcard members in Leeds. Itemised supermarket receipts were collected for a period of 28 d to estimate household food purchased from supermarkets and other retail outlets. Detailed demographic and dietary data were collected from each member of the family.

	Weight status of primary shopper		
	Lean (n=131) (BMI<25 kg/m²)	Overweight (n=83) (BMI≥25 kg/m²)	P value
Percentage energy from fat in food purchased (SD)	34.97 (6.78)	37.58 (6.12)	0.005
Mean daily purchase of fat (g) per person (adjusted for adults and children) (SD)	86 (34)	101 (37)	0.003
Mean daily purchase of energy (MJ) per person (adjusted for adults and children) (SD)	9.15 (2.51)	10.00 (2.69)	0.03

The Table shows a significant difference in the percentage energy derived from fat, and the absolute amounts of fat and energy purchased per person by lean and overweight shoppers. In addition a logistic regression analysis showed that families were 1.6 (95% CI: 1.4–1.8) times more likely to be classified as overweight for each unit increase in the BMI of the primary shopper, even when age, family size, sex and socio-economic status where adjusted for. This data suggests that overweight female supermarket shoppers may be an important focus for dietary interventions to prevent and manage the current epidemic of obesity occurring in UK families. Further work should be undertaken to confirm these findings.

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Determinants of plasma homocysteine in ischaemic heart disease patients. By P. TIGHE[1], M. WARD[1], O. FINNEGAN[2], H. MCNULTY[1], J.J. STRAIN[1], A.M. MOLLOY[3] and J.M. SCOTT[3], [1]*Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine, Northern Ireland, BT52 1SA,* [2]*Coronary Care Unit, Causeway Hospital, Coleraine, Northern Ireland, BT52 1HS and* [3]*Department of Biochemistry, Trinity College, Dublin, Ireland*

Elevated plasma homocysteine (tHcy) concentration is positively associated with an increased risk of cardiovascular disease with strong evidence supporting a causal link (Wald, 2002). Homocysteine metabolism is dependent on an adequate supply of the B-vitamins; folate, B_{12} and B_6. In healthy populations, folate is well established as being the most important modifiable determinant of tHcy concentration. Recent evidence from our own group has shown that once folate status has been optimised, tHcy becomes dependent on vitamin B_{12} (Quinlivan *et al*. 2002) and vitamin B_6 (McKinley *et al*. 2002). The importance of these nutrients in determining tHcy in ischaemic heart disease (IHD) patients is, however, less well documented. The aim of this study, therefore, was to compare B-vitamin status in IHD patients with age–sex matched controls and to examine whether nutritional determinants of tHcy differed between cases and controls.

IHD patients (*n* 39) were recruited from the cardiac out-patients' clinic, Causeway Hospital and healthy volunteers (*n* 38) were recruited from the local community. A 20 ml fasting blood sample was collected and analysed for the following biochemical parameters: tHcy (ImX), serum folate and serum B_{12} (microbiological assay), PLP (B_6; HPLC), riboflavin (EGRac) and MTHFR genotype (PCR). Dietary data were collected using 4 d food diaries and analysed for B-vitamin intake using the dietary analysis programme WISP (Tinuvel Software, v1.28).

	IHD group	Healthy group	*P*
Age	60.7 (10.4)	60.3 (11.6)	NS
% male	79	76	NS
BMI (kg/m^2)	29 (4.1)	28.7 (3.2)	
tHcy (μmol/l)	15.5 (7.4)	11.4 (2.5)	0.003
Serum folate (μg/l)	7.9 (4.5)	8.7 (4.6)	NS
Serum B_{12} (ng/l)	279 (103.1)	286 (74.8)	NS
PLP (nmol/l)	69.9 (42.5)	70.9 (28.3)	NS
Riboflavin (EGRac)	1.5 (0.16)	1.24 (0.13)	NS
Dietary folate (μg/d)	247 (94)	229 (75)	NS
Dietary vitamin B_{12} (μg/d)	3.7 (1.6)	3.7 (2.3)	NS
Dietary riboflavin (mg/d)	1.5 (0.5)	1.6 (0.7)	NS
Dietary vitamin B_6 (mg/d)	2.1 (0.6)	2.0 (0.4)	NS

Values are presented as mean (SD). Differences between groups were assessed using an independent *t*-test on log-transformed data.

Consistent with previous studies, tHcy concentration was found to be significantly higher in IHD patients compared to controls. No differences were observed in either the intakes or status of the relevant B-vitamins between the two groups. Serum folate, serum B_{12} and age were found to be the major determinants of tHcy concentration in both groups. No other variable was found to be a significant determinant of tHcy. MTHFR genotype did not appear to influence tHcy concentration; however, this was probably due to the size of the sample investigated.

In conclusion, tHcy was significantly higher in IHD patients compared with healthy controls; however, the nutritional determinants of tHcy concentration were not found to be significantly different between the two groups.

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Impact of definitions of eating events on energy intake and body weight status: analysis of the UK Women's Cohort Study. By B.A. MCNULTY and V.J. BURLEY, *Nutrition Epidemiology Group, The Nuffield Institute for Health, University of Leeds, 71–75 Clarendon Road, Leeds LS2 PL*

It has been suggested that one of the potential contributors to the rising prevalence of obesity is snacking. Research conducted on this area has shown contrasting outcomes, some studies suggest that eating frequency is inversely related to body weight, other studies contradict this. One possible reason for these conflicting results could be due to the definitions of eating events used (Gatenby, 1997). This study aimed to investigate whether the definitions of eating events affected the relationship between eating pattern and body weight status in middle-aged women.

The women studied formed a subsample of the UK Women's Cohort Study, which is a national, 10 year investigation of diet and cancer in 35 000 women initially aged 35–69 years. The baseline data on the cohort were obtained with a 217-item FFQ, with additional questions on health and lifestyle. A second contact was undertaken (Phase 2), 2–5 years after baseline, with all 35 000 women being sent a 4 d food diary and a further health and lifestyle questionnaire. A random sample of 385 women's diaries was used in this analysis. Eating events recorded in the food diaries were categorised by meals, snacks and drinking occasions, using strict criteria. A 'meal' was defined as an eating episode occurring at conventional meal times (breakfast, lunch or evening meal). Snacks were defined as an eating event containing solid or semi-solid food that occurred outside of conventional meal times, but that was separated from the next event by at least fifteen minutes. Drinks included all beverage types including alcoholic beverages and water. A measure of eating frequency was obtained by summing these events. Full analysis of the food diaries was conducted using DANTE, an in-house ACCESS-based diary analysis program based on the *UK Composition of Foods*. Self-reported weight and height were used to calculate body mass index (BMI). The women were divided into tertiles of eating events, and analysis of variance was conducted on the relationship between each tertile of eating frequency, snacking, number of main meals, drinking and BMI. This analysis was conducted before and after the exclusion of low energy reporters (EI/BMR<1.2).

On average the women consumed 2.8 main meals, two snacks and three (energy-containing) drinks per day. This gave a mean total eating frequency of 7.8 times per day. Total eating frequency was found to be significantly correlated with snacking frequency (r=0.506, P<0.01) and with drinking frequency (r=0.809, P<0.01) but not with the number of main meals consumed.

Mean (SD)	Low frequency	Medium frequency	High frequency	Linear trend
Total eating frequency	5.8 (0.8)	7.7 (0.5)	10.1 (1.3)	
Number of main meals	2.3 (0.3)	3.0 (0.1)	3.3 (0.1)	
Number of snacks	0.9 (0.4)	1.9 (0.3)	3.2 (0.7)	
Number of drinking occasions	1.3 (0.6)	2.9 (0.4)	5.0 (1.2)	
BMI by snacking group	25.0 (4.8)	25.0 (4.8)	23.9 (3.7)	P=0.06
BMI by snacking group[#]	22.9 (3.1)	23.3 (3.3)	23.3 (3.1)	NS
Energy intake by snacking group[#]	1917 (295)	2018 (291)	2090 (380)	P=0.02

[#]Excluding under-reporters.

Analysis of the full data set appeared to suggest that the most important variable in terms of the relationship between eating pattern and obesity was snacking. Frequent snackers had a lower BMI despite higher energy intakes. However, this relationship was no longer apparent after excluding energy under-reporters, and analysis using total eating frequency and snacking frequency gave similar results. Further analysis, including physical activity levels, is warranted.

Gatenby SJ (1997) *British Journal of Nutrition* **77**, S7–S20.

Dietary habits of the homeless. By S.G. ARTHUR, A.F. HACKETT and S.M. MAXWELL, *Faculty of Education, Community and Leisure, Liverpool John Moores University, Barkhill Road, Liverpool L17 6BD*

Morbidity and mortality rates are high amongst homeless people. An inadequate diet has been identified as a factor in the worse health of the homeless. Diets of homeless people have been shown to be high in intakes of saturated fat but deficient in many micronutrients, such as vitamins A, C and E, selenium, potassium and zinc (Evans & Dowler, 1999). Liverpool has a relatively large homeless population, some of whom (ninety at the time of study) are provided with employment assistance by the 'Big Issue in the North' Trust. The aim of this study was to examine the diet of male Liverpool-based vendors of the *Big Issue* magazine. The dietary intake of twenty-two male volunteers, aged 24–39 years, who lived in hostels (27%), with family or friends (45%) or slept rough (27%) was assessed using a 24 h recall, and analysed using Microdiet (University of Salford).

Dietary habits were very varied, in part due to the method used, but the range of nutrient intake reflected the extreme intakes of food; as one vendor said 'sometimes I eat a lot, sometimes a little'.

Daily nutrient intake Macronutrient	Mean	95%CI	% Energy	Range	Daily nutrient intake Micronutrient	Mean	95%CI	Range
Energy (MJ)	13.1	10.2–16.0		3.5–27.1	Vitamin A (μg)	949	637–1260	9–69
Total fat (g)	126	92–160	36	28–305	Vitamin C (mg)	62	4–119	0–619
Saturated fat (g)	45	28–62	13	3–158	Vitamin E (mg)	3.1	2–4.1	0–9
Monounsaturated fat (g)	36†	25–48	10	3–99	Vitamin D (μg)	2.7	1.3–4.2	0–10
Polyunsaturated fat (g)	12.7†	8–17	4	2–38	Folate (μg)	191*	88–294	48–1116
Carbohydrate (g)	373	287–458	48	91–721	Iron (mg)	16.7	8.5–24.9	1–87
Total sugars (g)	165	117–212	20	16–412	Potassium (mg)	3639	2554–4724	649–12291
Protein (g)	91	63–120	12	18–263	Selenium (μg)	29*	16–42	0.4–125
Alcohol (g)	32	6–58	7	0–213	Calcium(mg)	1048	622–1474	115–4695
NSP (g)	7.8†	4–11		0–26	Zinc (mg)	10.6	7–14	2–38

†Mean intake below DRV; *mean intake below RNI.

Takeaway foods, in particular chips, made a considerable contribution to the diet. The consumption of fruit and vegetables was very low. The item making the largest contribution to saturated fat was whole milk; to unsaturated fats, NSP and potassium it was chips; to sugars it was white sugar; to vitamin C it was orange juice; to vitamin E it was potato crisps; to folate it was lager; to iron it was bran flakes and to selenium it was tuna. Some extreme eating habits were observed which accounts for the range of intakes reported, often due to the fact of not being able to store food.

The nutrient intake was found to be similar to that of the homeless in London (Evans & Dowler, 1999) but higher than that of homeless men in Paris (Malmauret *et al*. 2002). A study of homeless men, women and children living in hostels in the Northwest found low intakes of key nutrients but relatively high intakes of fat (Coufopoulos, 1997) which contrasts with the high mean energy intakes and relatively low fat intakes of these males. An improved diet might be achieved by reducing the intake of chips, whole milk, alcohol and sugar in favour of fresh fruit, vegetables, wholegrain cereals and fish; however, the convenience and cost in monetary terms and implications for energy intake must also be considered. Practical issues and lifestyle factors should be taken into consideration when making any recommendations.

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How much is enough? Estimates of portion sizes of wholegrain foods. By A.R. JONES[1], R.H. GRIMSHAW[1], D.P. RICHARDSON[2] and C.J. SEAL[1], [1]*Human Nutrition Research Centre, School of Agriculture, Food and Rural Development, University of Newcastle, Newcastle upon Tyne NE1 7RU and* [2]*DPR Nutrition Limited, 34 Grimwade Avenue, Croydon, Surrey CR0 5DG*

Wholegrain foods are an important source of nutrients and phytoprotective substances. Increased intake of wholegrain foods has been associated with a reduced risk of several chronic diseases including heart disease, certain cancers and type 2 diabetes (Richardson, 2000, 2003). In contrast to dietary guidelines in the USA, which advise an increase in consumption of wholegrain foods (at least three servings per day), there are no specific recommendations relating to the consumption of wholegrain foods in the UK. Furthermore, findings suggest that intakes within the UK population are extremely low, although data on consumption levels are limited (Lang *et al*. 2003).

The present study has used data from five investigations previously completed in the Newcastle-upon-Tyne area to explore frequency of wholegrain food consumption and portion sizes of those wholegrain foods consumed. Dietary evaluations were completed using food diaries or food frequency questionnaires; in all cases portion sizes were estimated using the *Photographic Atlas of Food Portion Sizes* (Nelson *et al*. 1997).

In total, 1780 dietary records were collected from 827 subjects (352 males, 475 females) aged over 16 years. Consumption of wholegrain foods, including all foods that contained 20% or more wholegrain ingredient(s) by weight accounted for 3.2% of total measurements (derived from forty-two different food codes). Mean portion sizes (g) were calculated for each food and are reported here for selected foods by gender.

When comparing portion sizes of selected foods to the suggested US guidelines shown in the Table, it is clear that the amounts consumed can represent several US servings, thus indicating the ease with which dietary guidelines can be achieved. However, frequency of consumption within this population was low overall, with individuals preferring refined alternatives. This is in agreement with nationally representative data and is well below US recommendations. Initial findings from focus groups exploring perceptions of wholegrain products showed that knowledge and awareness of these foods was poor. In particular, participants were unaware of any dietary guidelines regarding wholegrain foods, and, once informed of US recommendations, subjects stated that they were ambiguous and unclear.

Food	US Food Guide Pyramid serving size		Mean portion size consumed (g)		Number of pyramid servings consumed	
	Description	Weight (g)	M	F	M	F
Wholemeal bread	1 slice	28.0	91.7	69.5	3.3	2.5
Whole wheat spaghetti*	Half a cup	70.0	250.2	181.4	3.6	2.6
Brown rice*	Half a cup	97.5	235.0	197.8	2.4	2.0
Bran flakes	One ounce	28.4	52.9	44.4	1.9	1.6
Cheerios	One ounce	28.4	44.6	33.2	1.6	1.2

*Cooked weights; M=males, F=females.

It is important to emphasise the role of wholegrain foods as a key component of a healthy diet. Promotion of increased wholegrain consumption requires positive, practical and simple educational messages similar to those which have been used by the 'five-a-day' scheme for fruit and vegetables. It is essential that advice on portion sizes of wholegrain foods is accurate and practical if action is to be effective.

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The effect of satiety on perceptions of usual food portion size. By L.J. BEASLEY, *Liverpool John Moores University, IM Marsh Campus, Barkhill Road, Liverpool L17 6BD*

Food diaries with estimated portion sizes are used extensively in nutritional surveys. Food photographs are frequently used with food diaries in order to estimate the quantity of food consumed as recorded in the food diary. Nelson *et al.*'s (1997) '*Food Portion Sizes: A Photographic Atlas*' consists of photographs of different portion sizes of a variety of commonly consumed foods. These photographs can be used to estimate portion sizes in the subject's food record and therefore establish their average nutrient intake.

Evidence suggests that subjects often experience difficulties in recalling and quantifying dietary information accurately and may be influenced consciously and/or unconsciously by various factors, resulting in under-/over-reporting when completing their food diary and establishing portion size from food photographs (Wirfält, 1998). Nelson *et al.* (1996) reported large variations in the estimation of self-served portion sizes from food photographs, with small portion sizes generally being overestimated and large portion sizes underestimated.

One factor that has not been investigated is whether subjects' satiety at the time of viewing the food photographs affects perception of their usual portion size. To investigate this, a sample of 115 undergraduate students was recruited. Satiety was assessed using a hunger-satiety scale (Roth 1993). Subjects were shown photographs of chips, fruit salad, rice and cheesecake at two levels of satiety (unfed and fed) on two different occasions 1 week apart, and were asked to indicate which portion size they would usually eat. Eight photographs were shown, 1=smallest, 8=largest portion. The results show the average photograph number chosen.

Results indicate that satiety did appear to have an effect on people's perceptions of their average portion size, with subjects generally indicating larger portion sizes when they were unfed than after they had consumed a Mars bar and can of Pepsi. The paired-samples *t*-test indicated that there was a significant difference in the perceived usual portion sizes of chips (P=0.001), rice (P=0.018) and cheesecake (P=0.001), and a non-significant difference in fruit salad (P=0.176) indicated by subjects when unfed than when fed, as shown in the Table.

Portion size	Chips (n=59, P=0.001)		Rice (n=59, P=0.018)		Fruit (n=59, P=0.176)		Cheesecake (n=59, P=0.001)	
	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed
Mean	4.97	4.22	3.10	2.69	5.54	5.19	3.71	3.08
SEM	0.21	0.24	0.18	0.18	0.26	0.28	0.27	0.25
SD	1.629	1.876	1.386	1.355	1.977	2.161	2.051	1.950

Significance: P value <0.05.

Difference between portion sizes: unfed–fed (% subjects).

	Chips	Rice	Fruit	Cheesecake
Zero	32	27	32	36
Larger	46	56	37	47
Smaller	22	17	31	17

This study suggests that satiety may affect some people's perception of usual portion size.

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Analysis of the nutritional intake of abstaining and relapsing alcoholics. By M.J. CHAO, M. NELSON, V.R. PREEDY and T.J. PETERS, *Department of Nutrition and Dietetics, 150 Stamford Street, London SE1 8WA*

Various studies have shown that the nutritional intakes of alcoholics are poor, although most of these studies have been carried out on hospitalised patients (Nicolas *et al.* 1993; Preedy & Peters, 1993). There are no comprehensive and recent assessments of nutritional intake in ambulatory alcohol misusers in the UK, both in relapse and remission. Food intake in current alcoholics is low compared with abstaining alcohol misusers.

Food consumption was assessed with three repeats of 24 h recall and a 174-item food frequency questionnaires (FFQ) dietary assessments. All abstaining patients had not drunk ethanol-containing beverages for at least 4 months, whereas relapsing alcoholics consumed at least 100 g of ethanol per day. Additional data analyses included anthropometric parameters of nutritional intake.

24 h recalls and the FFQs gave reproducible and consistent results. The results showed that abstaining alcoholics had a significantly greater mean BMI, percentage body fat, and waist:hip ratios than the relapsing alcoholics. Both the abstaining and relapsing alcoholics had reported total energy intakes below the estimated average requirement (EAR) of 10.60 MJ/d based on the comparative analysis between the nutritional data from the mean 24 h recall with the EAR of energy for the UK. In the FFQ, the abstaining group reported significantly lower intakes of vitamin B_2 (P=0.004), nicotinic acid (P=0.001) and folic acid (P=0.002), and significantly higher intakes of vitamin C (P=0.004) and vitamin E (P=0.046) than the relapsing group. In the mean 24 h recall, the abstaining group reported a significantly lower intake of folic acid (P=0.008), and a significantly higher intake of vitamin E (P=0.043) than the relapsing group.

In general, alcohol misusers have impaired nutritional intakes as compared with the UK's RNI. Both the abstaining and the relapsing alcoholics had inadequate intakes of one or more macro- or micronutrients. Micronutrients showed few consistent differences in intake between the two groups although vitamin E was consistently lower and folic acid consistently higher in the relapsing patients. The higher folic acid intake in the relapsing alcoholics may be due to their high consumption of beers and lagers, which contain significant amount of dietary folate.

	FFQ				**24 h recall**				***P****	
	Abstaining (n=12)		Relapsing (n=21)		Abstaining (n=12)		Relapsing (n=21)		Abstaining *v.* relapsing	
Micronutrients	Mean	SE	Mean	SE	Mean	SE	Mean	SE	FFQ	24 h recall
Vitamin A (μg/d)	630	91	587	104	372	54	345	170	0.782	0.908
Vitamin B_1 (mg/d)	2	0.2	2	0.1	1	0.1	1	0.4	0.908	0.090
Vitamin B_2 (mg/d)	1	0.1	2	0.3	1	0.2	2	0.2	**0.004**	0.067
Vitamin B_3 (mg/d)	15	1	29	3	34	8	33	8	**0.001**	0.935
Vitamin B_{12} (μg/d)	4	1	4	1	2	1	2	0.3	0.830	0.843
Vitamin C (mg/d)	83	17	33	7	93	23	49	29	**0.004**	0.302
Vitamin E (mg/d)	5	1	3	0.1	4	1	2	0.1	**0.046**	**0.043**
Folic Acid (μg/d)	247	20	455	44	157	25	282	30	**0.002**	**0.008**

*Based on unpaired *t*-test.

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A study of breast-feeding in the south-east region of the Islamic Republic of Iran. By M. SARGAZI[1,2], F. KHOSHABI[1], F. HORMOZI[1], F. FAKHIREH[3], M. SAEB[1], A. MALEKI[1], M. FIROOZKOOHI[1], H. SHAHRYARI[1], A. HACKETT[2] and S. MAXWELL[2], [1]*Zahedan University of Medical Sciences, Zahedan, Iran,* [2]*John Moores University, Liverpool L17 6BD and* [3]*Institute for Standard and Research of Sistan and Balouchestan, Zahedan, Iran*

Although the incidence of malnutrition has declined gradually (United Nations Children's Fund, 2002), it is still a worldwide problem and a contributing factor in nearly 60% of deaths among children in developing countries (World Health Organization, 2002; International Institute for Population Sciences and ORC Macro, 2000). There is good agreement that breast-feeding is good for the growth and health of infants and in less developed countries it may be the only way to provide complete nutrition for sustaining neonates' growth during the first 4–6 months of life (Slusser & Power, 1997). Malnutrition is also linked to an increased risk of death in children with diarrhoea and acute infections of the lower respiratory system (Rice *et al.* 2000; Froozani *et al.* 1997). In the last decade, an attempt has been made by the Iranian Ministry of Health and Medical Education and local health authorities to improve the condition of both children and mothers living in the country by increasing the number of regional and local health centres as well as by providing better facilities and services for the families, e.g. breast-feeding education.

A study has been carried out to investigate the prevalence of breast-feeding in relation to socio-economic conditions in 541 families residing in the south-east region of Iran, Sistan and Balouchestan, Zahedan, during 1996. Data were collected using face-to-face interviews with mothers and a questionnaire.

The results show that about 75% of the infants were breast-fed, of which 55% were exclusively breast-fed. Only 12.4% were fed using powdered milk and about 6% were fed using fresh animal milk i.e. cow and goat. Of the breast-fed infants, more than 50% were breast-fed for a duration of 12–24 months and 10% of them for more than 24 months. Fewer than 31% of breast-fed infants stopped before the age of 12 months and 24% when the infants experienced diarrhoea.

No reduction in breast-feeding was observed as the family size increased. Indeed more than 50% of the breast-fed children were from families with six or more members. A similar relationship was observed with family income and about 65% of the breastfed children were from families with the lowest incomes. About 40% of the breast-fed children had a mother who had had at least four previous pregnancies. About 50% of the mothers in the breast-fed group were educated, but most of them did not work and remained at home.

In conclusion, apart from economic conditions, the mother's social conditions seem to be the main reason for the high rate of breast-feeding among the children of the south-east area of Iran.

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Thiamin intake and status of nulliparous women in the first trimester of pregnancy and breakfast-cereal consumption. By G.A. REES, Z.M. BROOKE and W. DOYLE, *Institute of Brain Chemistry and Human Nutrition, London Metropolitan University, 166–220 Holloway Road, London N7 8DB*

Low red-cell thiamin has been associated with intrauterine growth restriction (Heinze & Weber, 1990). We have previously reported low thiamin status in 34% of pregnant women and associations between thiamin status and birth outcomes (Rees *et al*. 2002). Associations between maternal nutrient intake in the first trimester and birth weight have been found for many nutrients, including thiamin (Doyle *et al*. 1990).

This work explores the relationship between maternal thiamin intake and status in the first trimester of pregnancy in nulliparous women. Participants were recruited during booking-in at the Homerton Hospital antenatal clinic as part of a larger study looking at the relationship between nutritional status and birth outcomes (Rees *et al*. 2002). Participants provided 5 ml of blood and were given a 4 d diet diary to complete. Instructions were given to complete the diary using household measures. Thiamin analysis was performed by the MRC, HNR using the erythrocyte transketolase (ETK) method, based on the procedure by Vuilleumier *et al*. (1990). 36/69 diaries were returned – a response rate of 52%. Nutritional analysis was performed using Foodbase programme.

Thiamin intake was negatively correlated with ETK activation coefficient (ETKAC) levels (P=0.009, r=−0.454). Women were divided into two groups according to their thiamin status (normal ETKAC 1.0–1.14, n=24; low-status ETKAC >1.14, n=12). Women with normal thiamin status had significantly higher daily thiamin intakes (mean 2.17 mg/d) than women with low thiamin status (mean 1.26 mg/d; P=0.01). The mean daily intake of thiamin was 1.78 mg (SD 1.87). Two women experienced nausea and had low energy intakes and thiamin intakes below the RNI of 0.8 mg/d.

Caucasian women (n=26) had significantly higher mean thiamin intakes than non-Caucasians (n=10) (P=0.028). Women in non-manual occupations (n=17) consumed more thiamin per day than the unwaged and manual-occupation women combined (n=19) (P=0.004).

Breakfast-cereal consumers' (n=24) daily thiamin intake was significantly higher than the women who did not eat breakfast cereal (n=12) (P=0.041). The breakfast-cereal consumers' mean ETKAC levels (1.10) were significantly lower than the non-breakfast-cereal consumers (1.17) (P=0.015). Whilst there was no statistically significant difference in daily thiamin intake, women consuming two or more bowls of breakfast cereal per week had significantly lower mean ETKAC levels (1.08) than the women consuming less than two bowls of breakfast cereal per week (1.17) (P=0.003). 50% of non-Caucasians ate breakfast cereal compared with 75% of Caucasians.

This was a small-scale study in a population at risk of poor nutrition and low birth weight. It is of note that many women had low thiamin status with seemingly adequate thiamin intakes in the first trimester of pregnancy. Fortified foods such as breakfast cereals make an important contribution to the thiamin intake of some women. The challenge is to reach ethnic minorities who are at greater disadvantage and who may not consume fortified foods or supplements. We are now conducting a large supplementation study in our ethnically diverse population.

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Assessment of food 'quality' in Saudi Arabian premenopausal and postmenopausal women: implications for bone health. By S.O. KHOJA[1], J.A. KHAN[1] and S.A. NEW[2], [1]*Biochemistry Department, King Abdul Aziz University, PO Box 1540, Jeddah 21441, Kingdom of Saudi Arabia and* [2]*School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH*

The importance of assessing diet in population groups is to enable estimation of food consumption patterns, to determine nutrient intake and to explore the effects of diet on health. The problem in many Middle-Eastern countries, including Saudi Arabia, is the lack of any food composition tables which would enable accurate estimates of nutrient intake to be made. As a first step to develop such dietary methodologies as part of a bone-health study, we have undertaken some preliminary work to assess the extent of food 'quality' in Saudi Arabian women using the pyramid model.

As part of our ongoing bone-health study in 212 Saudi Arabian women (Khoja *et al*. 2002), a total of twenty premenopausal and twenty postmenopausal women living in the city of Jeddah were involved in this dietary substudy. They were aged between 20–30 years and 45–60 years, respectively, and had not suffered from any known condition and were not taking any medication likely to affect bone metabolism. Information on dietary intake of each individual was obtained using 3 d estimated food diaries. The amount of food consumed (in grams) for the five food groups was calculated for each subject: (I) bread, cereals and potatoes; (II) fruit and vegetables; (III) meat, fish and protein alternatives; (IV) milk and dairy products; (V) fatty and sugary foods. Intakes were converted to frequency of consumption (times per day) by dividing food groups by average portion sizes. Full instructions were provided to subjects and the recording period included a weekend day. Regular contact was also made to ensure compliance.

As shown in the Table, the average consumption of portions (times/d) in both premenopausal and postmenopausal women of the following food groups compared with the food pyramid were: (I) bread, cereals and potatoes (1.88, 1.87) *v*. (6–11) respectively; (II) fruit and vegetable group (2.3, 3.5) *v*. (5–9) respectively; (III) meat, fish and protein alternatives (1.4, 1.8) *v*. (2–3) respectively; (IV) milk and dairy products (1.3, 1.67) *v*. (2–3) respectively; (V) fatty and sugary foods (1.5, 1.8) *v*. 'sparingly', respectively. The average consumption of portions (times/d) of beverages was 1.5 and 1.8respectively.

	Premenopausal		Postmenopausal	
Food group	Amount (g/d)	Daily Frequency	Amount (g/d)	Daily Frequency
Bread and other cereals	234 ± 92 (106–443)	1.88	232 ± 91 (110–480)	1.87
Fruit and vegetables	237 ± 143 (5–411)	2.3	357 ± 192 (29–870)	3.5
Meat and alternative	173 ± 107 (5–411)	1.4	221 ± 113 (60–502)	1.8
Milk and dairy food	130 ± 52 (15–232)	1.3	162 ± 64 (33–255)	1.67
Fatty and sugary foods	85 ± 50 (14–211)	1.47	73 ± 52 (4–177)	1.2
Beverages	339 ± 225 (0–730)	1.5	396 ± 172 (132–780)	1.8

Mean ± SD (range), average consumption of portions (frequency = times/d).

These results suggest that the 'quality' of food consumed by Saudi Arabian women does not follow the recommended food guidelines. Further analysis of a full population data set is required to confirm these observations, which will assist in the development of food composition information.

Financial support from the King Abdul Aziz University (KAAU) is gratefully acknowledged.

Khoja SO, Khan JA, Maimani AR & New SA (2002) *Proceedings of the Nutrition Society* **61**, 112A.

Is salt an important risk factor for osteoporosis? A systematic review of the evidence. By C. TILNEY[1], D.J. TORGERSON[2], M. AL-JANABI[3] and S.A. NEW[1], [1]*Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH,* [2]*York Trials Unit, Department of Health Sciences, University of York, York YO10 5DD and* [3]*Department of Nuclear Medicine, King's College Hospital, London SE5 9RS*

The effect of salt on health has been gaining increasing prominence in the literature (SACN, 2003). The evidence as to whether salt is an important risk factor for bone health remains controversial. The aim of this investigation was to use a systematic review of published scientific evidence to quantify the exact nature of the relationship between salt, sodium and bone health.

A literature search was conducted using a number of specific databases, including the National Library of Medicine (PubMed), The Cochrane Collaboration (Cochrane), Bath Information and Data Services (BIDS), Ingenta, the US National Library of Medicines Bibliographic Database (Medline) and Science Direct, as well as manual searches of a wide range of nutrition and bone journals. Searches were limited to papers published from 1960 to the present. The studies identified were then analysed by investigating the correlation coefficients between salt/sodium intake and urinary calcium excretion and bone metabolism markers. An overall correlation coefficient was calculated to give a mean *r* value for the different urinary excretions. The standardised effect size of separate individual studies was also calculated, which provided a measure of the standard deviation difference between groups of data within one study. The coefficient of determination was used to measure the proportion of the variability of one variable that is accounted for by the variability in another and is a calculation of the square of the *r* value.

In total, nineteen studies were identified as permitting successful comparisons, within the following categories; urinary calcium/creatinine (Ca/Cr); urinary sodium/creatinine (Na/Cr); urinary hydroxyproline/creatinine (Hyp/Cr). Correlations between the different studies varied considerably, with the results of studies ranging from 0.34 to 0.91 for urinary Ca/Cr to Na/Cr ratios, 0.28 to 0.8 for the urinary Ca to urinary Na excretion and 0.25 to 0.63 for urinary Na/Cr to urinary Hyp/Cr. The overall *r* values ranged from 0.34 for urinary Ca, 0.49 for urinary Ca/Cr and 0.33 for urinary Hyp/Cr. The coefficient of determination also varied markedly between the different studies, ranging on average between 12.25 and 84.64%. The overall coefficient of determination ranged from 10.89% for urinary Hyp/Cr to urinary Ca/Cr, 12.25% for urinary Hyp/Cr to urinary Na/Cr and 24% for urinary Ca/Cr to urinary Na/Cr. A total of ten studies could not be successfully analysed due to lack of information provided on the control groups, lack of detail about experimental design, critical discrepancies in scientific protocol and differences in the units of measurement of markers of urinary excretion.

Studies showed a clear association between increased dietary sodium and increased urinary Ca excretion such that a 100 mmol increment in daily sodium intake was associated with an average loss of urinary Ca of approximately 1 mmol in free-living normocalciuric healthy populations. However, too many of the studies were short-term and hence long-term adaptation to higher Na intakes could not be assessed. Only a limited number of studies investigated bone metabolism and, in most of those, urinary Hyp/Cr was measured. This marker is now widely regarded as an unreliable biochemical marker of bone resorption since it is affected by dietary intake. Few studies used the newer markers of pyridinium cross links of collagen, serum osteocalin, serum bone specific alkaline phosphatase, urinary pyridinoline and deoxypyridinoline and the urinary C-terminal or N-terminal peptides. Most were of short-term duration and showed large inter and intra-individual responses to different dietary sodium loads. Very few studies have examined other indices of bone health such as bone mineral density or bone quality and no studies have looked at fracture risk.

Thus, based on the current scientific evidence available, the question as to whether salt intake is an important risk factor for osteoporosis remains unanswered and further studies are urgently required.

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Estimates of acid–base balance in the diets of older Scottish women. By C.J. PRYNNE and C. BOLTON-SMITH, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL*

Osteoporosis is a significant public health problem resulting in fractures and increased morbidity and mortality in the older population. It is characterised by a net loss of calcium from bone and a loss of micro-architectural structure that increases fragility and fracture risk. The response to the consumption of a high-acid diet may be bone resorption to yield calcium carbonate (Bushinsky, 2001). This buffers the metabolic acidosis and may be a specific cause of bone mineral loss in the elderly, whose ability to excrete acid is reduced. Lower rates of bone mineral loss and higher bone mineral density in older men and women have been associated with greater intakes of fruit and vegetables (New *et al*. 1997; Tucker *et al.* 1999). In this study, food intake was determined by a validated food frequency questionnaire (New *et al*. 1997) in 244 women aged over 60 years who were recruited to the Dundee Bone and Vitamin Intervention Study. Dietary acid–base balance was calculated by two methods, indirect net acid excretion (NAE_{ind}), based on the method of Remer (Remer & Manz, 1994), and protein/potassium ratio (Frassetto *et al.* 1998). A comparison was made between the two methods by Pearson correlation. Multiple regression analysis was carried out to estimate the contribution of the weights of foods eaten to the variance in $NAE_{ind.}$

NAE_{ind} mEq			Protein/potassium ratio g/mEq			Pearson	
Mean	SD	IQR	Mean	SD	IQR	*r*	*P*
54.7	11.2	47.9, 61.1	0.92	0.13	0.83, 0.99	0.81	<0.001

NAE_{ind} calculated using protein & phosphorus (acidic) and potassium & magnesium (alkaline) intakes.

The concordance between the two methods was poorer for subjects with high acid values. These subjects tended to be heavier, with greater total food consumption and also higher phosphorus intakes that contributed to the calculation of NAE_{ind}.

	Cereals/products	Milk/products	Meat and fish	Potatoes	Other vegetables	Fruit
B	+0.038	+0.018	+0.112	−0.046	−0.021	−0.032
SE	0.004	0.002	0.007	0.011	0.004	0.003
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

B= unstandardised coefficient; *P*=independent significance. Adjusted R^2=0.68.

The estimates of dietary acid–base balance were very close to those calculated from the diets of 101 teenage girls, assessed using a 7 d diary (C.J. Prynne *et al*., unpublished data) and showed that the dietary acid load was lower with a high consumption of potatoes, other vegetables and fruit. However, with the traditional British food pattern, particularly in this older age group, a higher intake of potatoes and vegetables was also associated with a higher intake of meat and fish. These latter foods contribute to the acid load; although positive effects of protein intake on bone mineral density have been reported in this age group too (Hannan *et al*. 2000). Milk products, another contributor to acid load, are also a major source of dietary calcium. Whilst the case to do so is not yet clear, any effort to reduce the acid load of the diets in this age group will need to be carefully considered with a view to maintaining energy, protein and calcium intakes to ensure bone and general health.

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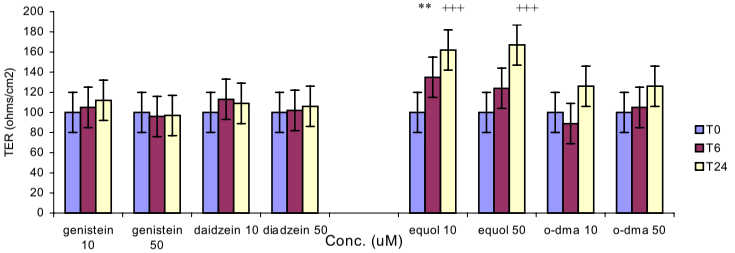
Effect of soya isoflavones and metabolites on paracellular permeability in various breast cell lines. By C.C. FLANAGAN, P.J. MAGEE and I.R. ROWLAND, *Northern Ireland Centre for Diet and Food (NICHE), University of Ulster at Coleraine, Cromore Road, Coleraine BT52 1SA*

Phytoestrogen isoflavones are a diverse group of multifunctional compounds found in soya foods, which have a possible role in the prevention of breast cancer (Adlercreutz & Mazur, 1997). The soya isoflavonoids genistein and daidzein are extensively metabolised by intestinal microflora. In the case of daidzein there is considerable individual variation in metabolism to metabolites equol and *O*-DMA (Rowland *et al*. 2000). In the present study the effect of genistein, daidzein and the metabolites equol and *O*-DMA on epithelial permeability was studied on three breast cell lines – MCF-7 (oestrogen-positive, cancerous), MDA-MB-231 (oestrogen-negative, cancerous), and MCF-10A (oestrogen-negative, non-cancerous). Increasing paracellular permeability has been associated with tumour promotion. Paracellular permeability can be assessed *in vitro* by measuring resistance to the flow of an electric current (transepithelial electrical resistance). Reduced resistance is associated with increased permeability and reduced epithelial integrity. The compounds were tested using a six-well plate culture technique and transepithelial resistance (TER) was measured using the EVOM epithelial voltohmeter. Cells were exposed to the compounds at concentration of 0, 10 and 50 µM in media free from endogenous oestrogens (Welshons *et al*. 1992) and incubated for 48 h; readings for TER were recorded at 0, 6 and 24 h. Isoflavones had no effect on TER in MCF-10A cells. In MDA-MB-231 cells genistein and daidzein had no effect on TER. With the daidzein metabolites there was a tendency to increase TER, particularly with *O*-DMA but the results were not significantly different from the untreated cells. In MCF-7 cells minor non-significant effects of diadzein and genistein were observed. Equol was associated with significant improvement in TER at both concentrations. A small non-significant increase in TER was also seen with *O*-DMA (Fig, 1). The results from this study are consistent with previous work indicating the isoflavones have a potential protective role in breast cancer. Our results suggest the inhibition of tumour promotion is a target of isoflavone action. The differential effects of daidzein and its metabolites, equol and *O*-DMA, suggest that gut micro-flora metabolism is important in the anti-cancer effect of isoflavones and that individual variation in equol production may play a role in determining breast cancer risk.

Fig 1. Equol, but not genistein or diadzein increased Ter in MCF-7 cells



Results expressed as a % of untreated cells at each time point.
Significant differences from T_0-T_6 *p<0.05 **p<0.01 ***p<0.00
Significant differences from T_0-T_{24} +p<0.05 ++p<0.01 +++ p<0.00

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Increased fruit and vegetable consumption increases the ferric reducing ability of plasma (FRAP) of free-living postmenopausal women. By S.R. AREFHOSSEINI, S. HIGGINS and C.A. EDWARDS, *Human Nutrition Section, Division of Development Medicine, University of Glasgow, Yorkhill Hospitals, Glasgow G3 8SJ*

Women are at greater risk for coronary heart disease (CHD) after the menopause (Rich-Edwards *et al*. 1995). Most epidemiological evidence indicates that diets rich in antioxidants such as vitamins C, E and carotenoids may reduce the risk of CHD by protecting against the adverse effects of free radicals (Ness & Powles, 1997). The aim of this study was to investigate the influence of increasing fruit and vegetable consumption on the 'antioxidant power' of the plasma as measured by the ferric reducing ability of plasma (FRAP) assay (Benzie & Strain, 1996).

Eight healthy postmenopausal women (age 56.1 (SD 6.9) years); BMI 25.2 (SD 2.9) kg m^{-2}) took part in this study. Habitual diet was assessed by a 7 d weighed intake. On the basis of the results, subjects were advised to make dietary changes to comply with the current dietary guidelines including advice to increase fruit and vegetable consumption to five portions or 400 g daily. Subjects were asked to follow this diet for 4 weeks, in a free-living situation. Fasting blood samples were obtained at baseline and after 1 and 4 weeks of the dietary intervention, and FRAP was measured. This method measures the ability of antioxidants in plasma to reduce the ferric component (Fe^{3+}) of a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to the ferrous form (Fe^{2+}), which is accompanied by the formation of a blue colour which can be measured at 593 nm. The CV of FRAP assay was 2.2%.

As shown in the Table, subjects significantly increased their intake of fruit and fruit and vegetables after 1 week of the dietary intervention. However, there were no significant changes in vegetable intake. There was a significant increase in FRAP level (mM) after 1 and 4 weeks of the dietary intervention. There were statistically significant correlations between the percentage of change in FRAP and the percentage change in fruit intake and in fruit and vegetable intake over 4 weeks (r=0.79, P=0.036 and r=0.86, P=0.014, respectively; Spearman correlation). These significant correlations remained even after adjustment for energy intake.

	Habitual (*n* 8)		**After 1 week (*n* 8)**		***P****	**After 4 weeks (*n* 7)**		***P***
	Mean	SD	Mean	SD		Mean	SD	
Vegetables (g)	59.2	15.4	58.5	15.6	0.889	55.8	16.0	0.735
Fruit (g)	291.2	90.3	400.5	65.5	**0.05**	375.0	27.2	0.237
Fruit & vegetables (g)	350.5	89.3	459.0	68.7	**0.025**	430.8	32.4	0.31
FRAP (mM)	0.55	0.04	0.58	0.04	**0.012**	0.58	0.03	**0.028**

* Difference between habitual and week 1; ** Difference between habitual and 4 weeks by Wilcoxon rank test.

This study has demonstrated that increased consumption of fruit and vegetables after general dietary advice induced a significant rise in the 'antioxidant power' of plasma as measured by FRAP. This was mostly associated with an increase in fruit intake.

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Genetic determinants of iron status. By Y. PASDAR, M. NELSON and J. POWELL, *Department of Nutrition and Dietetics, King's College London, 150 Stamford Street, London SE1 9NN*

Iron deficiency is the most common worldwide nutritional problem. In the last decade of the 20th century we are faced with an expansion of knowledge in both genetics and nutrition. The importance of the interaction between genes and nutrients in normal growth and development, and in the prevention and treatment of a number of chronic diseases is recognized. Heterozygocity for the C282Y and H63D mutations of the HFE gene may possess a selective advantage. HFE mutations increase iron absorption and the mean transferrin saturation, transferrin receptor and ferritin levels are slightly increased in heterozygotes (Whitfield *et al*. 2000), suggesting that HFE mutations may protect against iron depletion and iron deficiency anaemia. The aim of this study was to assess the role of HFE polymorphisms and dietary factors in variations in iron status.

Venous blood samples were obtained from sixty-nine British adolescent boys aged 11–14 years and fifty-five respondents aged 9–87 years from a UK-wide sample of low-income families. Genomic DNA was isolated from whole blood and analysed for the C282Y and H63D HFE mutations. Heterozygotes for each mutation and compound heterozygotes were screened by a new denaturing high performance liquid chromatography (DHPLC) technique, using the transgenomic nucleic acid fragment analysis system (WAVE). Blood samples were analysed for haemoglobin, haematocrit, MCV, MCHC, ferritin, transferrin saturation, transferrin receptor and total iron-binding capacity.

Genotype frequencies found among the 124 subjects studied were as follows: C282Y/H63D compound heterozygotes 2 (2%); C282Y heterozygotes 8 (6%); H63D heterozygotes 23 (19%), and 91 (73%) with no detectable mutation.

Dietary data based on 3 d food checklists (fifty-one adolescent boys) or 4 × 24 h recalls (thirty-five low-income respondents) were available for eighty-six of the subjects who had provided blood samples. The Table shows mean (SE) values for the ratios of ferritin and haemoglobin to dietary iron intake either as measured or energy-adjusted.

		H63D mutation			**C282Y mutation**		
		Absent	Present	*P**	Absent	Present	*P**
	n	65	21		79	7	
Ferritin: iron intake as measured	Mean	7.44	10.39	0.241	8.11	8.63	0.896
	SE	1.18	2.44		1.15	2.85	
Ferritin: iron intake energy adjusted	Mean	7.09	10.28	0.172	7.90	7.55	0.924
	SE	1.09	2.30		1.07	2.51	
Haemoglobin: iron intake as measured	Mean	1.58	1.69	0.512	1.58	1.98	0.339
	SE	0.08	0.09		0.06	0.39	
Haemoglobin: iron intake energy adjusted	Mean	1.54	1.64	0.388	1.56	1.67	0.544
	SE	0.06	0.08		0.05	0.23	

*Differences according to unpaired *t*-test.

There were no significant differences (unpaired *t*-test) between the ratios of ferritin and haemoglobin to dietary iron intake (as measured and energy adjusted) according to the presence or absence of H63D and C282Y mutations. With the exception of the C282Y-ferritin:iron intake energy-adjusted ratio, however, the ratios suggest that there are maybe higher levels of iron status for a given level of iron intake in the presence of the HFE mutations, but the small sample size means that the study had limited statistical power. We plan to conduct further analyses of iron status in relation to HFE mutations and dietary intake of iron and other factors likely to affect iron absorption in order to improve our understanding of the role of genetics in the prediction of iron status.

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Estimation of mean intakes of fourteen classes of dietary phenols in a population of male shift workers. By E. WOODS, M.N. CLIFFORD, M. GIBBS, S.M. HAMPTON, J. ARENDT and L.M. MORGAN, *School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH*

There is growing evidence that diets rich in fruit and vegetables offer protection from certain cancers, and some forms of vascular disease. Such diets are comparatively rich in phenols, polyphenols and tannins, and these substances are efficient antioxidants. There is some evidence that these properties may help explain the health benefits associated with such diets. It is known that shift workers are more susceptible to cardiovascular disease and it was therefore of interest to investigate their intake of dietary phenols.

The data reported here have been obtained from a food composition database produced at the University of Surrey (Gosnay *et al*. 2002), and subsequently updated, and 3 d diet diaries for a population of fifty male shift workers (aged 27–57 years) on offshore oil-industry installations. Twenty-six day-shift diaries and twenty-two night-shift diaries (12 h shifts 06.00–18.00–06.00 hours) were analysed and estimates were prepared for the consumption of fourteen classes of dietary phenols, i.e. hydroxybenzoic acid derivatives, cinnamates (including chlorogenic acids), anthocyanins, dihydrochalcones, flavanols, flavanones, flavonols, flavones, isoflavones, proanthocyanidins, stilbenes, derived polyphenols (characteristic of processed commodities such as black tea), and lignans. Data were examined for normality of distribution and day-time consumption compared with night-time consumption. The day-time data for this population of males were also compared with the female population previously studied.

The overall estimated mean total phenols intake for the male shift workers was 1.05±0.42 g/d, with the day-shift value being 0.99±0.36 g/d and the night-shift value being 1.12±0.47 g/d. The majority of these phenols (over 60%) were derived from black tea and coffee, making derived polyphenols (0.16 g/d) and cinnamates (0.67 g/d) the dominant subgroups consumed. The total flavonoids consumption was 0.21 g/d. A comparison of day-shift with night-shift values failed to demonstrate any statistically significant differences although mean anthocyanins, mean flavanones, and mean cinnamates intakes were respectively 73%, 23% and 22% higher in the diets of night-shift workers, associated with a greater consumption of juices (including citrus) and coffee on night shifts.

The data from Gosnay *et al*. (2002), recalculated using the revised food composition database, gave a mean total-phenols intake of 0.78±0.41 g/d for females aged 20–30 years, which is significantly lower (P=0.02) than the day-shift value reported here for an older population of males, possibly reflecting a lower overall energy intake in the females. Although not reaching statistical significance, the males consumed more cinnamates and flavanones and less isoflavones, reflecting greater consumption of coffee and citrus and lower consumption of soya products.

It is clear from this study, as from that of Gosnay *et al*. (2002), that coffee and black tea are major sources of dietary phenols in these two very different populations, and as such the possible dietary significance of cinnamates and derived polyphenols are as deserving of the same levels of attention as that currently afforded to flavonoids.

Gosnay SL, Bishop JA, New SA, Catterick J & Clifford MN (2002) *Proceedings of the Nutrition Society* **61**, 125A.

***In vivo* antioxidant status in a population of vegetarians and omnivores.** By S. HALDAR[1], Y.A. BARNETT[1], J. POWELL[2], J. FLETCHER[2], D. TALBOT[2] and I.R. ROWLAND[1], [1]*Northern Ireland Centre for Food and Health (NICHE), School of Biomedical Sciences, University of Ulster, Coleraine, County Londonderry, BT52 1SA and* [2]*Unilever Research, Colworth Laboratory, Sharnbrook, Bedford, MK44 1LQ*

Epidemiological evidence suggests a reduced incidence of chronic diseases, such as heart disease and cancer, and a reduction in mortality from these diseases in long-term vegetarians when compared to those on a mixed, omnivore diet (Changclaude *et al.* 1992; Appleby *et al.* 1999). Since free radical damage to biological macromolecules is a major contributor to the aetiology and pathogenesis of heart disease and cancer, the epidemiological findings in vegetarians may be in part attributed to the differences in the levels of antioxidants between vegetarians and omnivores.

The aim of this study was to determine *in vivo* antioxidant status in the blood of vegetarians and omnivores. Thirty-seven vegetarians and sixty-four omnivores were all healthy, non-smokers between the ages of 20 and 64 years. Vegetarians were defined as those who did not consume any meat, chicken, fish or any other flesh foods more than six times during the year. All the subjects were recruited predominately from the University of Ulster campuses and the rest from around Northern Ireland. The mean age for the vegetarians was 35.3 years (range: 20-64), and for the omnivores was 38.7 years (range: 22-63). Measurements were carried out on one fasting blood sample. The total antioxidant capacity of plasma was measured using the FRAP assay (Benzie & Strain, 1996). Erythrocyte glutathione peroxidase (GPX), superoxide dismutase (SOD), glutathione transferase (GST) activities, erythrocyte reduced glutathione (GSH) concentration and plasma levels of vitamin C, uric acid (UA), zinc, α-tocopherol, retinol, lutein, β-carotene and lycopene were also measured.

Parameter	Vegetarians (n=37)		Omnivores (n=64)		P value*
	Mean	SE	Mean	SE	
FRAP (µmol/l)	1124.49	28.66	1148.66	20.71	0.49
GPX (U/gHb)	70.28	3.61	88.86	3.51	0.10
SOD (U/gHb)	1393.14	23.51	1372.05	26.87	0.56
GST (U/gHb)	3.80	0.16	3.65	0.17	0.56
GSH (µmol/gHb)	5.53	0.15	5.33	0.11	0.31
Vitamin C (µmol/l)	71.28	4.00	72.92	4.04	0.79
UA (µmol/l)	280.84	10.14	286.10	8.09	0.69
α-tocopherol (µmol/l)	39.06	1.34	37.65	0.96	0.39
Retinol (µmol/l)	2.63	0.13	2.55	0.08	0.62
Lutein (µmol/l)	0.47	0.03	0.40	0.02	0.04
β-carotene (µmol/l)	0.97	0.09	0.88	0.07	0.44
Lycopene (µmol/l)	1.19	0.08	1.05	0.05	0.13
Zinc (µmol/l)	18.21	0.50	18.89	0.37	0.27

*Statistical analysis was performed using independent samples t-test.

Plasma carotenoids were approximately 10% higher in vegetarians than in omnivores, although the difference was statistically significant only for lutein (P≤0.05). In omnivores, GPX was approximately 25% higher than in vegetarians, although the value did not reach statistical significance (P=0.10). Activities of other antioxidant enzymes in erythrocytes, total antioxidant capacity of plasma and levels of other antioxidants in blood were similar in both the diet groups. Overall, the results suggest that an omnivorous diet can be as healthy as a vegetarian diet in terms of antioxidant status. In addition, there could be factors other than antioxidant status that might be contributing to the reduced risk of chronic diseases in vegetarians.

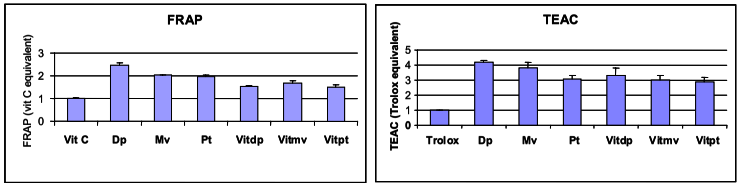
Appleby PN, Thorogood M, Mann JI & Key TJA (1999) *American Journal of Clinical Nutrition* **70**, 525S–531S.
Benzie IFF & Strain JJ (1996) *Analytical Biochemistry* **239**, 70–76.
Changclaude J, Frentzelbeyme R & Eliber U (1992) *Epidemiology* **3**, 395–401.

Antioxidant activity and cell effects of anthocyanins and vitisins from red wine. By M. García-Alonso[1,2], R. Turner[1], J.C. Rivas-Gonzalo[2], A.M. Minihane[1], G.H. Rimbach[1] and S. de Pascual-Teresa[1], *Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, Reading, RG6 6AP and* [2]*Unit of Food Science and Nutrition, School of Pharmacy, University of Salamanca, Spain*

Red wine is a major source of anthocyanins, such as malvidin, petunidin and delphinidin. Epidemiological studies suggest that a moderate intake of anthocyanins might be associated with a protection against chronic diseases, including cancer and atherosclerosis. During red wine maturation, there is a loss of anthocyanins and it appears that other pigments, so-called vitisins, are formed through the interaction of the original anthocyanins with pyruvic acid. However, the antioxidant and cellular activity of anthocyanins and vitisins remains largely unknown.

Antioxidant properties of the test components were assessed *in vitro* using the TEAC and FRAP assays. In order to determine whether the antioxidant properties of anthocyanin and vitisin affect their cellular activity, nitric oxide production (measured by the Greiss reaction) in RAW 264.7 macrophage stimulated with IFN-γ plus LPS and pre-treated with anthocyanins and vitisins was carried out. The uptake of the neutral red dye was used to measure cell viability. All the test compounds showed an antioxidant activity at least double that of vitamin C in the FRAP assay and three times greater than that of trolox in the TEAC assay. Delphinidin showed the highest antioxidant activity of all the tested compounds, followed by malvidin and petunidin, with a similar activity. Both in the FRAP and TEAC assay the vitisins exhibited lower antioxidant activity than their parent compounds.

FRAP and TEAC values for the anthocyanins and vitisins (Dp: delphinidin; Pt: petunidin; Mv: malvidin; Vitdp: vitisin of delphinidin; Vitpt: vitisin of petunidin; Vitmv: vitisin of malvidin; vit C: vitamin C).



Pre-treatment of RAW 264.7 macrophages with up to 100 mM of the test compounds did not affect cell viability. Twenty-four hours post-treatment with IFN-γ plus LPS, murine macrophages showed a significant increase ($P \leq 0.05$) in the levels of NO_2^- in the culture medium from 5 to ~100 μM/10^6 cells. Pre-treatment of RAW 264.7 with malvidin, petunidin, delphinidin and their corresponding vitisins for 6 h did not affect NO production in activated macrophages.

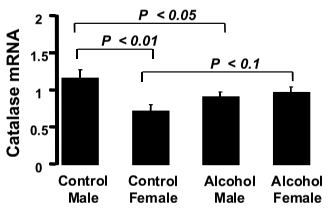
Current data indicate that the chemical interaction between anthocyanins and pyruvic acid during wine ageing slightly decreases the antioxidant activity of anthocyanins. However, the antioxidant activity of the test compounds was not accompanied by a suppression of NO production in activated macrophages, suggesting that anthocyanins may exert their antioxidant effect via a different molecular mechanism.

The chronic effects of alcohol feeding on mitochondrial and cytosolic superoxide dismutase, catalase and glutathione peroxidase mRNA levels in skeletal muscle: is there a gender difference in response? By T. Nakahara[1], R. Hunter[2], R. Rajendram[2], K. Hashimoto[3], M. Hirano[3], C.R. Martin[4] and V.R. Preedy[2], [1]*Kyushu University Ropponmatsu, Fukuoka, Japan* [2]*King's College London, London WC2R 2LS* [3]*Hizen National Mental Hospital, Saga Japan and* [4]*University of York, York YO10 5DD*

Chronic alcohol ingestion causes a well-defined skeletal muscle lesion, i.e., *alcoholic myopathy,* which is possibly the most prevalent skeletal muscle disease in the Western Hemisphere (Preedy *et al*. 2001 *a,b,c,d*). Females are particularly susceptible to this pathology. Although the causative agent is known, the precise sequence of events between alcohol ingestion and the development of the myopathy is ill-defined. Various studies have suggested that the myopathy is exacerbated or precipitated by impaired antioxidant status. In the liver, another major organ affected by alcohol, mRNA levels of the manganese-containing superoxide dismutase (SOD) increase, possibly as a compensatory response. We hypothesised that similar responses occur in mRNAs encoding antioxidant enzyme in alcohol-exposed skeletal muscle. We also hypothesised that greater changes would occur in female rats.

To test this we measured the mRNA levels of both mitochondrial-containing (Mn-SOD) and the cytosolic copper/zinc containing superoxide dismutase (Cu,Zn-SOD), catalase and glutathione peroxidase (GPX) in a well-characterised model of chronic alcoholic myopathy. We fed male and female rats nutritionally complete diets containing ethanol as 35% of total calories (treated) or isocaloric amounts of the same diet in which ethanol was replaced by isocaloric glucose (controls). At the end of 6 weeks, rats were killed and mRNA analysed in representative skeletal muscle by reverse transcription–polymerase chain reaction (RT-PCR) with an endogenous internal standard, GAPDH.

Alcohol feeding caused skeletal muscle myopathy as defined by muscle weight reductions. However, the mRNA levels of Mn-SOD, Cu,Zn-SOD and GPX were unaffected by alcohol feeding.



Catalase mRNA levels in skeletal muscle of myopathic rats
Male or female rats were fed nutritionally complete liquid diets containing 35% of total dietary energy as ethanol or identical amounts of the same diet in which ethanol was replaced with isoenergetic glucose (controls). Data are mean±SEM (n=8–10).

In contrast, catalase mRNA decreased in muscle of male rats, whereas there was a tendency for catalase mRNA levels in female rats to increase. For catalase mRNA, there was an overall significant interaction between alcohol and gender (two-way ANOVA; P<0.01).

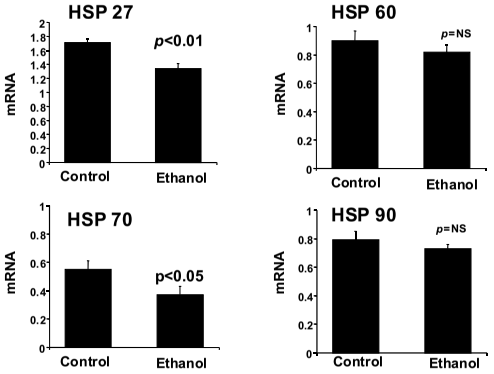
In conclusion, there is no evidence that the mRNA levels encoding the important antioxidative enzymes Mn-SOD, Cu,Zn-SOD and GPX are increased in skeletal muscle of myopathic rats, although there are changes in catalase mRNA which is gender-specific.

Preedy VR, Adachi J, Peters TJ, Worrall S, Parkkila S, Niemela O, Asano M, Ueno Y, Takeda K, Yamauchi M, Sakamoto K, Takagi M, Nakajima H & Toda (2001*a*) *Alcoholism, Clinical and Experimental Research* **25**, 54S-59S.
Preedy VR, Adachi J, Ueno Y, Ahmed S, Mantle D, Mullatti N, Rajendram R & Peters TJ (2001*b*) *European Journal of Neurology* **8,** 677-687.
Preedy VR, Paice A, Mantle D, Dhillon AS, Palmer TN & Peters TJ (2001*c*) *Drug and Alcohol Dependency* **63**, 199-205.
Preedy VR, Peters TJ, Adachi J, Ahmed S, Mantle D, Niemela O, Parkkila S & Worrall S (2001*d*) Alcohol in Health and Disease, pp. 243-259. New York: Marcel Dekker Inc.

The effects of chronic alcohol exposure on the mRNA and protein levels of skeletal muscle heat-shock proteins. By T. NAKAHARA[1], R HUNTER[2], A. MCARDLE[3], C.S. BROOME[3], K. HASHIMOTO[4], M. HIRANO[4], C.R. MARTIN[5] and V.R. PREEDY[2], [1]*Kyushu University Ropponmatsu, Fukuoka, Japan,* [2]*King's College London, London, WC2R 2LS* [3]*The University of Liverpool, Liverpool, L69 3BX* [4]*Hizen National Mental Hospital, Saga Japan and* [5]*University of York, York, YO10 5DD*

Alcoholic myopathy is characterised by loss of skeletal muscle protein (including the main force-generating proteins such as myosin) and impaired protein synthesis (Preedy *et al.* 2001*a,b,c,d*). However, the intervening steps between alcohol ingestion and myopathic lesions are unknown. A number of studies have proposed that heat-shock proteins may increase either as a stress response or as a compensatory reaction in response to alcohol. Although many of the aforementioned studies have been carried out *in vitro*, there are no previous studies on skeletal muscle exposed to alcohol *in vivo*. To address this, we proposed that similar increases occur in heat-shock proteins in skeletal muscle of myopathic rats.

We measured HSP mRNA and protein in male Wistar rats (0.1 kg BW) fed alcohol (as 35% of total calories) for 6 weeks. Comparative analysis was made on rats pair-fed identical amounts of the same diet in which ethanol was replaced by isocaloric glucose. At the end of the study period, hind limb muscle was analysed for HSPs 27, 60, 70 and 90 by reverse transcription–polymerase chain reaction (RT-PCR) with an endogenous internal standard, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH). HSP protein levels were measured by SDS-PAGE and Western blotting.



Heat-shock protein mRNA levels in skeletal muscle of ethanol-fed rats

HSP mRNA levels were measured by RT-PCR and expressed relative to GAPDH. Data are expressed as mean±SEM (n=8–10). P values pertain to differences between controls and ethanol groups.

The results showed that at the end of 6 weeks, the mean levels of all HSPs mRNA declined with chronic alcohol ingestion, although statistical significance was only obtained for HSPs 27 (P<0.01) and 70 (P<0.05). This pattern of changes was reflected in HSP protein content, as assessed by Western blotting, although statistical significance was not achieved.

We conclude that in the chronic ethanol-fed rat, muscle HSPs do not increase, demonstrating the need for caution when translating *in vitro* events to the *in vivo* situation. Reduced HSP proteins may impair post-translational chaperoning of newly synthesised skeletal muscle proteins, which may possibly contribute to alcoholic myopathy.

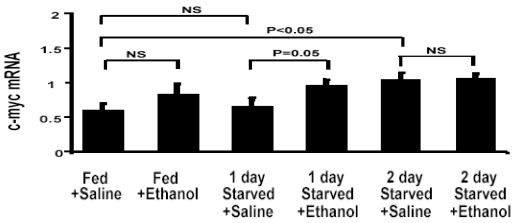
Preedy VR, Adachi J, Peters TJ, Worrall S, Parkkila S, Niemela O, Asano M, Ueno Y, Takeda K, Yamauchi M, Sakamoto K, Takagi M, Nakajima H & Toda (2001*a*) *Alcoholism, Clinical and Experimental Research* **25**, 54S-59S.
Preedy VR, Adachi J, Ueno Y, Ahmed S, Mantle D, Mullatti N, Rajendram R & Peters TJ (2001*b*) *European Journal of Neurology* **8,** 677-687.
Preedy VR, Paice A, Mantle D, Dhillon AS, Palmer TN & Peters TJ (2001*c*) *Drug and Alcohol Dependency* **63**, 199-205.
Preedy VR, Peters TJ, Adachi J, Ahmed S, Mantle D, Niemela O, Parkkila S & Worrall S (2001*d*) Alcohol in Health and Disease, pp. 243-259. New York: Marcel Dekker Inc.

The effects of acute alcohol exposure on skeletal muscle *c-myc*, *p53* and *Bcl-2* mRNA and the modulating influence of starvation. By T. NAKAHARA[1], R. HUNTER[2], K. HASHIMOTO[3], M. HIRANO[3], M. KOLL[2], C. R. MARTIN[4] and V.R. PREEDY[2], [1]*Kyushu University Ropponmatsu, Fukuoka, Japan,* [2]*King's College London, London SE1 9NN,* [3]*Hizen National Mental Hospital, Saga Japan and* [4]*University of York, York YO10 5DD*

Skeletal muscle damage is a frequent manifestation of alcohol misuse (Preedy *et al.* 2001*a,b,c,d*). Increased expression of proto-oncogenes (i.e. *c-myc*) may be a causative process in the development of alcohol-induced muscle disease, possibly via activating apoptosis or an uncharacterised transcriptional pathway. There is also some evidence to suggest that starvation exacerbates the deleterious effects of alcohol on muscle.

We hypothesised that (1) increases in *c-myc* mRNA levels occur in muscle exposed to alcohol; (2) prior starvation will cause further increases in *c-myc* mRNA expression in response to ethanol; and (3) other genes involved in apoptosis (i.e. *p53* and *Bcl-2*) would also be affected by alcohol. To test this, we measured *c-myc*, *p53* and *Bcl-2* mRNA levels in skeletal muscle of fed or starved (1 or 2 d) male Wistar rats (approx. 0.15 kg) dosed acutely (2.5 h) with ethanol (75 mmol/kg body weight). Control rats were treated identically with saline (0.15 mol/l NaCl). At the end of the study, rats were killed and *c-myc*, *p53* and *Bcl-2* mRNA was analysed in skeletal muscle by reverse transcription–polymerase chain reaction (RT-PCR) with an endogenous internal standard, GAPDH.

The data showed that (1) in normally fed rats dosed for 2.5 h, ethanol had no effect on muscle *c-myc* mRNA; (2) starvation *per se* increased *c-myc* mRNA levels and at 1 d potentiated the acute effects of ethanol, indicative of a sensitisation response; and (3) other mRNAs were insensitive to alcohol and starvation. The only effect seen with *p53* mRNA levels was a decrease in muscle of rats starved for 1 d compared with fed rats. There was no significant effect on *Bcl-2* mRNA in any of the experimental conditions.



c-myc mRNA levels in skeletal muscle of fed and starved rats dosed with ethanol.

c-myc mRNA levels were measured by RT-PCR and expressed relative to GAPDH. Data are displayed as mean±SEM (n=8–10).

The changes in *c-myc* mRNA in starved rats dosed with alcohol may well represent a pre-apoptotic effect or even a non-specific cellular stress response to alcohol.

Preedy VR, Adachi J, Peters TJ, Worrall S, Parkkila S, Niemela O, Asano M, Ueno Y, Takeda K, Yamauchi M, Sakamoto K, Takagi M, Nakajima H & Toda (2001*a*) *Alcoholism, Clinical and Experimental Research* **25**, 54S-59S.
Preedy VR, Adachi J, Ueno Y, Ahmed S, Mantle D, Mullatti N, Rajendram R & Peters TJ (2001*b*) *European Journal of Neurology* **8,** 677-687.
Preedy VR, Paice A, Mantle D, Dhillon AS, Palmer TN & Peters TJ (2001*c*) *Drug and Alcohol Dependency* **63**, 199-205.
Preedy VR, Peters TJ, Adachi J, Ahmed S, Mantle D, Niemela O, Parkkila S & Worrall S (2001*d*) Alcohol in Health and Disease, pp. 243-259. New York: Marcel Dekker Inc.

An investigation of the effects of black tea (*Camellia sinensis* (L)) on postprandial glycaemia in healthy humans. By J. BRYANS[1], P.A. JUDD[2] and P.R. ELLIS[1], [1]*Nutrition, Food and Health Research Centre, King's College London, London SE1 9NN and* [2]*Lancs. PG School of Medicine and Health, Preston PR1 2HE*

Tea polyphenols may have a beneficial role in attenuating postprandial glycaemia. Isolated tea polyphenols have been shown *in vitro* to inhibit the enzymes involved in carbohydrate digestion, inhibit the sodium-glucose co-transporter and enhance insulin secretion. It is not known whether these effects are exerted in humans consuming a normal tea drink. A randomised cross-over study was conducted to investigate whether an instant black tea drink could reduce the postprandial response to oral ingestion of 75 g of glucose in healthy subjects.

Eighteen healthy volunteers (aged 24–45 years, mean BMI 23.9 kg/m^2) participated in the study. The test beverages were: a control drink (water, 75 g glucose), a caffeine drink (water, 0.052 g caffeine, 75 g glucose) and an instant black tea drink (water, 1 g tea, 75 g glucose). The total volume of each drink was 250 ml. The caffeine drink was included to control for any potential confounding effects of caffeine in the tea beverage. Subjects consumed each drink following an overnight fast on three separate study days. Serial blood samples were taken by repeated venepuncture after fasting (0 min) and then at 30-min intervals up to 2.5 h, and plasma was analysed for glucose and insulin concentrations.

Instant black tea significantly reduced plasma glucose and increased plasma insulin concentrations in the latter stages of the postprandial curve compared with the control and caffeine drinks. Repeated measures ANOVA demonstrated a significant treatment × time effect for both glucose (P<0.05) and insulin (P<0.01). Contrast testing with Bonferroni adjustment showed significant differences in glucose concentrations between the tea drink and the control and caffeine drinks at 120 min (see Table). Significant increases in insulin were found after tea compared with the control and caffeine drinks at 90 min (P<0.01) and at 150 min (P<0.05) for caffeine only. Significant increases in insulin concentration were found after caffeine compared with tea at 30 min (P<0.01) and 120 min (P<0.05).

Incremental† plasma glucose concentrations (mean ± SEM, mmol/l) for all drinks

Drink	30 min	60 min	90 min	120 min	150 min
Control	3.48 ± 0.45	1.60 ± 0.18	0.97 ± 0.21	0.51 ± 0.22	−0.42 ± 0.31
Caffeine	3.14 ± 0.29	1.75 ± 0.27	1.03± 0.19	0.32 ± 0.22	−0.40 ± 0.23
1 g tea	3.42 ± 0.41	1.65 ± 0.26	0.66 ± 0.10	−0.78 ± 0.23*	−0.17 ± 0.18

* Significant difference (P<0.01) between the tea drink and the control and caffeine drinks.
† Incremental = mean test value – mean fasting value

The reduction in plasma glucose and increases in plasma insulin concentrations observed in this study occurred in the latter stages of the postprandial curve, possibly reflecting the time required for the polyphenols or their metabolites to become bioavailable. The findings suggest that instant black tea reduces postprandial glucose, possibly via an insulinotropic effect.

This study was funded by a grant from ISFE, Switzerland. The tea was a gift from Brooke Bond UK.

Hii CS & Howell SL (1985) *Journal of Endocrinology* **107**(1), 1–8.
Matsumoto N, Ishigaki F, Ishigaki A, Iwashina H & Hara Y (1993) *Bioscience Biotechnology Biochemistry* **57**(4), 525–527.

Influence of soya isoflavone consumption on plasma total antioxidant capacity in young women. By E.J. LYNN and H. WISEMAN, *Nutrition, Food and Health Research Centre, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN*

Isoflavones (IF) consumed in soya have been shown to decrease plasma concentrations of an F_2-isoprostane 8-*epi*-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$), a biomarker of lipid peroxidation (Wiseman *et al.* 2000). A randomized crossover study was used to compare diets enriched with soya foods with diets supplemented with IF capsules in twenty-two subjects, for 2 weeks with a 4 week washout. Soya drinks and other soya products were used, providing approx 60 mg/d IF (approx 38 mg genistein and 22 mg daidzein equivalents). An IF-containing soya supplement was used, providing 60 mg/d IF (40 mg genistein, 16 mg daidzein and 4 mg glycitein equivalents). Following approval by the Research Ethics Committee at King's College London, twenty-three young healthy women (mean age 22.9 years, SD 3.3 years) were recruited from the student population at King's College London, and gave written informed consent. Twenty-two subjects completed both treatment periods. Blood samples were collected at baseline and at the end of each treatment period. Plasma total antioxidant capacity has been measured. Plasma concentrations of 8-epi-$PGF_{2\alpha}$ and vitamin E, and also white cell oxidative DNA damage, will be determined.

Measurement of plasma total antioxidant capacity was made using the ferric reducing/antioxidant power (FRAP) assay, according to the method of Benzie & Strain (1996). Heparinized plasma samples were stored at −70°C for between 52 and 70 d prior to analysis, and three replicate plasma samples were analysed.

	Age	**Baseline**		**IF in soya food**		**IF in soya supplement**	
	(years)	FRAP value (μmol/l)		FRAP value (μmol/l)		FRAP value (μmol/l)	
Tertile	Mean	Mean	SD	Mean	SD	Mean	SD
Lower (n=7)	24.0	717*	72	747‡	74	759	96
Middle (n=7)	21.2	847*	19	834	54	849	61
Upper (n=8)	24.0	921	41	944	106	950	70

*P<0.001 compared with upper tertile (independent samples t-test); ‡P<0.05 change from baseline (paired t-test).

The data were stratified into tertiles on the basis of baseline antioxidant status (FRAP values). There was a significant difference (P<0.001) between tertiles. A significant increase in FRAP value was observed in the lower tertile following the soya food treatment (P<0.05) compared to baseline. Following treatment with the soya supplement, although there was an increase in FRAP value compared with baseline, it failed to reach significance. No significant difference between the treatments compared to baseline was observed in either the middle or the upper tertiles. There was no significant difference in FRAP values between treatments compared to baseline, for the unstratified data.

These results suggest that only subjects with a low baseline antioxidant status (FRAP values in the lower tertile), appear to benefit from the antioxidant properties of soya IF. Furthermore, consumption of IF within the food matrix in soya foods appears to be a more effective antioxidant treatment (Wiseman *et al.* 2000), in terms of increased plasma total antioxidant capacity, than consumption of IF extracted from the food matrix in soya supplements.

Benzie IFF & Strain JJ (1996) *Analytical Biochemistry* **239**, 70–76.
Wiseman H, O'Reilly JD, Adlercreutz H, Mallet AI, Bowey E.A, Rowland IR & Sanders TAB (2000) *American Journal of Clinical Nutrition* **72**, 395–400.

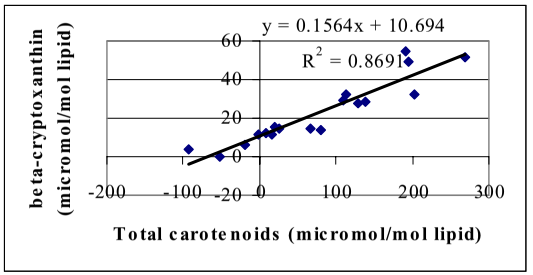
A linear relationship exists between the absorption of xanthophylls by individuals and their absorption of carotenes following enhanced consumption of fruits and vegetables. By M.H. GORDON, A.F. WALKER and W.G. ROBERTS, *Hugh Sinclair Unit of Human Nutrition, The University of Reading, PO Box 226, Whiteknights, Reading RG6 6AP*

Fruits and vegetables contain a wide range of nutrients and phytochemicals including several carotenoids. Carotenoids are incorporated into mixed lipid micelles and absorbed by the mucosa of the small intestine via a passive diffusion-driven process. There is known to be a wide variation in the bioavailability of carotenes (carotenoids with a hydrocarbon structure) between individuals. However, transport and clearance of carotenes and xanthophylls (carotenoids containing polar groups) differ in several key aspects. Carotenes are found primarily in the core of LDL, while xanthophylls are more evenly distributed between LDL and HDL, and are believed to be surface components (Parker, 1997).

Analysis of plasma carotenoids was included in a prospective study to investigate the effects of five portions of fruits and vegetables per day. Eighteen smokers were recruited, and their diets were supplemented with 4 × 1 g fish-oil capsules (0.83 g of docosahexaenoic acid and 1.2 g of eicosapentaenoic acid per day) for 9 weeks. After 3 weeks, they consumed an additional five portions of fruits and vegetables per day, and then they returned to their normal diet for the last 3 weeks of the study. Fruits and vegetables supplied comprised orange juice peach, grapes, apple, strawberries, clementines, plums, continental leaf salad, onions, tomato soup, mixed frozen vegetables and red pepper.

Fasting blood samples were taken at the ends of weeks 0, 3, 6 and 9. Plasma levels of the carotenes β-carotene, α-carotene and lycopene and the xanthophylls lutein, zeaxanthin and β-cryptoxanthin were analysed by HPLC.

When fruit and vegetable intake was increased between weeks 3 and 6, the small increase in the mean plasma concentration observed for lycopene did not reach statistical significance (+23.8 μmol/mol lipid; P=0.10) but paired t-tests showed significant increases in the mean plasma concentrations of lutein (+6.5 μmol/mol lipid; P=0.007) and β-cryptoxanthin (+22.9 μmol/mol lipid; P<0.001). Wilcoxon signed rank tests also identified significant increases in plasma concentrations of α-carotene (+6.2 μmol/mol lipid; P=0.006) and β-carotene (+18.4 μmol/mol lipid; P=0.047).



Increase in fasting plasma levels of β-cryptoxanthin plotted against the total increase in plasma total carotenoid levels for eighteen individuals following enhanced consumption of fruits and vegetables.

When the increase in the plasma concentration of each carotenoid was plotted against the increase in the total plasma carotenoid concentration for each individual, reasonable linear correlations were found as shown in the Figure, with R^2 values for β-carotene, α-carotene, lycopene, lutein + zeaxanthin and β-cryptoxanthin corresponding to 0.71, 0.63, 0.78, 0.63 and 0.87. This suggests that individuals who absorb carotenes well also absorb xanthophylls well. We conclude that differences in the pharmacokinetics of absorption and clearance of carotenes and xanthophylls did not cause differential effects in the variation of fasting plasma carotenoid levels between individuals.

Parker RS (1997) *European Journal of Clinical Nutrition* **51**, S86–S90.

Variation in dietary supplement use in perimenopausal and early postmenopausal Scottish women. By H.M. MACDONALD[1,3], S.A. NEW[2] and D.M. REID[1,3], [1]*Osteoporosis Research Unit, University of Aberdeen, Woolmanhill Hospital, Aberdeen AB25 1LD,* [2]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH and* [3]*Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen AB25 2ZD*

Dietary supplement use may make a major contribution to individual diets in terms of total nutrient intake and could potentially influence disease outcome. Little is known about whether dietary supplements are taken continuously over a period of years and whether they form an integral part of diet in the long-term. However, many epidemiological studies routinely add nutrients from dietary supplements to obtain total dietary intake. For our research we were particularly interested in nutrients that might influence bone health: calcium, vitamin D, vitamin C, which have been positively associated with markers of bone health, and retinol which it has been suggested may be a risk factor for osteoporosis (Michaelsson *et al.* 2003).

Subjects were selected from the Aberdeen Prospective Osteoporosis Screening Study (APOSS) of over 5000 women who had a bone mineral density scan in 1990–3 and again in 1997–2000. A subset of 1064 women had their diets assessed in 1993 by food frequency questionnaire (FFQ) (validated using 7 d weighed intake records and markers of antioxidant status) and the diets of 898 of these women were reassessed at the second visit (85% response rate). At both visits details were obtained on dietary supplement use.

Overall, 37% women took dietary supplements at the second visit compared with 25% at the baseline visit. The majority took cod liver oil (the latter providing vitamin D and retinol) or evening primrose oil (containing vitamin E). As shown in the Table, there was a wide variation in the quantities taken for all supplements. The mean intake of nutrients from supplement use exceeded the RNI for vitamin C (3.5 to 4-fold) and retinol (1.5 fold) and was equivalent to 46-55% of the RNI of calcium. Although overall there was an increase in the number of women (between 60 and 100%) taking each supplement, most of this increase came from new users, since fewer than half the women taking supplements at baseline were still taking them at the second visit.

Supplement	Baseline visit					Follow-up visit					Both visits	
	n	%	Mean	SD	Range	*n*	%	Mean	SD	Range	*n*	%
Calcium (mg) RNI 700 mg	43	5	325	277	20–900	67	8	383	259	20–1000	18	2
Vitamin C (mg) RNI 40 mg	80	9	141	293	10–2000	133	15	162	224	25–1060	39	4
Vitamin D (μg) RNI 0/10 μg	120	13	4.4	2.2	2.5–15	211	24	6.0	2.9	1.25–22.5	61	7
Vitamin E (mg)	129	14	12.2	23.1	0.33–167	193	22	30.9	63.3	0.33–310	57	6
Retinol (μg) RNI 600 μg	104	11	886	323	400–2400	206	23	944	346	800–2400	53	6

Although dietary supplements can add to the total nutrient intake of the diet, usage may be sporadic and their contribution to the diet may be less than that assumed by measurement on a single occasion. Further work is required to establish the importance of dietary supplements to the diet. In particular, with the large number of women taking supplements at the second visit, it is important to establish whether sporadic, short-term use of supplements has any detrimental effect on bone health, and whether, as in the case of retinol, high intakes consumed even on an occasional basis pose a problem for the future.

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Effect of prebiotics on mineral bioavailability in rats. By L.M. YBARRA[1], N.M.B. COSTA[2], P.R. CECON[3] AND C.L.L.F. FERREIRA[1], [1]*Departamento de Tecnologia de Alimentos,* [2]*Departamento de Nutrição e Saúde and* [3]*Departamento de Informática, Universidade Federal de Viçosa, Viçosa, MG 36571-000, Brazil*

There is a growing interest in functional foods, especially concerning vitamins and minerals. Calcium-fortified foods may increase calcium ingestion by over 200% of the dietary reference intakes, which may reduce iron absorption (Whiting & Wood, 1997). The use of prebiotics could be an alternative choice to improve mineral bioavailability (Takahara *et al*. 2000; Scholz-Ahrens *et al*. 2002) regardless of its intake. The aim of this study was to evaluate the effects of fructooligosaccharide (FOS; (Raftilose® P95) on iron, calcium and magnesium bioavailability, as well as the interaction of calcium with iron and magnesium bioavailability in rats. Seventy weaning male Wistar rats were divided into seven experimental groups (n=10) in a 2 × 3 + 1 factorial design. The treatments consisted of a low-iron control diet (AIN-93G-based diet with 14 mg iron/kg diet, corresponding to 58% of the rat's nutritional requirements), and FOS diet (control + 1% Raftilose® P95). Each of these two treatments provided three levels of calcium (2.5, 5.0 and 10.0 g as $CaC0_3$/kg diet). Another group was kept on an AIN-93G control diet (Reeves *et al*. 1993). Test diets were administrated for a period of 28 d. Blood samples were collected by tail bleeding at the end of experiment for haemoglobin and haematocrit analysis. The animals were killed under CO_2, and their right femurs were removed for calcium and magnesium determinations. The statistical analysis was carried out using SAEG software (UFV, 1993), by Student t-tests and regression analysis. FOS supplementation showed different effects only on the high-calcium (10 g/kg) diets. At this level of calcium, FOS intake resulted in an increased calcium (p<0.01) and magnesium (p<0.02) absorption, and showed no effect on iron bioavailability (p>0.05) (Table 1). FOS supplementation did not prevent anaemia development. Prebiotics may effect mineral absorption through effects of microbial fermentation in the intestines, leading to a reduction in pH, which will increase cation concentrations stimulating passive absorption (Ohta *et al*., 1995); increase in absorptive surface due to gut proliferation resulting from increased short chain fatty acid concentrations (Tooping, 1996); and formation of low molecular weight compounds, such as calcium acetate and lactate, which are more easily absorbed by the ionised cell membranes (Trinidad *et al.*, 1993). The levels of dietary calcium had no effect (p>0.05) on bone calcium levels, compared with the control diet with the recommended calcium level (AIN-93G). The increase in dietary calcium content had negative effects on haemoglobin, haematocrit and on bone magnesium (p<0.01) levels. The negative effect of calcium on magnesium absorption was partially counterbalanced by FOS incorporation (10 g/kg) in the diet. These results indicated a negative effect of high calcium intake on iron and magnesium absorption. FOS supplementation (10 g/kg) had no effect on anaemia development in animals fed iron-deficient diets, but increased magnesium and calcium absorption. FOS was most effective when dietary calcium was high.

Table 1 – Effect of FOS' effect on the haemoglobin (g/dL), haematocrit (%), calcium (mg/g bone) and magnesium (mg/g bone) in rats feed diets with different calcium levels.

Diet/	Haemoglobin (g/dL)			Haematocrit (%)			mg magnesium/g bone			mg calcium/ g bone		
Ca level (g/kg)	2.5	5.0	10.0	2.5	5.0	10.0	2.5	5.0	10.0	2.5	5.0	10.0
Control	8.86^{ns}	8.22^{ns}	6.15^{ns}	37.40^{ns}	37.00^{ns}	31.00^{ns}	1.65^{ns}	1.51^{ns}	0.93***	83.62^{ns}	90.85^{ns}	83.79**
FOS	8.41	8.43	5.77	36.20	34.70	27.10	1.62	1.48	1.07	84.63	88.93	92.79

Control = AIN-93G diet with 14 mg iron/kg; FOS = Control + 1%Raftilose® P95
^{ns} not significant at 5% using Student t-test, for values in same column.
** significant at 1% through t-test.
*** significant at 2% through t- test.

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Magnesium bioavailability is not affected by consumption of an infant formula supplemented with long-chain polyunsaturated fatty acids in rats. By M.P. VAQUERO[1], M. VELDHUIZEN[2] and B. SARRIA[1], [1]*Department of Metabolism and Nutrition, Instituto del Frío CSIC, C/José Antonio Novais 10 and* [2]*Department of Biotechnology, Escuela Técnica Superior de Ingenieros Agrónomos, Politécnica University, 28040 Madrid, Spain*

Beneficial effects of supplementing infant formulas with long-chain polyunsaturated fatty acids (LCPUFA) of the series n-3 and n-6, on visual and neural development in preterm infants have been reported whereas in term infants neutral or positive outcomes have been described (Gibson & Makrides, 1999). It has been hypothesized that the supply of LCPUFA may be most critical during the first postnatal months (Birch *et al*, 2002). However, there is controversy as far as other effects are concerned, such as incorporating LCPUFA may affect mineral bioavailability. Iron absorption was found to be unaffected using a rat model, haemoglobin did not change, but the erythrocytic concentrations of iron (Vaquero *et al.* 2001*a*), copper and zinc increased (Vaquero *et al*. 2001*b*). Neonates are susceptible to hyper- and hypomagnesmia. Magnesium absorption was not affected by the consumption of diets rich in linoleic acid although thermal treated fats appear to alter magnesium bioavailibility (Pérez-Granados *et al*. 1999). However, data concerning the possible influence of LCPUFA on magnesium bioavailability in infant formulas are lacking.

Therefore, the aim of this study was to discover the possible effects of LCPUFA supplementation of an infant formula on magnesium bioavailability using the balance technique in growing rats. Three isoenergetic diets were prepared, with two of them containing the unsupplemented (F), and supplemented (SF) infant formula as the fat source (150 g/kg), and the third being a control diet based on the American Institute of Nutrition recommendations (AIN-93) except for the fat level (C). The diets were given to weaning rats for 28 d. Food intake and body weight were monitored, and during the last week faeces and urine were collected to calculate magnesium absorption and retention.

Food intake and body weight evolution did not show significant differences between F and SF, but in both groups were found to be significantly lower (P<0.05) compared to C. During the balance week, mean food intake was g /d: F, 13.9 (SD 1.8); SF, 12.9 (SD 1.3); C, 15.8 (SD 2.06) and magnesium intake was significantly lower in F and SF compared to C. However, magnesium absorption (A) and retention (R) were similar in the three groups. These parameters were not significantly affected by the LCPUFA supplementation and nor were the absorption and retention efficiencies.

	Infant formula F (n 12)		**Infant formula SF** (n 10)		**Control** (n 12)		ANOVA
	Mean	SD	Mean	SD	Mean	SD	P
Ingested Mg (mg)	6.38[a]	0.84	5.98[a]	0.61	7.34	0.96	0.001
Absorbed Mg (mg)	5.48	0.91	5.15	0.61	4.89	0.63	NS
Urinary Mg (mg/d)	2.26	1.09	1.68	0.80	1.51	0.74	0.058
Absorbed/ingested (%)	87.1[a]	5.6	86.0[a]	3.3	67.0	7.1	0.000
Retained (mg/d)	3.21	0.96	3.47	0.73	3.38	0.87	NS
Retained/absorbed (%)	50.3	15.5	58.3	12.4	46.0	10.2	NS

[a] Significantly different from Control (Bonferroni test).

These results indicate that consumption of a diet containing an infant formula supplemented with LCPUFA compared to the unsupplemented formula does not affect magnesium bioavailability in growing rats.

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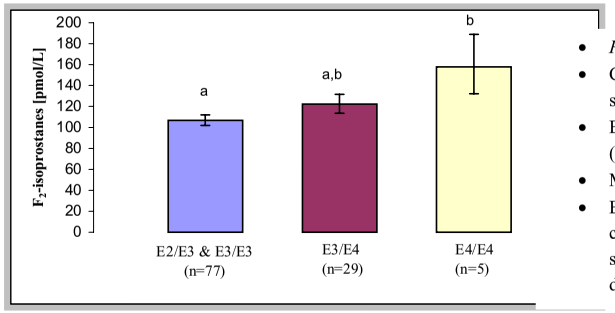
Apolipoprotein E genotype as a determinant of plasma F_2-isoprostane levels. By A.M. MINIHANE[1], Y. HU[2], M. DIETRICH[2], G.H. RIMBACH[1], J.D. MARROW[3], E. MATTHEWS[1], G. KEYOUMU[1], L. PACKER[4] and G. BLOCK[2], [1]*School of Food Biosciences, University of Reading, Reading, RG2 6AP,* [2]*University of California, Berkeley, CA, USA,* [3]*School of Medicine, Vanderbilt University, Nashville, TN, USA and* [4]*School of Pharmacy, University of Southern California, Los Angeles, CA, USA*

Apolipoprotein E is a protein integrally involved in lipid transport and the clearance of lipoproteins form the circulation. It is a polymorphic protein with three common alleles, apoE2, apoE3 and apoE4.

Population studies have demonstrated that apoE4 carriers (25–27% of the UK population) are at higher risk of developing diseases characterised by oxidative damage such as coronary heart disease (CHD) and Alzheimers Disease (AD). This increased risk has traditionally been attributed to the higher circulating cholesterol levels in this group. However, a recent study observed a higher incidence of CHD outcomes in apoE4 carriers relative to non-carriers even after correcting for plasma lipids, with the authors suggesting that a lower antioxidant potential of the apoE4 protein may be partly responsible (Humphries *et al*. 2001).

In the current study the association between apoE genotype and lipid peroxidation, as assessed by plasma F_2-isoprostane, was determined. A sample of 111 healthy adult smokers (sixty-three female, forty-eight male; seventy-two white, thirty-nine black; mean age 45 (SD 12) years), recruited for an intervention trial, were included in the analysis. ApoE genotype was determined by PCR amplification of the polymorphic region of DNA, followed by restriction enzyme digestion and separation of the fragments by PAGE. Plasma F_2-isoprostanes were measured by gas chromatography/mass spectrometry. The inter-group differences were assessed by ANOVA with adjustment for sex, race, age and body mass index (BMI). Subjects with an E2/E4 genotype (*n* 2) were excluded and non-E4 individuals (E2/E3, E3/E3) (*n* 77) pooled for the analysis. F_2-isoprostane levels were 19% higher in the E4 carriers (E3/E4, E4/E4) (*n* 34) relative to the combined E2/E3 plus E3/E3 group (P=0.04).



- P=0.04
- Controlled for age, sex, race and sex
- Bars represent mean (SEM)
- Model r^2=0.40
- Bars without a common letter are significantly different (P<0.05)

When E3/E4 and E4/E4 were analysed as separate group, as demonstrated in the figure above, F_2-isoprostane levels were 1.14 and 1.48 times higher in the heterozygous and homozygous groups respectively (P=0.04).

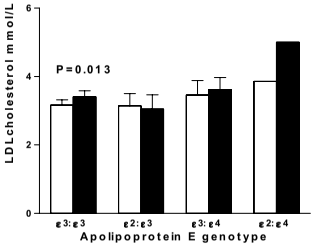
The current study suggests that in smokers apoE4 carriers have higher levels of lipid peroxidation as assessed by plasma F_2-isoprostane levels. Further work examining the impact of apoE genotype on a range of markers of oxidative stress in smokers and non-smokers is merited.

Humphries SE, Talmud PJ, Hawe E, Bolla M, Day IN & Miller GJ (2001) *Lancet* **358**, 115–119.

The influence of apolipoprotein E genotype on LDL cholesterol in middle-aged men and women, following supplementation with a low dose of docosahexaenoic acid. By H.E. THEOBALD[1], S.E. HUMPHRIES[2] and T.A.B. SANDERS[1], [1]*Nutrition, Food and Health Research Centre, Franklin-Wilkins Building, King's College London, London SE1 9NN,* [2]*Centre for the Genetics of Cardiovascular Disease, British Heart Foundation Laboratories, Rayne Building, Royal Free and University College London Medical School, University Street, London WC1E 6JJ*

We previously reported that an intake of ~0.7 g docosahexaenoic acid (DHA) supplied as algal triacylglycerol (DHASCO), compared with placebo resulted in a significant increase in LDL cholesterol (Theobald & Sanders, 2001). Minihane *et al.* (2000) in a *post hoc* analysis of subjects with the atherogenic lipoprotein phenotype given fish oil, suggested that the LDL cholesterol elevating effects were confined to carriers of the *ε4* allele. We have conducted further analyses of the response to DHA with respect to apolipoprotein E genotype. The subjects were thirty-eight middle-aged, healthy, mildly hyperlipidaemic men and women. The study had a cross-over design with each treatment period lasting 3 months separated by a 4-month wash out period.

Apolipoprotein E genotype was determined using the 'salting out' method following isolation of genomic DNA from white blood cells (Bolla *et al.* 1999). Of the thirty-eight subjects who completed the study, twenty-three were homozygous for the *ε3* allele, nine heterozygous for the *ε4* allele and five subjects were heterozygous for the *ε2* allele (one of whom was heterozygous for *ε2*:*ε4*). The frequency of the *ε4* and *ε2* alleles were not significantly different from that reported from other UK studies (Humphries *et al*. 2001). Comparisons of the responses according to genotype were made using analysis of covariance with adjustments for age, BMI and gender. The figure shows the adjusted response of LDL cholesterol to DHA treatment according to apolipoprotein E genotype (mean values, error bars show SEM, open bars = pre-treatment, filled bars = post-DHA treatment). LDL cholesterol increased by 5.9% (P=0.017) in subjects homozygous for the *ε3* allele and 5.7% (P=0.13) in subjects heterozygous for the *ε4* allele. There were four individuals with the genotype *ε3*:*ε2*, which was too few for meaningful statistical analysis.



The results of the present study show that in healthy but moderately hypercholesterolaemic men and women, 0.68 g of DHA increases LDL cholesterol similarly both in subjects homozygous for the *ε3* allele and in subjects heterozygous for the *ε4* allele. Further research is needed to investigate the mechanism involved in the LDL-elevating effect of algae-source DHA.

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Differential effects of *n*-3 polyunsaturated fatty acids on lymphocyte activation. By M.D. MESA, S. KEW, S. TRICON, R. BUCKLEY, A.M. MINIHANE and P. YAQOOB, *School of Food Biosciences, The University of Reading, Whiteknights, Reading RG6 6AP*

Fish oil, rich in *n*-3 polyunsaturated fatty acids (PUFA), has been shown to suppress a number of parameters of immune function, including *ex vivo* lymphocyte proliferation, cytotoxic T lymphocyte activity, natural killer cell activity and the production of cytokines in laboratory animals. A number of human studies also suggest that *n*-3 PUFA have immunomodulatory actions. However, the majority of these studies have examined the combined effects of the long-chain *n*-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). At present, it is not clear whether the immunomodulatory effects of fish oil are due to EPA or DHA or a combined effect of these *n*-3 PUFA. The aim of the current study was to determine the effects of supplementation of healthy volunteers with either EPA or DHA on the expression of CD69, an early lymphocyte activation marker, by peripheral blood mononuclear cells (PBMC).

In a placebo-controlled, double-blind parallel study, forty-two healthy subjects aged 24–66 years, were randomised to supplementation with either placebo (olive oil), EPA (4.7 g/d) or DHA (4.9 g/d) for 4 weeks. Total fat intake from capsules was 9 g/d. Blood samples were taken pre- and post-supplementation, blood was diluted 1:1 with culture medium and then cultured for 24 h in the presence or absence of 6 μg/ml concanavalin A (Con A). For the determination of CD69 expression on lymphocytes, the stimulated, diluted whole blood (200 μL) was incubated with fluorescent-labelled anti-CD69 and anti-CD3 monoclonal antibodies for 30 min at 4°C, erythrocytes were lysed, leukocytes fixed, and the DNA stained according to the manufacturer's instructions. Cell preparations were analysed by flow cytometry. Lymphocytes were identified as being CD3-positive and both the percentage of lymphocytes expressing CD69 (percentage positive) and the median fluorescence intensity (MFI; related to the number of CD69 molecules expressed per T lymphocyte) were determined.

		% CD69 positive T lymphocytes				Expression of CD69 (MFI)			
		−Con A		+Con A		−Con A		+Con A	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Placebo	Pre-	1.7	0.2	22.8	1.8	306	25	1019	49
	Post-	1.3	0.1	22.8	1.5	305	42	1059	65
EPA	Pre-	1.6	0.2	24.2	1.9	329	58	1192	68
	Post-	1.7	0.2	28.4	2.3	265	29	1122	53
DHA	Pre-	1.3	0.1	23.2	2.8	301	37	1162	78
	Post-	1.5	0.2	25.0	3.9	192*#	7	1034	52

There were no differences in expression of CD69, either as percentage of positive cells or as MFI, between the treatment groups at baseline (one-way ANOVA; see Table). At end of supplementation, unstimulated T lymphocytes from subjects who had been supplemented with DHA demonstrated a significantly lower level of expression of CD69 (MFI) compared with the placebo group (*one-way ANOVA and *post-hoc t*-test with Bonferroni correction, $P<0.05$). The level of expression of CD69 by unstimulated T lymphocytes in subjects from the DHA group was also significantly lower than at baseline (#paired *t*-test, $P<0.01$). There was a trend towards a decrease in CD69 MFI for unstimulated T lymphocytes in the EPA group, but this was not statistically significant. The change from baseline (pre-supplementation) of CD69 expression (MFI) on T lymphocytes stimulated by Con A (data not shown) was significantly greater in subjects supplemented with DHA than those on placebo (one-way ANOVA and *post-hoc t*-test with Bonferroni correction, $P<0.02$). There was no effect of EPA on change in CD69 expression from baseline. These results suggest that DHA suppresses T lymphocyte activation to a greater degree than EPA and that this effect is apparent even in cells which have not been exposed to mitogenic stimulation.

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Hypotriacylglycerolaemic effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). By R. TURNER, R. BUCKLEY, J.E. LUFF and A.M. MINIHANE, *School of Food Biosciences, University of Reading, Whiteknights, Reading, RG6 6AP*

There is significant evidence to indicate that the consumption of fish oils is cardioprotective in the vast majority of individuals, with the proposed protective mechanisms including an improved blood lipid profile (Kris-Etherton *et al*. 2003). The primary active components are thought to be the two long-chain polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, these fatty acids are traditionally consumed together and only limited published research is available on the differential effects of EPA versus DHA on plasma lipoprotein metabolism.

In the current placebo-controlled parallel study, participants (n=42) consumed either EPA (4.9 g/d), DHA (4.7 g/d), or an olive-oil placebo for a period of 4 weeks. The groups were randomised at baseline for age, body mass index (BMI), gender and total cholesterol (TC). Fasting blood samples were collected at baseline and 4 weeks for the analysis of plasma TC, triacylglycerol (TAG), HDL-cholesterol and apolipoprotein levels. The main findings are outlined in the Table.

	EPA (SEM)		**DHA** (SEM)		**Placebo** (SEM)		Signif. between groups†
	0 weeks	4 weeks	0 weeks	4 weeks	0 weeks	4 weeks	
TAG (mM)	1.18 (0.19)	0.92 (0.15)*	1.16 (0.19)	0.72 (0.07)*	1.26 (0.16)	1.17 (0.17)	0.038
TC (mM)	5.52 (0.29)	5.38 (0.26)	5.03 (0.17)	4.75 (0.21)	5.32 (0.26)	5.30 (0.27)	0.383
HDL-C (mM)	1.74 (0.14)	1.78 (0.14)	1.51 (0.21)	1.57 (0.12)	1.40 (0.10)	1.45 (0.11)	0.974
$ApoA_1$ (μg/ml)	1499 (85)	1485 (75)	1446 (79)	1359 (70)	1378 (50)	1421 (59)	0.094
$ApoC_3$ (μg/ml)	130.6 (15.9)	138.3 (12.2)	114.1 (17.4)	107.9 (15.4)	109.5 (8.7)	109.8 (8.1)	0.688
ApoE (μg/ml)	32.6 (1.7)	37.5 (1.8)*	27.6 (2.2)	31.7 (2.4)*	30.5 (1.6)	31.4 (1.7)	0.194
ApoE:TAG ratio	35.6 (5.3)	53.2 (9.2)*	29.4 (3.2)	48.7 (6.1)*	29.1 (4.8)	33.4 (5.6)	0.012
$ApoA_1$:HDL ratio	926 (121)	890 (111)	1003 (81)	917 (78)*	1078 (92)	1052 (82)	0.746

Values are means (SEM). †Analysis by ANOVA and * at 4 weeks indicates a significant ($P<0.05$) within-treatment change.

There was no significant difference between any of the groups at baseline for any of the outcomes of interest. The group mean (SEM) age and BMI were 46.5 (2.0) years and 25.6 (0.5) kg/m^2, respectively. There were twenty-two females and twenty males on the study. No significant effect of the treatments on circulating cholesterol levels were evident. However, a significant effect of treatment on TAG was observed (P=0.038), with a significant difference between the DHA and placebo groups (P=0.026). Within-group reductions of 39% (P=0.003) and 22% (P=0.006) were observed in the DHA and EPA groups, respectively. Although no significant inter-group treatment effects on apolipoprotein concentrations were evident, a significant within-group decrease in the $ApoA_1$:HDL ratio was evident in the DHA group only (P=0.018).

The current study suggests that DHA is more effective than EPA in reducing plasma TAG. However, further studies using more physiological doses of fatty acids are needed.

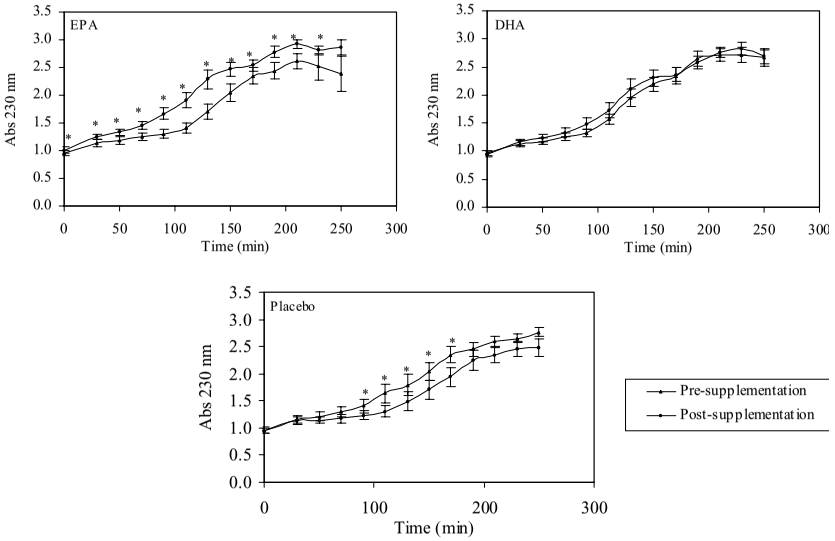
The capsules for the study were supplied by Ocean Nutrition, Nova Scotia, Canada.

Kris-Etherton PM, Harris WS & Appel LJ (2003) *Arteriosclerosis, Thrombosis and Vascular Biology* **23**, e20–e31.

Effects of EPA versus DHA on the oxidizability of low-density lipoprotein. By M.D. MESA, R. BUCKLEY, A.M. MINIHANE and P. YAQOOB, *School of Food Biosciences, The University of Reading, Whiteknights, Reading RG6 6AP*

Evidence suggests that fish oil, rich in the *n*-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), protects against cardiovascular disease. However, paradoxically, enrichment of low-density lipoprotein (LDL) with *n*-3 PUFA is suggested to enhance its susceptibility for oxidation, which may enhance its atherogenicity. It is not clear whether the pro-oxidant effects of fish oil are mediated equally by EPA and DHA. The aim of this study was therefore to determine the separate effects of EPA and DHA on the susceptibility of LDL for oxidation.

In a placebo-controlled, double-blind parallel study, thirty-six healthy subjects aged 24–66 years, were randomized to supplementation with either placebo (olive oil), EPA (4.7 g/d) or DHA (4.9 g/d) for 4 weeks; thirty-five subjects completed the study. Blood samples were taken pre- and post-supplementation. LDL was isolated and 100 µg protein/ml of LDL was oxidized with 25 µM Cu_2SO_4. Rates of oxidation were monitored by measuring the formation of conjugated dienes by spectrophotometry at 230 nm.



Values are shown as mean (± SEM). $P<0.05$.

Supplementation with olive oil decreased the susceptibility of LDL to oxidation (paired *t*-tests; see Figure), which may be due to the antioxidant activities of phenolic compounds within olive oil. Supplementation with EPA significantly increased the susceptibility to oxidation (paired *t*-tests; see Figure). However, DHA had no effect on LDL oxidizability relative to baseline (paired *t*-tests; see Figure). These results suggest that EPA and DHA within LDL are not equally susceptible to oxidation and that EPA is more prone to oxidation than DHA.

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Docosahexaenoic acid lowers blood pressure in middle-aged men and women. By H.E. THEOBALD[1], P.J. CHOWIENCZYK[2] and T.A.B. SANDERS[1], [1]*Nutrition, Food and Health Research Centre, Franklin-Wilkins Building, King's College London, London SE1 9NN,* [2]*Department of Clinical Pharmacology, St. Thomas' Hospital, King's College London, London SE1 7EH*

Cross-sectional studies suggest that the habitual intake of long-chain *n*-3 fatty acids is associated with lower blood pressure (Bonaa *et al*. 1990). Meta-analyses of randomised controlled trials conclude that an intake of ~3 g long-chain *n*-3 fatty acids/d exerts hypotensive effects, and this is more evident in subjects with initially elevated blood pressure (Geleijnse *et al*. 2002). These conclusions were drawn from studies using mixtures of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Few studies have examined the effects of DHA alone and none have examined intakes below 1 g/d, which is closer to the daily intake in cross-sectional studies.

The present study reports the influence of 0.7 g DHA/d on resting heart rate and blood pressure compared with an olive oil placebo. The study was a randomised double blind, placebo-controlled cross-over trial with each treatment period lasting 3 months with a 4-month washout period, conducted in nineteen men and nineteen women aged 40–65 years. Blood pressure (BP) and heart rate were measured with subjects supine and at rest in a temperature-controlled clinical investigation unit (26°C±1°C), using a Dinamap® monitor (Critikon Company LLC, USA). The table shows heart rate and systolic and diastolic blood pressures before and after each treatment phase.

	DHA (*n* 38)				**Placebo** (*n* 38)				*P* value for *F*
	Pre-DHA		Post-DHA		Pre-placebo		Post-placebo		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Systolic BP (mmHg)	116.7	2.3	114.6	1.9	117.8	1.9	116.7	2.3	P=0.166
Diastolic BP (mmHg)	71.3	1.4	69.1*	1.2	72.4	1.1	72.6	1.5	P=0.003
Heart rate (beats/min)	71.6	1.4	70.4	1.2	71.9	1.5	72.4	1.5	P=0.526

*P=0.01 compared placebo with Bonferroni adjustment for multiple comparisons.

Both systolic and diastolic blood pressure fell following the DHA treatment but only the reduction in diastolic BP (3.3 mm Hg, 95% CI −1.3, −5.3), compared with placebo was statistically significant. These results suggest that an intake of DHA within the range provided by the diet has a blood-pressure-lowering effect. *Post hoc* analyses suggested that the reduction in diastolic BP was inversely related to habitual blood pressure. Future studies are needed to confirm these findings using a larger sample size in subjects with mild to moderately elevated blood pressure. Although, the reduction in blood pressure was modest, in population terms this would be expected to have a significant impact on the incidence of stroke and CHD (Law *et al*. 1991).

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Gene expression changes following supplementation with sodium selenite analysed by a custom-made 'selenoprotein' array. By S. VILLETTE[1], S. DILLON[2], F. MCARDLE[2], J.R. ARTHUR[3] and J.E. HESKETH[1], [1]*School of Cell and Molecular Biosciences, University of Newcastle, Newcastle-upon-Tyne NE1 7RU,* [2]*Department of Medicine, University of Liverpool, Liverpool L61 3GA and* [3]*The Rowett Research Institute, Aberdeen AB21 9SB*

Selenium (Se) is essential for a wide range of biochemical processes. There is debate over whether the decrease in selenium intake of the UK population over recent years has implications for health (Rayman, 2002). Se is incorporated into a range of selenoproteins during translation and there is evidence that when Se supply is limiting there are cellular mechanisms which essentially prioritise selenoprotein synthesis (Hesketh & Villette, 2002). Genomic approaches present the opportunity to examine effects of Se supply on expression of a range of selenoproteins rather than individual genes.

In this study we have used a custom-made macroarray to study expression of the majority of known human selenoprotein genes, as well as genes encoding proteins implicated in selenium metabolism and a limited number of genes whose products are associated with arachidonic acid metabolism and immune response. Two separate, independent supplementation trials were carried out in which normal healthy individuals (male, female aged 35-50, blood Se < 1μM) received either 50 or 100 μg selenium as sodium selenite per day, or a visually identical placebo. Macroarray gene expression analysis was carried out on samples from both trials. Blood samples from the subjects were taken at days 0 and 42 in Trial 1 or days 0 and 105 in Trial 2, in both cases when the supplementation ceased. Lymphocytes were purified from the blood samples and RNA extracted using TRIzol reagent. RNA from four sets of three volunteers (two placebo, two Se-supplemented) in Trial 1 were pooled by mixing 2 μg of RNA and from the resulting 6 μg 400 ng of total RNA used for synthesis of ^{32}P-labelled cDNA probe by reverse transcription. The probes isolated from pre- and post-supplementation samples were hybridised overnight in parallel to two identical custom-made 'selenoprotein' Atlas™ arrays. After washing, specifically bound probe was detected using a phosphoimager and the two array scans were analysed and compared using AtlasImage v2.7 software. Differential gene expression was assessed by calculation of the ratio of signal intensity for the post-supplementation sample to signal intensity for the pre-supplementation sample, both corrected for background, and normalised by taking into account changes in intensity of signals corresponding to a set of 'housekeeping genes'. A change of equal to or greater than two-fold was considered significant.

Only a small number (three) of genes showed consistent changes in the placebo group. In contrast, supplementation with 50 μg selenium as sodium selenite caused a wider change in the pattern of lymphocyte expression with thirteen genes showing changes. RNA samples from volunteers in Trial 2 were not pooled but analysed individually; again expression of more genes (7–18) was altered following supplementation (in this case with 100 μg selenium as sodium selenite) than following placebo (1–7 genes). Thus, using either pooled or individual samples, macroarray analysis shows that Se supplementation increases/decreases expression of a range of genes. Initial analysis indicates that the genes affected include those that code for proteins associated with Se metabolism, arachidonate/lipoxygenase metabolism and thyroid hormone/immune function rather than the selenoproteins themselves.

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The effect of zinc supplementation in human subjects on the expression in small intestine of the zinc transporter hZTL1. By R.A. CRAGG, S.R. PHILLIPS, J.M. PIPER, F.C. CAMPBELL, J.S. VARMA, J.C. MATHERS and D. FORD, *Schools of Cell and Molecular Biosciences and Clinical Medical Sciences, Human Nutrition Research Centre, University of Newcastle upon Tyne, Kings Road, Newcastle upon Tyne NE1 7RU*

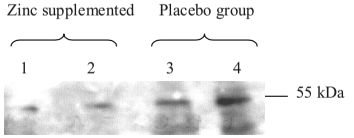
We previously reported the cloning of an intestinal zinc transporter hZTL1 which is located at the apical membrane of the human enterocyte (Cragg *et al.* 2003) and which mediates zinc uptake when heterologously expressed in the *Xenopus laevis* oocyte system (Cragg *et al.* 2002), indicating a potential role for hZTL1 in regulating the absorption of dietary zinc.

The present study was undertaken to examine the effect of zinc supplementation on hZTL1 expression in the human intestinal mucosa. Seventeen volunteers (six males; average age 51.5 years (range 33–76 years), mean BMI 25.3 kg/m^2 (range 15.1–43.5 kg/m^2)) were recruited to a randomised double-blind cross-over study. All subjects had an ileostomy with minimal resection of the ileum, allowing non-invasive access to the intestinal mucosa. Subjects were given a placebo or supplement of Zn (25 mg/d given as zinc sulphate) for 14 d, followed by a 4-week washout before taking the alternative supplement. Blood samples were taken before and after each trial and pinch biopsies (3–4 × 1 mm^3) of ileal mucosa collected via the stoma at the end of each trial. In addition, 7 d food diaries with portion sizes quantified by a food photographic atlas were completed during each trial.

Mean serum zinc levels measured by ICP were 16.6 (SEM 0.5) μmol/l after placebo and 20.0 (SEM 1.6) μmol/l after zinc supplement (P=0.08 by paired, two-tailed Student's t-test). Mean dietary intake of Zn (including the supplement) was 7.1 (SEM 0.5) mg/d during the placebo trial period and 32.1 (SEM 0.5) mg/d during the Zn-supplemented trial period. The level of hZTL1 protein in pooled samples was quantified by Western blot analysis using an anti-hZTL1 antibody (Cragg *et al.* 2003).

A semi-quantitative measure of hZTL1 mRNA expression in poly-A$^+$ RNA prepared from pooled biopsies was obtained by RT-PCR terminated during the linear phase of product amplification.

Densitometric analysis of the hZTL1-specific protein band on a Western blot, based on analysis of two separate loadings of each pooled sample, revealed a 1.8-fold decrease in hZTL1 protein expression following zinc supplementation. No difference in hZTL1 mRNA levels between the two groups was observed by visual inspection of the hZTL1-specific PCR product generated from quantities of mRNA that yielded β-actin-specific bands of equal intensities.



Western blot of protein from pooled biopsies of human small intestinal mucosa after electrophoresis under denaturing conditions. Lanes 1 and 3 contained 10 μg of sample and lanes 2 and 4 contained 20 μg of sample, probed with anti-hZTLI antibody.

The data indicate that control of hZTL1 expression in the human small intestine in response to changes in dietary zinc is at the translational or post-translational level. The pattern of regulation by zinc of hZTL1 demonstrated in this study is consistent with maintenance of zinc homeostasis through controlled, hZTL1-mediated intestinal absorption of zinc.

This work was supported by BBSRC grant 13D/11012.

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DNA methylation and S-adenosylmethionine:S-adenosylhomocysteine (SAM:SAH) ratio in colorectal carcinogenesis. By G. VARELA-MOREIRAS, M.P. GONZÁLEZ, R. PÓO and E. ALONSO-APERTE, *Facultad de Ciencias Experimentales y de la Salud, Universidad San Pablo-CEU, Crta. Boadilla del Monte Km. 5.3, 28668 Boadilla del Monte (Madrid), Spain*

Colorectal cancer is the second most common cause of cancer-related death in both men and women. However, it has been also estimated that 50–75% of these cases are preventable, either through screening and early detection or through nutritional methods or chemoprevention. Folate, B_6 and B_{12} deficiencies have been associated with increased rates in the development of certain types of cancers, mainly colorectal. Additionally, studies conducted in cell cultures, animal models and humans suggest that folate may modulate colorectal carcinogenesis. Its role maybe associated with the role tetrahydrofolate in maintaining DNA and RNA methylation, as well as in thymidylate and purine synthesis, essential for DNA synthesis and repair. However, there have been no studies in humans related to other methionine cycle markers that could influence the effect of folate on the aetiology/prevention of colorectal carcinogenesis. The ratio of SAM:SAH is frequently used as an indicator of cellular methylation capacity, whereby a decrease in this ratio predicts reduced cellular methylation potential. Folate insufficiency leads to a decrease in SAM synthesis, which may compromise SAM-dependent methylation reactions, but also to an increase in cellular concentrations of SAH. In humans, folate insufficiency is associated with hypomethylation of lymphocyte DNA, and increased risk for a variety of cancers. It is unclear, however, from the SAM:SAH ratio whether substrate insufficiency, product inhibition or both are required to negatively affect cellular methylation capacity.

Thus, we analysed the global DNA methylation and S-adenosylmethionine (SAM)/S-adenosylhomocysteine (SAH) colonic mucosa concentration in biopsies from patients previously diagnosed with adenocarcinoma of the colorectum (n=45, twenty-eight men with a mean age of 71;1 years, and seventeen women with a mean age of 70;6 years). Inclusion and exclusion criteria were used, and a consent form was obtained for each participant. At the time of surgery, for each individual we obtained biopsies of the adenocarcinomas and biopsies of normal-appearing mucosa, in order to perform an intra-individual comparison with malignant samples. Significant colonic DNA global hypomethylation (P <0.05) was found in injured tissue *v.* control. Results for colonic SAM and SAH are also presented:

Samples	**SAM (nmol/g)**		**SAH (nmol/g)**		**SAM:SAH**	
Total	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Normal mucosa	18.1	1.62	5.4	0.49	5.0	0.63
Adenocarcinoma	33.8 [a]	2.68	12.0 [a]	1.36	3.6 [b]	0.38
Men	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Normal mucosa	19.7	2.41	4.5	0.73	6.6	0.99
Adenocarcinoma	34.6 [a]	10.9	10.9 [a]	1.45	3.7 [c]	0.47
Women	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
Normal mucosa	13.7	2.44	6.7	0.65	2.6	0.60
Adenocarcinoma	31.7 [a]	4.16	13.4 [a]	2.96	3.7	0.79

Significantly different from normal mucosa: [a] p <0.05, [b] p =0.08, [c] p =0.05

A lower 'methylation' ratio (SAM:SAH) was observed in pathological tissue *v.* control, mainly due to a much higher SAH concentration. The effect is also related to sex, with a marked decrease only observed in men, although SAH and SAM colonic concentrations in women are also significantly increased in tumours, when compared to normal mucosa. These data clearly support the hypothesis of the importance of altered DNA methylation, as a major pathway towards cancer. It would be desirable in the future to evaluate whether the increased DNA damage could be compensated for by an increase in DNA repair.

The effect of dietary calcium supplementation on short-term zinc kinetics in elderly osteoporotic women. By N.M. LOWE[1], M.C. IP[1], C.I.A. JACK[2], N. CARMICHAEL[2], W.D. FRASER[3] and M.J. JACKSON[2], [1]*Department of Biological Sciences, University of Central Lancashire, Preston PR1 2HE,* [2]*Department of Medicine and* [3]*Department of Clinical Chemistry, University of Liverpool, Liverpool L61 3GA*

It is well established that zinc plays an important role in the maintenance of optimal bone health, as a structural component of bone mineral (Eberle *et al.* 1999), and in a functional role as a cofactor for enzymes such as alkaline phosphatase (Yamaguchi, 1998). Calcium supplements are frequently prescribed as part of the treatment regimen for patients diagnosed with osteoporosis. Paradoxically, previous research has indicated that calcium may interfere with and reduce the absorption of zinc, particularly if phytate is present, due to the formation of insoluble complexes which renders the zinc unavailable for absorption (Davies & Nightingale, 1975).

A stable isotope tracer study was designed to investigate the effect of calcium supplementation (1 g given daily as $CaCO_3$) on Zn kinetics in a group of twelve elderly female patients diagnosed with osteoporosis. The patients, aged 62–77 years, were in good general health, and not taking any medication or nutrient supplements at the time of study. Isotopes of zinc were administered intravenously (0.3 mg of ^{70}Zn) and orally (1.8 mg of ^{67}Zn) at the start of the study and after a 4-week period of calcium supplementation. Blood samples were taken at specific time intervals and the plasma separated by centrifugation and stored at −20°C. After removal of the organic plasma components by microwave digestion, the zinc was extracted from the sample by ion exchange chromatography (Lowe *et al.* 2000) and the isotope enrichment was measured by ICP-MS. Preliminary analysis of short-term zinc kinetics (120 min) of five of the patients has been completed using a two-compartment model, where pool *a* is composed primarily of plasma zinc, and pool *b* is composed of a proportion of liver zinc (Lowe *et al.* 1998).

Time point	**Qa** (mg) Mean (SEM)	**Qb** (mg) Mean (SEM)	**kaa** (/min) Mean (SEM)	**kbb** (/min) Mean (SEM)	**Fab** (mg/min) Mean (SEM)
Baseline	3.53 (0.8)	21.2 (3.6)	0.1489 (0.033)	0.0136 (0.002)	0.272 (0.05)
After Ca supplementation	3.16 (0.5)	16.7 (3.7)	0.0831 (0.003)	0.0096 (0.004)	0.128 (0.03)

The data revealed that calcium supplementation had no significant effect on the size of pool *a* (Qa), the size of pool *b* (Qb) or the fractional turnover rate of pool *b* (kbb). However there was a 44% fall (P=0.06) in the fractional turnover rate of the plasma zinc compartment (kaa) post-calcium-supplementation, associated with a 53% fall (P=0.06) in the flux of zinc moving between the two compartments (Fab). These changes failed to achieve statistical significance but suggest that calcium supplementation may alter the uptake of zinc from the plasma by the liver. Further kinetic analyses will be carried out to characterise these apparent changes more extensively.

Financial support from NHS Executive North West R&D Directorate is gratefully acknowledged.

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Differential effects of selenium supply on expression of thioredoxin reductase isoforms in different tissues. By L.K. CROSLEY[1,2], F. NICOL[1], J.E. HESKETH[2] and J.R. ARTHUR[1], [1]*The Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB and* [2]*School of Cell and Molecular Biosciences, University of Newcastle, Newcastle upon Tyne NE1 7RU*

Selenium is essential in biological systems when present as selenoproteins containing the amino acid selenocysteine. An adequate supply of selenium, and thus selenocysteine, is critical for the optimal synthesis of selenoproteins such as the glutathione peroxidases and iodothyronine deiodinases. These enzymes have several important cellular roles such as protection against free radical attack (Brigelius-Flohe, 1999) and catalysing active thyroid function (Arthur *et al*. 1993). Thioredoxin reductases are selenoproteins that fulfil another function, namely to maintain intracellular redox status as part of the thioredoxin system (Sun *et al*. 1999).

The UK intake of selenium has decreased over the last 25 years to half of the UK Reference Nutrient Intake (Rayman, 2000). The response of thioredoxin reductase expression to decreased selenium supply has not been fully investigated and it is essential to determine whether there is a differential tissue and/or isoform response. The aim of this work is to study how the expression of two isoforms of thioredoxin reductase (TR1, the cytosolic form, and TR2, the mitochondrial form) respond to altered dietary Se intake.

Male, weanling , Rowett Hooded Lister rats (6 per group) were given diets containing 0.008, 0.033, 0.063 or 0.11 mg selenium/kg and the levels of TR1 and TR2 protein and their corresponding mRNAs were determined in liver, heart, kidney and testis by ELISA and Northern hybridisation. The levels of the two isoforms were differentially affected, with TR1 being more sensitive to Se depletion than TR2. Hepatic TR1 protein levels in rats maintained on the severely deficient (0.008 mg Se/kg) diet fell to 20% of those fed the selenium-adequate (0.11 mg Se/kg) diet (126.19±17.92 vs 616.05±45.13 ng/ mg protein); however, the TR2 protein levels in the same animals decreased to 31% of adequate levels (101.06±6.01 vs 319.91±15.49 ng/ mg protein). Renal TR1 and TR2 levels fell by 25% and 38%, respectively, in the rats maintained on the 0.008 mg Se/kg diet compared with those fed the 0.11 mg Se/kg diet (42.67±5.93 vs 168.55±12.96 and 65.94±3.77 vs 170.43±15.87 ng/ mg protein). TR2 protein levels in heart and testis also showed less sensitivity to decreased dietary selenium than TR1 protein levels. There were also tissue-specific effects with heart and testis TR1 and 2 protein levels showing either less response or no response to decreased dietary selenium compared with liver and kidney. Except in the liver, measurements of TR1 and TR2 mRNA abundance levels did not show parallel changes to the protein measurements, and even in the liver the fall in protein was greater than those in mRNA (43% less mRNA in the animals fed the 0.008 mg Se/kg diet compared with those maintained on the 0.11 mg Se/kg diet). We hypothesise that there is decreased translation of the mRNAs, and an increase in their instability, under conditions of Se depletion and that there is a differentially greater effect on TR1 than TR2. The data support and extend the concept that there is tissue-specific control of selenoprotein expression that effectively 'prioritises' the synthesis of selenocysteine towards certain selenoproteins, in this case TR2 rather than TR1.

L.K.C. was a recipient of a BBSRC studentship. The work was also supported by Scottish Executive Environment and Rural Affairs Department (SEERAD).

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Expression of the sodium-dependent vitamin C transporters SVCT1 and SVCT2 in vascular cells. By R.M. OGBORNE, S.A. RUSHWORTH and M.A. O'CONNELL, *MRC Human Nutrition Research, Fulbourn Road, Cambridge CB1 9NL*

The early stages of vascular disease are characterised by leucocyte adhesion to the endothelium, migration into the intima of the artery wall and a subsequent pro-inflammatory response leading to foam cell formation and plaque rupture (Libby, 2002). Vitamin C has been proposed as a beneficial antioxidant in vascular disease progression, but clinical trials have provided conflicting results, highlighting the requirement for further molecular studies aimed at assessing its efficacy in preventing the early stages of vascular disease development. The main physiological form of vitamin C is L-ascorbic acid and this is taken up into cells by one of two recently identified sodium-dependent transporters, SVCT1 and SVCT2, which express stereo specificity for L-ascorbic acid (Daruwala *et al*. 1999). The oxidised form of vitamin C, dehydroascorbic acid, is taken up by the glucose receptors GLUT1 and GLUT3 and reduced back to ascorbate by intracellular mechanisms. SVCT1 receptor has been localised to a limited number of tissues, including the intestine, with SVCT2 more ubiquitously expressed (Wang *et al*. 1999).

The aim of this study was to determine SVCT1 and SVCT2 expression on human vascular cells, including primary lymphocytes, monocytes and endothelial cells (HUVEC). THP-1, a human monocytic leukaemia cell line, and Jurkat D1.1, a human acute T-cell leukaemia cell line, were also examined to determine whether they were suitable models of monocytes and lymphocytes for further studies. Caco-2 cells, derived from a human colonic adenocarcinoma, were included as positive controls, as they have previously been found to express both types of receptor. Reverse-transcriptase PCR (RT-PCR) was performed to determine qualitative mRNA expression of SVCT1 and SVCT2 in the various cell types. Real-time PCR was then used to determine relative quantitative mRNA receptor expression, normalised against GAPDH expression. SVCT2 protein expression was examined by protein separation using SDS-PAGE, followed by Western blotting.

SVCT2 mRNA was expressed in all cell types examined by RT-PCR. SVCT1 mRNA was detected in primary human monocytes and lymphocytes, and Jurkat D1.1, but was undetectable in THP-1 cells and HUVEC by this method. However, real-time PCR demonstrated that both THP-1 cells and HUVEC expressed SVCT1 at low levels. SVCT2 mRNA expression was higher in all vascular cell types than SVCT1, with highest levels of both receptors found in primary lymphocytes. SVCT2 protein was expressed in lymphocytic cells, HUVEC and Caco-2 cells. These results demonstrate that SVCT2 is the major L-ascorbic acid receptor in vascular cells. Studies are underway to examine regulation of these receptors by L-ascorbic acid and pro-inflammatory stimuli in vascular cells.

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Selenoprotein gene expression in an intestinal cell line during selenium depletion: a macroarray approach indicates effects on SelW and glutathione peroxidase 1. By V. PAGMANTIDIS[1], S. VILLETTE[1], G. BERMANO[2], J. BROOM[2], J.R. ARTHUR[3] and J.E. HESKETH[1], [1]*School of Cell and Molecular Biosciences, University of Newcastle, Newcastle-upon-Tyne NE1 7RU,* [2]*University of Aberdeen, Medical School, Aberdeen, AB24 3FX and* [3]*The Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB*

The micronutrient selenium (Se) is incorporated into a range of selenoproteins involved in numerous biochemical processes within the body. There is some debate that the decrease in selenium intake of the UK population over recent years may have implications for health (Rayman, 2002). It is therefore of interest to define how the expression of the various selenoproteins alters when Se supply is suboptimal. There is evidence that when Se supply is limiting there are cellular mechanisms which prioritise selenoprotein synthesis (Hesketh & Villette, 2002). Se deficiency leads to decreases in both protein and mRNA levels of some selenoproteins; this is thought to be due to decreased mRNA translation and a subsequent increased mRNA degradation (Bermano *et al*. 1996*a*). Genomic approaches present the opportunity to examine effects of Se supply on expression of a range of selenoproteins rather than individual genes. In this study, we have used a custom-made macroarray to study expression of a range of selenoprotein genes.

Caco-2 cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum until they reached confluence. They were then maintained in serum-free medium with either insulin (5 μg/ml) and transferrin (5 μg/ml) (Se-deficient cells) or insulin, transferrin and selenium as sodium selenite (7 ng/ml) (Se-replete cells). Under such conditions both Caco-2 and rat hepatoma H4 cells provide a model of Se depletion (Bermano *et al*. 1996*b*; Pagmantidis *et al*. 2002). Total RNA was extracted, and 3.5 μg of it was used for synthesis of ^{32}P-labelled cDNA probe by reverse transcription. RNA isolated from Se-supplemented and Se-depleted cells was hybridised overnight in parallel to two identical custom-made 'selenoprotein' Atlas™ arrays. The array contained cDNA probes corresponding to the majority of known human selenoproteins, proteins implicated in selenium metabolism and a limited number of genes whose products are associated with arachidonic acid metabolism and immune response. After washing, specifically bound probe was detected using a phosphorimager and the two array scans were analysed and compared using the AtlasImage v2.7 software. In order to allow for any differences in labelling efficiency between RNA samples, normalisation was performed by taking into account changes in intensity of signals corresponding to the 'housekeeping genes'. Differential gene expression was assessed by calculation of the ratio of signal intensity for the Se-deficient cells to signal intensity for the Se-adequate cells, both corrected for background, and normalised as described above. A change of equal to or greater than two-fold was considered significant.

The results confirmed those found previously by Northern hybridisation (Pagmantidis *et al*. 2002), namely that the mRNA levels for GPX1 and GPX4 decreased under Se depletion, whereas for GPX2 there was no statistical significant change. In addition, expression of SelW was found to decrease in Se depletion. In conclusion, the major changes in selenoprotein gene expression at the mRNA level seen in Caco-2 cells during Se depletion were the marked decrease in abundances of SelW and glutathione peroxidase 1 mRNAs. It is likely that this reflects the instability of these mRNAs under conditions of low Se supply. The relationship of these changes to potential roles of selenium in the prevention of colon cancer is under investigation.

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Rayman MP (2002) *Proceedings of the Nutrition Society* **61**, 203–215.

Effects of selenium deprivation on the survival of lymphoid and colonic cell lines in culture. By M.J. KING[1], R. HEYWOOD[1], V. PAGMANTIDIS[1], J.C. MATHERS[2] and J.E. HESKETH[1], [1]*School of Cell and Molecular Biosciences,* [2]*Human Nutrition Research Centre, School of Clinical Medical Sciences, University of Newcastle upon Tyne, Agriculture Building, Kings Road, Newcastle Upon Tyne NE1 7BU*

Selenium (Se) is an essential micronutrient in the human diet. Suboptimal Se intake has been linked to an increased risk of cancer (Clark *et al*. 1996) and increased susceptibility to viral attack (Beck *et al*. 1998). Low Se levels may also contribute to decreased immune response, since dietary Se supplementation resulted in enhanced T-cell and natural killer cell activity (Kiremidijan-Schumacher *et al*. 1994). Se is incorporated into functional proteins, including the glutathione peroxidase (GPx) family of antioxidant enzymes which contribute to the cells' protective mechanism against oxidative stress. For example, overexpression of GPx4 *in vitro* resulted in increased resistance to cholesterol hydroperoxides (Hurst *et al*. 2001). Increasing Se supply in culture results in increased expression and activity of glutathione peroxidases in a hierarchical manner (Bermano *et al*. 1996).

The ability of Se to increase cellular protection against oxidative stress was studied in a human T-cell line, Jurkat E6.1. The cells were cultured with (Se+) or without (Se−) supplementation with 7 ng/ml sodium selenite for 48 h. Cells were challenged for 24 h with a range of tert-butyl hydroperoxide (t-BuOOH) concentrations and their viability assessed by counting live and dead cells using Trypan Blue. Controls were cells cultured under Se+ or Se− conditions without addition of t-BuOOH. Cell viability was expressed graphically as a percentage, and the LD50 was calculated. Se-supplemented T-cells were able to survive higher doses of t-BuOOH as summarised in the Table, when compared to selenium-depleted cells. The human colonic cell line Caco-2 was also examined, and preliminary data showed that the mean LD50 of selenium-replete and selenium-depleted cells against t-BuOOH were similar to those for Jurkat cells. Similarly, there was a marked difference between survival of rat hepatoma cells cultured in Se+ and Se− conditions (B. Duperrier & J.E. Hesketh, unpublished observations) and these correlated with previously reported changes in selenoprotein gene expression (Bermano *et al*. 1996).

	Se supplemented		**Se depleted**	
Cell line	Mean LD50 (mM)	±SEM	Mean LD50 (mM)	±SEM
Jurkat E6.1	20	4	9	2
Caco-2	26	11	10	3

The lower LD50 values in both Se-depleted Caco-2 and Jurkat cells correlated with decreased GPx1 gene expression. GPx4 mRNA levels also decreased but to a lesser extent. Further studies, such as assessment of hierarchy of selenoproteins in peroxide-challenged cells, would further elucidate the role of selenoproteins in the antioxidant activities of these cell lines.

This work was funded by the BBSRC.

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Kiremidijan-Schumacher L, Roy L, Wishe HI, Cohen MW & Stotzky G (1994) *Biological Trace Element Research* **41**, 115–27.

Development of a method for quantifying radioactivity in proteins in sodium dodecyl sulphate-polyacrylamide gels. By S.B. ZHOU[1], M.J. BAILEY [1], M.J. DUNN[2], V.R PREEDY[1] and P.W. EMERY[1], [1]*Nutrition, Food and Health Research Centre, King's College London, London SE1 9NN and* [2]*Department of Neuroscience, Institute of Psychiatry, King's College London, London SE5 8AF*

Electrophoretic separation of proteins by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) is an important tool in proteomics. The regulatory mechanisms involved in protein expression can also be investigated by metabolic tracer techniques such as measuring the rate of incorporation of isotopically labelled amino acids into proteins *in vitro*. Detection and quantification of radioactively labelled proteins in gels has traditionally been accomplished by autoradiography. However, this is insufficiently sensitive for routine quantification of proteins with low abundance. An alternative approach would be to extract the proteins from the gels for subsequent analysis by liquid scintillation counting. Preliminary studies showed that elution of proteins using water or aqueous buffers resulted in low yields, but that gels could be dissolved by heating in hydrogen peroxide to produce a clear, stable solution that mixed efficiently with a standard liquid scintillation cocktail. We have therefore undertaken a systematic investigation into the reliability and reproducibility of using H_2O_2 to digest gels and extract radioactively labelled proteins for quantitative analysis.

We first demonstrated that addition of H_2O_2 had no effect on the count rates of [^{14}C]-bovine serum albumin (BSA) when counted in a conventional liquid scintillation system, and that the count rates remained stable for several days. Temperatures up to 70°C had no effect on the amount of radioactivity detected, but there was a significant reduction in count rates in samples incubated at 80°C. Aliquots containing 32–65 µg [^{14}C]-BSA were then run on one-dimensional SDS-PAGE gels and stained with Coomassie Brilliant Blue (CBB) R-250. The gels were cut into fragments of 1 cm × 1 cm and each fragment was incubated with 2 ml H_2O_2 at 40, 50, 60 or 70°C overnight then mixed with 8 ml scintillation fluid and counted. The recovery of radioactivity at 40, 50 and 60°C was 63%, 85% and 92% of that at 70°C, so all subsequent experiments used incubation at 70°C. The total radioactivity recovered represented 89% of the amount loaded in the case of 32 µg BSA and 92% of the amount loaded in the case of 65 µg BSA. The experiment was then repeated with ten aliquots of 50 µg [^{14}C]-BSA, with five lanes being stained with CBB and five lanes being stained with Sypro Ruby. Recovery was 90±4% (mean ± SEM, n=5) of the amount loaded in the lanes stained with CBB and 94±3% in the lanes stained with Sypro Ruby. In a final experiment, aliquots containing 200 µg protein from homogenates of rat liver that had been labelled by incorporation of ^{35}S-methionine were separated by two-dimensional gel electrophoresis (2-DE) using the method of Weekes *et al*. (1999). The gels were stained with Sypro Ruby and cut into fragments of 1 cm × 1 cm, treated with H_2O_2 and counted as above. The total amount of radioactivity recovered from duplicate gels was 91% and 89% of the amount loaded onto the second-dimension gels. A further 1.2% was recovered in the first-dimension strip and 8.2% was recovered in the fixing and destaining solutions. Thus 100% of the radioactivity loaded onto the second-dimension gel was accounted for.

We conclude that this method offers a reliable and reproducible way of quantifying radioactivity in complex mixtures of proteins that have been separated by 2-DE, thereby complementing existing proteomic techniques.

This work is supported by the Wellcome Trust and the BBSRC.

Weekes J, Wheeler CH, Yan JX, Weil J, Eschenhagen T, Scholtysik G & Dunn MJ (1999) *Electrophoresis* **20**, 898–906.

A systematic investigation into protein loss during two-dimensional gel electrophoresis. By S.B. ZHOU[1], M.J. BAILEY[1], M.J. DUNN[2], V.R PREEDY[1] and P.W. EMERY[1], [1]*Nutrition, Food and Health Research Centre, King's College London, London SE1 9NN and* [2]*Department of Neuroscience, Institute of Psychiatry, King's College London, London SE5 8AF*

Two-dimensional gel electrophoresis (2-DE) is an important tool for separating and identifying proteins within complex mixtures. Although the gels can be stained and scanned, the resulting data may not be truly representative of the abundance of proteins in the original mixture. This is partly because there are losses of proteins at various stages of the 2-DE procedure. However, there appear to be no data in the literature on the extent of these losses. We have therefore undertaken a systematic study to quantify the losses of protein during an established 2-DE procedure (Weekes *et al*. 1999).

Radioactively labelled proteins were used so that the amount lost at each stage could be quantified by liquid scintillation counting of aliquots of the various buffers and washing solutions. The amounts of radioactivity remaining in the immobilised pH gradient (IPG) strips and the second-dimension gels was quantified by digesting the strips or gels with hydrogen peroxide and liquid scintillation counting using a recently developed protocol (Zhou *et al*. 2003). The proteins that were investigated were [^{14}C]-bovine serum albumin and a homogenate of liver taken from rats that had been injected with [^{35}S]-methionine 15 min prior to killing. Proteins in the homogenate were precipitated with ice-cold acetone or 1.5% perchloric acid (PCA) and washed 8–12 times with acetone or PCA to remove the free amino acids. Measured amounts of protein were suspended in 8 M urea reswelling buffer and taken up into IPG strips. Some strips were taken for counting, to quantify losses during the reswelling stage, and the reswelling trays were washed and the washings counted. Further strips were counted after isoelectric focusing. The remaining strips were equilibrated with two 6 M urea equilibration buffers, and again some strips were counted to quantify the losses during the equilibration stage. Finally the strips were run out on second-dimension gels, fixed and stained with Sypro Ruby and the gels were then cut into fragments and counted. The cumulative proportions of radioactivity lost after each of the three main stages are shown in the Table as means of duplicate or triplicate assays.

Amount of protein loaded onto IPG strips	Isotope used, method of precipitating protein and washing off free amino acids, size of IPG strips used	Radioactivity lost after re-swelling step	Cumulative radioactivity lost after focusing step	Cumulative radioactivity lost after equilibration
45 µg	[^{14}C]-Albumin, washing with acetone, 18 cm IPG strip	25%	Not determined	36%
45 µg	[^{14}C] -Albumin, no washing, 18 cm IPG strip	20 %	Not determined	29%
200 µg	[^{35}S]-methionine liver protein homogenate, washing with acetone, 7 cm IPG strip	41%	55%	78%
400 µg	[^{35}S]-methionine liver protein homogenate, washing with PCA then with acetone, 18 cm IPG strip	53%	60%	77%
1000 µg	[^{35}S]-methionine liver protein homogenate, washing with acetone, 18 cm IPG strip	32%	49%	92%

There were major losses during the reswelling and equilibration stages, and in both cases the losses could be accounted for by radioactivity recovered in the buffers and washings. The losses appeared to vary with the nature of the proteins being analysed and the amount loaded. Recovery from the second-dimension gel was approximately 90% of the amount loaded, with the other 10% mainly in the fixing and destaining solutions. We conclude that any data on protein abundance derived from 2-DE should be regarded as no more than semi-quantitative.

This work is supported by the Wellcome Trust and the BBSRC.

Weekes J, Wheeler CH, Yan JX, Weil J, Eschenhagen T, Scholtysik G & Dunn MJ (1999) *Electrophoresis* **20**, 898–906.
Zhou SB, Bailey MJ, Dunn MJ, Preedy VR & Emery PW (2003) *Proceedings of the Nutrition Society* **62**, OCA/B.

A proteomic approach to measure protein expression in a model of muscle protein accretion. By M.J. BAILEY[1], J.A. WESTBROOK[2], M.J. DUNN[3], V.R. PREEDY[1] and P.W. EMERY[1], [1]*Nutrition, Food and Health Research Centre, King's College London, London SE1 9NN,* [2]*Proteome Sciences plc and* [3]*Department of Neurosciences, Institute of Psychiatry, King's College London, London SE5 8AF*

Muscle protein accretion occurs in a number of patho-physiological situations such as growth, hypertrophy and the fasting–feeding transition. However, it is not known whether this process is accompanied by concordant changes in the profiles of all cytoplasmic, contractile and cytoskeletal proteins. Recent developments in proteomics have raised the possibility that changes in a wide range of muscle proteins can be measured simultaneously. We have therefore used two-dimensional gel electrophoresis to quantify the changes in expression of a wide range of proteins in an established model of acute muscle protein accretion, i.e. muscle repair. Measurements were made 48 h after imposition of a surgical wound, when the overall rate of protein synthesis in the tissue is twice as high as normal (Emery & Ghusain-Choueiri, 1994).

Eight mature female rats were subjected to laparotomy under isofluorane anaesthesia. After 48 h the animals were killed and abdominal muscle samples were taken from the wound site (wounded) and 2 cm away from the wound site (control). The tissues were homogenised, proteins were separated by two-dimensional gel electrophoresis and the proteins were detected by silver staining. Normalised protein density was determined using PD Quest software on scans of the gels, and selected results are shown in the Table. From sixteen gels the mean number of protein spots detected was 461 (between 299 and 653 spots in each gel). It was possible to identify thirty-eight spots by matching with a previously published database of 100 muscle proteins (Yan *et al*. 2001). Of those, twelve were found to have significant differences ($P<0.05$) in expression when comparing wounded and control sites.

Protein	Mean protein density Control (a.u.)	Mean protein density Wounded (a.u.)	Percentage change	*P* value
Actin aortic (smooth muscle)	32884	22585	−3	0.031
Alpha actin	13975	36780	+163	0.001
Beta enolase	40399	17407	−57	0.014
Contraception-associated protein	3373	2343	−31	0.050
Creatine kinase	2341	1097	−53	0.030
Cypher 2 mouse	5291	2906	−45	0.020
Fructose bisphosphate aldolase	46602	26873	−42	0.046
Glyceraldehyde 3 phosphate dehydrogenase	2602	13381	+414	0.003
Heat-shock protein 27	9156	29834	+226	0.011
Triose phosphate isomerase	12664	6176	−50	0.003
Tropomyosin	3274	1751	−47	0.044
Voltage ion channel	4170	2185	−48	0.023

P values refer to differences between control and wounded as determined by paired *t*-test. a.u.= arbitrary units.

There were wide differences in the responses of different proteins to wounding. Both contractile proteins such as actin and non-contractile proteins such as triose phosphate isomerase showed significant changes in expression. Whilst there is a large overall increase in protein expression in this model, other individual proteins are shown to be of decreased expression. This demonstrates the complex and discordant changes occurring during the process of protein accretion. These data show the potential of proteomic methods to elucidate the differential expression of many proteins simultaneously.

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Differential sensitivity of HCT116 and HCT116 3–6 colon cancer cells to butyrate. By D.M. MARLAND[1], E.A.WILLIAMS[2], W. BAL[1], B.K. SHENTON[3] and J.C. MATHERS[1], [1]*Human Nutrition Research Centre, School of Clinical Medical Sciences, University of Newcastle upon Tyne, NE1 7RU,* [2]*Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Sheffield S5 7AU and* [3]*School of Surgical and Reproductive Sciences, University of Newcastle upon Tyne NE2 4HH*

Hereditary non-polyposis colorectal cancer (HNPCC) is a familial cancer, inherited in an autosomally dominant fashion due to a germline mutation in one of the genes responsible for DNA mismatch repair (MMR). MMR is important for maintaining genomic integrity and correcting errors which occur when copying DNA. The majority (~70%) of the MMR mutations in HNPCC patients are found in either *hMLH1* or *hMSH2*. Mutations in these genes are associated with microsatellite instability (MSI), which is defined as an alteration in the length of repeated DNA sequences.

The short-chain fatty acid butyrate, produced by bacterial fermentation of carbohydrate in the colon, has anti-neoplastic effects in colorectal cancer (CRC) cells, inducing cell cycle arrest, apoptosis and differentiation. Previous work has shown that MMR-deficient and -proficient CRC cells are differentially sensitive to butyrate. This study was designed to test the hypothesis that the MMR status of the CRC cell line causes differential sensitivity to butyrate.

The CRC cell lines HCT116 ($hMLH1^{-/-}$) and HCT116 with chromosome 3 replacement (HCT116 3–6) ($hMLH1^{+/-}$) were grown for 24 h in McCoy's 5A media prior to treatment with media containing 0 mM or 1 mM butyrate for 60 h. Media was changed every 24 h and cells were harvested, counted and cell cycle analysed every 12 h.

Fig. 1

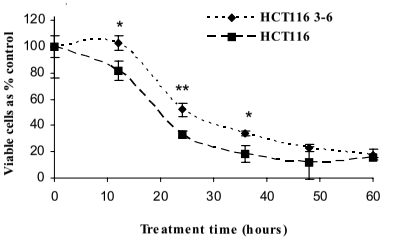
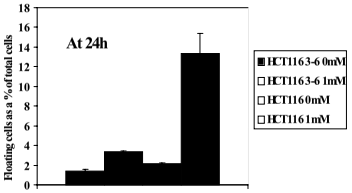


Fig. 2



*$P<0.05$, **$P<0.01$.

As expected, butyrate treatment reduced proliferation of both cell lines (Fig. 1). However, the HCT116 (MMR-deficient) cells were significantly more sensitive to the anti-proliferative effects of butyrate after 12 h, 24 h and 36 h of exposure. In both cell lines butyrate causes a shift in cell cycle such that there is an accumulation of cells in G0/G1 phase and a reduction of cells in S phase of the cell cycle. This effect occurred to a similar extent in both cell lines. Exposure of cells to 1 mM butyrate increased the percentage of total cells that were floating for both cell lines (Fig. 2). However, this effect was more pronounced in the MMR-deficient HCT116 cell line. Previous work showed that >80% of the floating cell population in the 1 mM butyrate treated flask were apoptotic for both cell lines. This study shows that in the absence of a functional *h*MLH1 gene, colon cancer cells are more sensitive to suppression of growth and induction of apoptosis by butyrate.

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Student Competition

The influence of dietary iron levels on the expression of iron transporter genes in rat colon. By K.L. JOHNSTON, C. SOROMANI and P.A. SHARP, *Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH*

The concerted action of two iron transport proteins in the duodenum is responsible for the absorption of dietary iron. Divalent metal transporter 1 (DMT1) is located in the apical membrane of enterocytes and is responsible for uptake from the diet, whereas IREG1 localizes to the basolateral membrane and mediates iron efflux. DMT1 exists as two splice variants, one with an iron-responsive element (DMT1+IRE) and one without (DMT1non-IRE), the former of which is regulated by cellular iron status. Only 10% of dietary iron is absorbed in the duodenum, which means that 90% reaches the distal small intestine and colon. Uptake of iron in the colon would presumably require the presence of both DMT1 and IREG1 proteins and thus the aim of this study was to measure the colonic expression of the genes encoding for these proteins in response to varying levels of dietary iron.

Three groups of four male Wistar rats (approximately 250 g) were fed either iron-loaded (20 g/kg), iron-deficient (<0.5 mg/kg) or iron-replete (50 mg/kg) diet for 7 d. After this time, rats were killed humanely, in accordance with UK Home Office legislation, and 5 cm sections of proximal and distal colon were removed and subjected to total RNA isolation using the TRIzol method. RT-PCR was performed using specific primers to measure transporter mRNA levels and all data were normalized to β-actin. Results were semi-quantified using Scion image software and are expressed as mean ± SEM. Statistical analysis was carried out using one-way ANOVA followed by Tukey's *post-hoc* test.

All three iron transporter genes were clearly expressed in both the proximal and distal colon. Consumption of iron-deficient diet resulted in a significant increase in DMT1+IRE (1.39±0.12 a.u.; control, 0.81±0.10 a.u., $P<0.01$) and DMT1 non-IRE (1.81±0.16 a.u.; control, 0.85±0.13 a.u., $P<0.01$) but not IREG1 (0.77±0.03; control, 0.66±0.15 a.u.) expression in the proximal colon. Rats fed iron-loaded diets had lower DMT1 IRE levels (0.34±0.03 a.u., $P<0.05$) but increased expression of IREG1 (1.07±0.09 a.u., $P<0.05$) in the proximal colon. DMT1 non-IRE levels were not affected by dietary iron loading. Iron transporter expression in the distal colon was not significantly different in any of the dietary groups.

These data suggest that the iron transport machinery essential for absorption in the duodenum is also expressed in the colon. The dramatic regulation of iron transporter genes by dietary iron in the proximal colon suggests that this region could be capable of absorbing dietary iron under certain physiological conditions. Experiments are currently under way to determine the cellular localization of DMT1 and IREG1 protein in the proximal colon to gain a better understanding of their role in colonic iron uptake.

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Screening for novel single nucleotide polymorphisms in gene regions corresponding to 3′untranslated regions of glutathione peroxidases 1 and 4 and thioredoxin reductase 1. By S. VILLETTE[1], L.K. CROSLEY[1], F. CAPLE[1], J.R. ARTHUR[2] and J.E. HESKETH[1], [1]*School of Cell and Molecular Biosciences, University of Newcastle, Newcastle-upon-Tyne NE1 7RU and* [2]*The Rowett Research Institute, Aberdeen AB21 9SB*

The decrease in selenium intake of the UK population over recent years may have implications for health (Rayman, 2002). Selenium (Se) has functions in a range of biochemical processes within the body and it is incorporated into selenoproteins as the amino-acid selenocysteine. This occurs during mRNA translation and requires a specific stem-loop structure (the SECIS) in the 3′untranslated regions (3′UTR) of the selenoprotein mRNAs. When Se supply is limiting there are cellular mechanisms which essentially prioritise selenoprotein synthesis (Hesketh & Villette, 2002). It appears that the different 3′UTRs from the selenoprotein mRNAs contribute to such prioritisation (Bermano *et al.* 1996) and it is therefore possible that subtle changes in 3′UTRs, such as those that result from single nucleotide polymorphisms, may affect selenoprotein synthesis. If this is the case, then such genetic differences between individuals may be responsible for variable response of individuals to Se supplementation. We have therefore screened gene regions corresponding to the 3′UTRs of glutathione peroxidases (GPx) 1 and 4 and thioredoxin reductase 1 for single nucleotide polymorphisms (SNPs). Lymphocytes were purified from blood samples taken from sixty-six normal healthy volunteers (male, female aged 35-50, blood Se < 1μM) in the Aberdeen area. DNA was extracted using TRIzol reagent. Regions corresponding to the 3′UTRs of the three mRNAs were amplified by PCR. In the case of GPX4 the PCR products from all sixty-six samples were purified and sequenced directly. The data show that the region of the predicted SECIS was a highly conserved with no variation. However, an allelic variation was observed at position 718(T/C) and this polymorphism occurred at a high frequency (TT 25%, TC41%, CC34%) (Villette *et al.* 2002). No variation was found at position 738 (predicted from genome data). The PCR products from the region corresponding to the GPX1 3′UTR could not be sequenced and so DNA-HPLC was used to screen a subset of thirty-eight samples and subsequently the GPX1 3′UTR region from four samples was subcloned and sequenced. There was no evidence for any allelic variation in the region corresponding to the predicted SECIS or the majority of the 3′UTR. DNA-HPLC analysis of PCR products derived from different regions of the TR1 gene show that several parts of the 3′UTR, including the predicted SECIS region, showed no evidence of heterozygosity, and thus any mutation or polymorphism. However, one region showed two potential polymorphisms and sequencing confirmed that there was sequence variation within this region. In conclusion, these data show that in GPX4 and TR1 genes there are SNPs that occur with regions that correspond to the 3′UTR. Since these 3′UTRs are crucial for Se incorporation, potentially these allelic variations may influence selenoprotein synthesis and Se requirements: their functional significance is now being investigated.

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Bermano G., Arthur JR & Hesketh JE (1996) *Biochemical Journal* **320**, 157–160.
Hesketh JE & Villette S (2002) *Proceedings of the Nutrition Society* **61**, 405–414.
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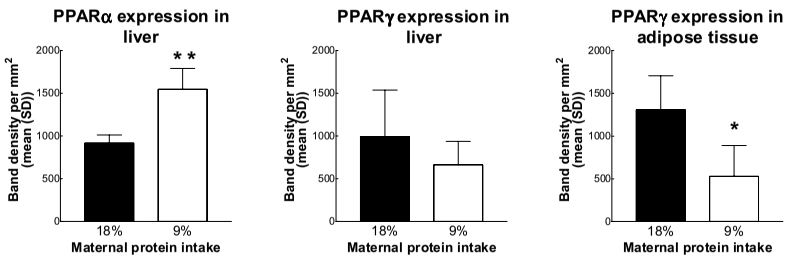
Maternal dietary protein restriction during pregnancy alters the expression of peroxisomal proliferator-activated receptors in the liver and adipose tissue of the offspring after weaning. By G.C. BURDGE[1], K.A. LILLYCROP[2], R.L. DUNN[1], E.S. PHILLIPS[2] and A.A. JACKSON[1], [1]*Institute of Human Nutrition and* [2]*Division of Biochemistry and Molecular Biology, Biomedical Sciences Building, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

Epidemiological studies and experiments using animal models show that restricted nutrient availability during development programmes the physiology of the fetus. Alterations to the activity of the renin–angiotensin pathway have been implicated as a cause of hypertension in the offspring of rats fed a low-protein diet during pregnancy (Sherman & Langley-Evans, 2000). Persistent changes to the activities of metabolic pathways imply altered gene expression. Programming of the activities of transcription factors may represent one potential mechanism for long-term changes in gene expression. Peroxisomal proliferator-activated receptors (PPARs) are ligand-activated transcription factors that play a pivotal role in fetal development, and in carbohydrate and fat metabolism in adults. We have investigated whether maternal protein intake during pregnancy in the rat alters the expression of PPARs in the offspring after weaning.

Pregnant rats were fed isoenergetic diets containing either 18% (w/w) or 9% (w/w) casein from conception to delivery (*n* 5 per dietary group), and then standard chow during lactation (Langley & Jackson, 1994). Pups (*n* 5 per dietary group, one per litter) were weaned at 28 d and killed at 33 or 34 d. Liver and abdominal adipose tissue was frozen immediately after removal. Expression of PPARα and PPARγ was determined by RT-PCR, and the corresponding bands on agarose gels were normalised against cyclophilin and quantified by densitometry.



Reduced maternal protein intake during pregnancy resulted in increased PPARα expression (70%, $^{**}P<0.0001$), but did not alter PPARγ expression, in the liver of the offspring. In contrast, PPARγ expression was reduced by 60% ($^{*}P<0.01$) in adipose tissue in the 9% protein group.

These results show that maternal protein intake during pregnancy produced both tissue and isoform-specific changes in PPAR expression in the offspring. Although the physiological significance remains to be determined, since PPARα regulates β-oxidation in liver and PPARγ promotes triacylglycerol storage in adipose tissue, such changes in PPAR expression suggest impaired control of lipid metabolism. Together these results support the hypothesis that one mechanism by which maternal diet may programme metabolic pathways in the fetus is by modifying the expression of transcription factors that regulate the activities of key enzymes.

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Effects of drinking an alkaline mineral water on renal calcium conservation and bone turnover in postmenopausal women. By S. SCHOPPEN[1], C. DE LA PIEDRA[2], A.M. PEREZ-GRANADOS[1], A. CARBAJAL[3] and M.P. VAQUERO[1], [1]*Department of Metabolism and Nutrition, Instituto del Frío, CSIC, C/Jose Antonio Novais 10, 28040 Madrid Spain,* [2]*Laboratory of Bone Physiopathology, Fundación Jimenez Díaz, Plz. Cristo Rey 1, Madrid, Spain and* [3]*Department of Nutrition and Bromatology I, Faculty of Pharmacy, Complutense University, Madrid, Spain*

The effects of the consumption of mineral water have been studied under the following aspects: bioavailability of minerals, effects on bone metabolism and osteoporosis risk according to calcium content, effects on the digestive tract, and effects on lipid metabolism and cardiovascular risk. Water favours the solubility of dietary components in general and exerts beneficial effects on constipation. Mineral waters, with a known mineral composition, can contribute to dietary intake of calcium, magnesium, potassium, etc. It is also known that calcium and magnesium from mineral waters are highly bioavailable, and certain mineral waters can be used as a supplement, representing a good way to improve intake of these minerals (Böhmer *et al*. 2000; Sabatier *et al*. 2002). Carbonated waters are rich in bicarbonate. We hypothesise that an alkaline mineral water, rich in sodium bicarbonate, could be considered an 'alkali-forming food' and could have a beneficial influence on bone metabolism and counterbalance the detrimental effects of 'acid-forming diets' rich in animal protein (New, 2002; Massey, 2003).

The present study was carried out with eighteen postmenopausal women on the Menopause Programme of the Area of Health and Consumption of the Madrid City Council. Their habitual dietary habits were determined by a modified dietary history, which was checked in the second period with a 3 d record. Women (*n*=18; mean age=53 years) included in the study had to have been amenorrhoeic for at least 1 year, healthy and not obese (BMI<30). No women was taking oestrogen replacement therapy, vitamin supplements, minerals and phytoestrogens or other medication known to affect bone and lipid metabolism. The study consisted of two intervention periods of 2 months each, in which women drank 1 litre daily of either the control mineral water (HCO_3^- 71.1; Ca^{2+} 25.2; Mg^{2+} 2.7; Na^+ 9; K^+ 1.4 mg/l) or the carbonated mineral water (HCO_3^- 2094.4; Ca^{2+} 43.6; Mg^{2+} 5.7; Na^+ 1116.5; K^+ 54.7 mg/l) (Vichy Catalán®). At the end of both periods, blood samples and 24-h urine were collected and serum separated. Urinary calcium and magnesium were determined by atomic absorption spectrophotometry and urinary phosphorus by colorimetry. Biochemical bone markers PINP and β-CTX were determined in serum by ELISA.

	Control mineral water period		Carbonated mineral water period	
	Mean	SD	Mean	SD
Energy intake (kcal)	2057	392	1877	301
Calcium intake (mg/d)	1085	471	1010	574
Magnesium intake (mg/d)	306	72	264	87
Phosphorus intake (mg/d)	1258	457	1140	336
24 h-urinary calcium (mg)	164.99	51.16	139.41*	55.52
24 h-urinary magnesium (mg)	77.84	32.37	79.22	31.27
24 h-urinary phosphate (mg)	0.83	0.18	1.03#	0.3
Urine pH	6.09	0.41	6.76	0.34

Significantly different from the control mineral water period: $^{*}P=0.037$, $^{\#}P=0.015$ (paired Student's *t*-test).

Dietary habits did not vary during the 4 months of the intervention study. The consumption of 1 litre of this carbonated mineral water for 8 weeks did not affect biochemical bone markers (PINP; β-CTX), Urinary calcium excretion was significantly decreased while phosphate excretion increased during the carbonated mineral water period. The results suggest that a moderate intake of an alkaline mineral water may help to conserve renal calcium.

This research was financed by Vichy Catalán. We thank Madrid City Council for its participation in the study.

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Dietary adequacy in a Swiss elderly population: developmental work of a Swiss Food Frequency Questionnaire (FFQ). By E. WYNN[1], M.A. KRIEG[1], J. CORNUZ[1], D.R WHITTAMORE[2], P. BURCKHARDT[1] and S.A. NEW[2], [1]*University Hospital (CHUV), 1011 Lausanne, Switzerland and* [2]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 5XH*

Assessment of dietary intake in specific populations is a critical component in the determination of the effect of nutritional factors on health. For the assessment of average long-term dietary intake in large numbers of individuals, food frequency questionnaires (FFQ) have emerged as a particularly useful tool, as they give a better approximation of usual long-term dietary intake than short-term records, can be self-administered and are relatively inexpensive to use. Prior to their use in studies, FFQ need to be adapted to the typical food consumption of the population under investigation and nutrients of specific interest to the metabolic system being examined need to be emphasised.

As part of an ongoing study into osteoporosis in a Swiss elderly population (SEMOF: Swiss evaluation of measurement of risk of osteoporotic fracture), the influence of dietary intake on bone health indices will be examined in a total of 500 ambulatory women from Lausanne. Since few dietary methodologies for populations exist within Switzerland, a dietary questionnaire is urgently required. This abstract presents the preliminary developmental work on a Swiss FFQ.

Dietary intake of fifty-one Swiss women (mean age 82 years, mean weight 61 kg) was assessed using a 4 d weighed record (WR). A dietician prior to starting the weighed record trained each woman and regular contact was made during and after the recording period to ensure compliance and competency. Food portion sizes were estimated using the SU-VI-MAX photo manual and all components of dietary intake were measured using Prodi 4.5^{R} software. The frequency of consumption of the following key food groups were calculated using the pyramid model: (1) bread, cereals and potatoes; (2) fruit and vegetables; (3) meat, fish and protein alternatives; (4) milk and dairy products.

Nutrients	RDA	Mean	SD	Range	Food groups	RDA	Average daily frequency of consumption
Energy (kJ)	7115-8370	6356	1504	3672–10299	Bread and cereals	3	2.95
Protein (g)	60.7	63.7	16.1	29–116.5	Vegetables	2–3	2.12
Fat (g)	56-77.8	60.7	20.5	18.5–119.9	Fruit	2–3	1.99
Calcium (mg)	1200	879.2	376.9	465.8–2765.8	Meat, fish and eggs	1	1.17
Phosphorus (mg)	800	1134.4	373	633.4–2654.1	Milk & dairy products	2–3	3.12
Magnesium (mg)	360	271.7	98.7	122.7–570.1			
Potassium (mg)	3000	2626	768	1097–4596			

As shown in the Table, the mean daily consumption of nutrients was lower (except for protein, fat and phosphorus) than the French RDA (Martin, 2001). Dietary intake compared to the RDA was 73.3% for calcium, 142% for phosphorus, 75.5% for magnesium and 88% for potassium. However, the average daily frequency of consumption of each food group was within the recommended frequencies of the Swiss Association for Nutrition's diet pyramid. The mean energy intake to BMR ratio for the group was 1.26 (SD +/- 0.33). This does indicate some under-reporting in the group, although it is important to note that the average age of women in this cohort was 82 years and hence the BMR equation may be somewhat overestimated.

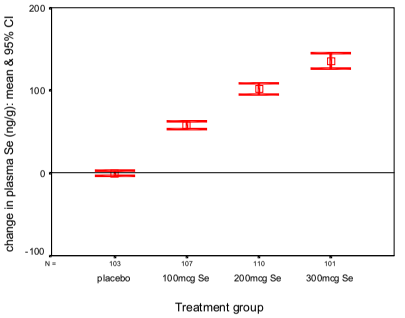
Further developmental work on the FFQ food list is required by calculation of the percentage contribution of food items as sources of bone health nutrients as well as total energy intake. The information collected from these weighed records will also enable determination of the FFQ food portion sizes.

Martin A (2001) *Apports nutritionnels conseillés pour la population française*. 3rd edn. France: Editions Tec & Doc.

The effect of selenium on mood and quality of life: results from the UK PRECISE Trial pilot study. By M.P. RAYMAN[1], M. WARREN-PERRY[1], J. CATTERICK[1], J. BLISS[2], E. HALL[2] and D. LAWRENCE[2], [1]*School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH and* [2]*Clinical Trials and Statistics Unit, Institute of Cancer Research, Sutton, Surrey, SM2 5NG*

There are a number of indications that selenium (Se) is important to the brain (Rayman 2000). Three published studies have shown an effect of Se supplementation or deprivation on mood, as measured by the POMS-BI (*Profile of mood states: bi-polar form*) questionnaire.

In the light of these reports, we investigated the effect of Se on mood in a study ancillary to the UK PRECISE (PREvention of Cancer by Intervention with SElenium) pilot study. In this double-blind, placebo-controlled intervention trial, 501 UK subjects aged 60–74 years were randomly allocated to receive 100, 200 or 300 µg Se/d as high-Se yeast or a placebo yeast. Mood was measured by the POMS-BI questionnaire at baseline and after 6 months of treatment. The POMS-BI responses can be expressed as a total mood score or broken down into six mood categories or subscales. The SF-36, a standardised, validated Health Survey ('quality of life') questionnaire that measures both mental (including categories that can be related to mood) and physical well-being, was also administered at these time points. Plasma Se was measured at baseline and 6 months. Se status rose significantly over the 6 months of treatment in all groups except the placebo group.



This randomised trial is by far the largest study to date to investigate the effect of Se on mood: 438 subjects (who completed the questionnaires at both time points) *v.* 91 subjects in total in the other studies. We found no significant difference (paired *t*-test) between mean total mood scores at baseline and 6 months in the individual treatment groups despite significant increases in plasma Se. The same was true for all of the mood subscales. There was also no significant difference (ANOVA P=0.681) in the change in total mood score (or mood subscale scores) across treatment groups. In support of our findings with the POMS-BI, we did not find any benefit of Se supplementation on any of the subscales of the SF-36.

Possible explanations for the different findings across studies include: the greater mean age of our subjects compared with those in previous studies; better baseline Se status in our cohort than in the study of Benton & Cook (1991); an alternative interpretation of Finley & Penland's (1998) data *viz.* that they may in fact have been observing regression to the mean in their study, as their low- and high-Se treatment groups were not equivalent in mood at the beginning of the study, the low-Se group having higher initial scores and the high-Se group having lower initial scores.

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Rayman MP (2000) *Lancet* **356**, 233–241.

The role of collagen hydrolysate on joint health. By S. OESSER, *Surgical Research of the Department of General Surgery and Thoracic Surgery of the University of Kiel, Michaelisstrasse 5, D-24105 Kiel, Germany*

Over the past few decades interest has expanded in the role of nutritional supplements as agents which may have a specific effect on disease pathophysiology. Collagen hydrolysate (CH) is one such supplement and enzymatic degraded collagen has been used in the treatment of degenerative diseases of the musculo-skeletal system. In recent years a number of clinical studies have shown the benefit of orally administered CH on joint health, especially on osteoarthritis (Moskowitz, 2000). The therapeutic mechanisms for the benefits, however, remain essentially unclear.

Experimental investigations have demonstrated intestinal absorption of CH in its high molecular form (Figure 1) with peptides up to 10 kDa as well as a preferential accumulation of these CH-derived fragments in cartilage tissue (Oesser *et al*. 1999).

Figure 1 - Qualitative absorption of collagen-hydrolysate

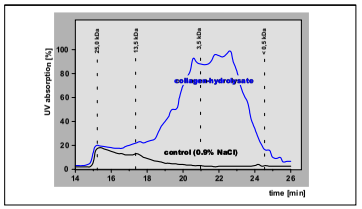
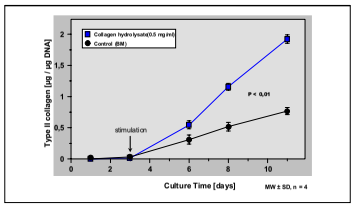
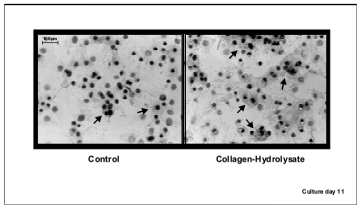


Figure 2 - Stimulation of type II collagen secretion



In current studies the influence of CH on the metabolism of mature chondrocytes has been investigated in a cell-culture model (Oesser & Seifert, 2003). It could be shown that the presence of CH in the culture medium led to a dose-dependent increase in type II collagen biosynthesis, whereas native collagen as well as collagen-free hydrolysates failed to stimulate the production of type II collagen in chondrocytes. These results clearly indicate a stimulatory effect of CH on the type II collagen biosynthesis of chondrocytes and suggests a possible mechanism for the regulation of collagen turnover in cartilage tissue (Figure 2). Moreover, utilizing immunocytochemical methods, it was demonstrated that in addition to an enhanced deposition of pericellular type II collagen in chondrocytes treated with CH (Figure 3), the amount of pericellular aggrecan was significantly increased as well, indicating that the cells were stimulated to synthesize a complete extracellular matrix (ECM).

Figure 3 - Immuncytochemical visualisation of type II collagen



Based on these results and clinical studies to date, CH might be of particular importance for the nutrition of cartilage tissue and might help to reduce degenerative alterations in the ECM. Moreover, the high safety profile of CH makes it especially attractive as a nutritional supplement in the prophylaxis of joint degeneration, as well as an agent with potential for therapeutic benefit in the treatment of osteoarthritis.

Moskowitz RW (2000) *Seminars in Arthritis and Rheumatism* **30**, 87–99.
Oesser S , Adam M, Babel W & Seifert J (1999) *Journal of Nutrition* **129**, 1891–1895.
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The role of hepatic nuclear factor 4-α (HNF4α) in mediating the effects of dietary saturated fatty acids on the expression of the microsomal triglyceride transfer protein gene. By T. VALLIM[1,2], J. LAMB[2], H.M. SIMS[1], A.J. BENNETT[2], M.A. BILLETT[2] D.A. WHITE[2] and A.M. SALTER[1], [1]*Schools of Bioscience and* [2]*Biomedical Science, University of Nottingham, Nottingham, LE12 5RD*

Microsomal triglyceride transfer protein (MTP) plays an essential role in the assembly of triacylglycerol-rich lipoproteins in the intestine and liver (White *et al.* 1998). Diets rich in saturated fatty acids (SFA) increase the expression of the MTP gene and thereby may increase the synthesis and secretion of very low-density lipoprotein (Bennett *et al.* 1995; Billett *et al*. 2000). SFA, in the form of acyl coenzyme A derivatives, have been suggested to regulate the activity of the nuclear transcription factor, HNF4α (Hertz *et al.* 1998). The aim of these studies was to investigate whether the effect of saturated fatty acids on MTP gene expression is mediated by HNF4α.

Figure 1

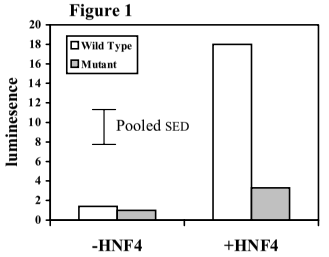
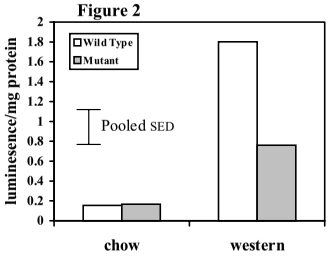


Figure 2



Analysis of the MTP promoter suggests the presence of three putative HNF4α binding sites. To investigate whether these sites play a role in regulating MTP gene expression, two versions of the promoter were linked to a reporter firefly luciferase gene. The first promoter construct contained all of the putative HNF4 binding sites (wild type), while in the second construct (mutated) each of these sites were mutated to prevent binding of HNF4. Constructs were transfected into primary cultures of hepatocytes either in the presence or absence of an HNF4α-expression vector and after 48 h the activity of luciferase was determined. The combined results of three individual experiments are shown in Figure 1. Increasing HNF4α expression upregulated MTP promoter activity and mutation of the three putative binding sites abolished this response (two-way analysis of variance indicated an interaction between type of construct and the presence or absence of the HNF4α expression vector, P=0.002).

In a second experiment, hamsters (two groups of three animals) were fed either normal chow or chow supplemented with 17.4%(w/w) fat, (both diets contained 0.06% cholesterol). The fat consisted of a mixture of beef tallow, corn oil and tripalmitin (containing approximately 42% saturated fatty acids, 18% monounsaturated and 17% polyunsaturated) approximating to a Western diet. Animals were fed for 3 weeks, hepatocytes were prepared and transfected with wild type or mutated constructs as above. Cells transfected with the wild type promoter (white bars) from fat-fed animals, expressed luciferase to a greater extent than those fed normal chow (Figure 2). This effect was significantly reduced in cells transfected with the mutated promoter (grey bar), indicating that the response to fat feeding was, at least partly, mediated by HNF4 (interaction between diet and construct, P=0.003).

We conclude that the effects of SFA on expression of MTP are partly mediated by HNF4.

This work was funded by BBSRC Project Grant D11993.

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Hertz R, Magenheim **J**, Berman I & Bar-Tana J (1998) *Nature* **392**, 512–516.
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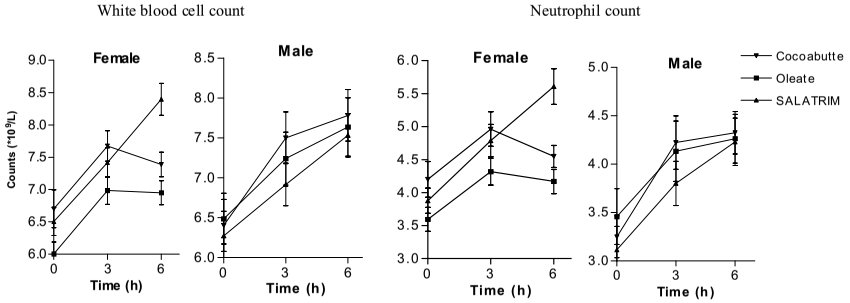
Postprandial leucocyte increase following a fatty meal is not related to FVII activation. By T.A.B. SANDERS[1], S.E.E. BERRY[1] and G.J. MILLER[2], [1]*Nutrition, Food and Health Research Centre, King's College London, 150 Stamford Street, London SE1 9NN and* [2]*Medical Research Council Cardiovascular Group, Wolfson Institute, St. Bartholomew's and the Royal London School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ*

Postprandial lipaemia induced by meals rich in oleic acid results in impaired endothelial function (Ong *et al.* 1999) and an increase in FVII coagulant activity (FVIIc) (Sanders *et al.* 2001). It has recently been suggested that the postprandial increase in leucocytes which occurs following a fatty meal may be responsible for the impairment in endothelial function (Van Oostrom *et al.* 2003) and it could also possibly explain the increase in FVIIc by providing an increased contact surface for activation of FVII. In a previously published study (Sanders *et al.* 2001), test meals containing oleate-rich sunflower oil or cocoa butter were found to result in similar postprandial increases in plasma triacylglycerol (TAG) concentration and to result in an increase in FVIIc and activated FVII concentration. However, a meal containing a structured stearic-acid-rich TAG (Salatrim) resulted in decreased postprandial lipaemia and no increase in FVIIc. We present previously unpublished data on the postprandial changes in blood counts from that study. The study was a randomized cross-over design and subjects received three test meals in random order. Full blood counts were measured at fasting and 3 h and 6 h following the meal. The results (mean values with SEM) for white blood cell (WBC) and neutrophil counts are shown for seventeen men and eighteen women aged 40–60 years.

WBC and neutrophil counts increased significantly ($P<0.0001$) following all three test meals but there was a significant gender × meal × time interaction ($P=0.006$ and $P=0.001$ respectively). The pattern



of response was similar in men following all three meals but in the female subjects the WBC and neutrophil count increased from 3 h to 6 h following the Salatrim meal but plateaued following the oleate and cocoa butter meals. Thus the increase in WBC and neutrophil counts was at least as great following the Salatrim meal as that following the oleate and cocoa butter meals but was not accompanied by the same increase in FVIIc. The postprandial increases in WBC and neutrophil count are probably a consequence of secretion of lymph associated with TAG-rich lipoproteins into blood.

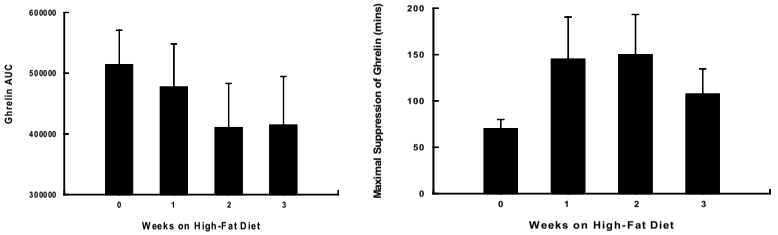
Ong PJ, Dean TS, Hayward CS, Della Monica PL, Sanders TA & Collins P (1999) *Lancet* **354**, 2134.
Sanders TA, Oakley FR, Cooper JA & Miller GJ (2001) *American Journal of Clinical Nutrition* 73, 715–721.
Van Oostrom AJ, Sijmonsma TP, Rabelink TJ, Van Asbeck BS & Cabezas MC (2003) *Metabolism* **52**, 199–202.

Suppression of the plasma ghrelin response during a 3-week fat-supplemented diet in healthy young men. By M.D. ROBERTSON[1], R.A. HENDERSON[2], G.E. VIST[2] and R.D.E. RUMSEY[2], [1]*Oxford Centre for Diabetes Endocrinology and Metabolism, University of Oxford, Oxford OX3 7LJ and* [2]*Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN*

Ghrelin, a 28-amino-acid peptide secreted primarily from the stomach, has been identified as the endogenous ligand for the growth hormone secretagogue receptor. Ghrelin stimulates hunger and is suppressed in the postprandial state and has been linked to both type 2 diabetes and obesity (Wang *et al.* 2002). Despite the link between adiposity and plasma ghrelin, postprandial regulation especially by dietary fat remains unclear. The aim of the present study was to investigate the effects of oral fat on plasma ghrelin levels before and during a short-term obesity-promoting diet.

With the approval of the local ethics committee, six healthy males (22–30 years; BMI 19-25 kg/m^2) underwent dietary intervention after completing diet and exercise diaries for 7 d. For three further weeks subjects followed their own exercise diary and their own diet diary, but supplemented with 88 g/d dietary fat (double cream and roasted peanuts). Oral fat tolerance tests (OFTT) were undertaken at baseline, 7, 14 and 21 d of fat supplementation.

The diet was changed from a mean of 29% to 45% energy intake from fat and there was a small weight gain noted each week ($P=0.009$). Ghrelin concentrations were significantly reduced during the baseline OFTT, with maximal suppression after 70 min ($P=0.033$). The postprandial ghrelin response (AUC) was significantly reduced following 2 weeks of fat supplementation ($P=0.005$) with earlier suppression noted after 3 weeks ($P=0.001$) (see Figure).



Postprandial triacylglycerol (TAG) concentrations were also significantly increased by fat supplementation ($P=0.009$) although no direct correlation was found between the ghrelin and TAG responses. The suppression of plasma ghrelin induced by increased dietary fat intake may be an initial metabolic signal in the aetiology of obesity.

Wang G, Lee H-M, Englander E & Greeley GH (2002) *Regulatory Peptides* **105**, 75–81.

Downregulation of hepatic mRNA expression of ghrelin is associated with maternal undernutrition during late gestation in the postnatal sheep. By E.A. BUTT, S. PEARCE, J. BISPHAM, M.E. SYMONDS and T. STEPHENSON, *Academic Division of Child Health, School of Human Development, University Hospital, Nottingham NG7 2UH*

Ghrelin, an endogenous ligand of the growth hormone (GH) secratagogue receptor, is primarily secreted from the stomach (Wang *et al.* 2002). Influences on the hypothalamic-pituitary axis also suggest a role in the secretion of (GH) (Masayasu *et al.* 2001), and providing a peripheral signal to the brain to stimulate food intake (Wren *et al.* 2001) and may determine hepatic sensitivity of insulin-like growth factor-1 (IGF-1) to GH. IGF-1 mRNA abundance in the fetal liver is nutritionally regulated (Brameld *et al.* 2000), and the extent to which IGF-1r and ghrelin mRNA abundance are downregulated by nutrient restriction in late gestation has not been established. This present study was undertaken to examine the effects of maternal undernutrition during late gestation, on the mRNA expression of IGF-1, its receptor (IGF-1r) and ghrelin.

Thirteen twin-bearing ewes of similar body weight were entered into the study. Six were nutrient-restricted (NR; fed 50% of total energy requirements) over the final month of gestation and seven controls (C) were fed to 100% of requirements, as calculated to produce a 4.5 kg lamb at term. One lamb from each ewe was then tissue-sampled at 1 d of age and one at 30 d of age. The relative abundance of IGF-I, its receptor and ghrelin mRNA to 18S rRNA were determined using RT-PCR. Results are given as means with their standard errors in arbitrary units (a.u.) and are expressed as a percentage. Differences in nutritional treatments were analysed using Tukey's *post hoc* test for ANOVA.

Sampling age	**Liver weight** (g)		**IGF-1** (a.u.)		**IGF-1r** (a.u.)		**Ghrelin** (a.u.)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1 day C	128.2	11.6	437	16	37	12.9	185	28.4
NR	82*	7.6	88*	26	78*	7.7	50**	11
30 days C	437	25.5	144	12	71	8.3	215	16.1
NR	415	21.7	415*	22	54	18.5	47**	22

Significantly different from control at * $P<0.05$, ** $P<0.001$ level, using ANOVA, Tukey's *post hoc*.

NR offspring had smaller liver at 1, but not 30 d of age, despite no difference in body weight between groups. Ghrelin mRNA abundance was consistently lower in the liver of NR offspring. In contrast, at 1 d IGF-1 mRNA was reduced but its receptor upregulated. These adaptations may have contributed to the retardation of liver mass by 1 month of age when IGF-1, but not receptor, mRNA was higher in NR offspring. In conclusion, maternal nutrient restriction over the final month of gestation restricts liver growth and may increase hepatic sensitivity to IGF-1. As a consequence 'catch-up' growth of the liver occurs after birth. The extent to which this adaptation may be related to altered ghrelin sensitivity remains to be established.

E.A. Butt was supported by a BBSRC Scholarship.

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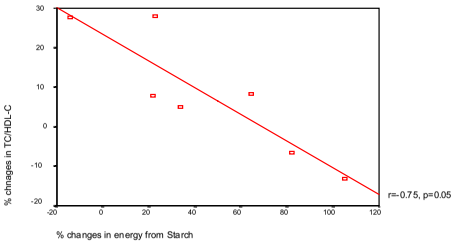
Masayasu K, Hiroshi H & Hisayuki M (2001) *Trends in Endocrinology and Metabolism* **12**, 118–122.
Wang G, Heung-man L, Englander E & Greeley GH (2002) *Regulatory Peptides* **105**, 75–81.
Wren AM, Seal LJ, Cohen MA & Brynes AE (2001) *Journal of Clinical Endocrinology and Metabolism* **86**(12), 5992–5995.

Relationship between changes in percentage energy from dietary carbohydrate and changes in plasma lipid concentrations in healthy postmenopausal women. By S.R. AREFHOSSEINI, S. HIGGINS and C.A. EDWARDS, *Human Nutrition Section, Division of Development Medicine, University of Glasgow, Yorkhill Hospitals, Glasgow G3 8SJ*

Coronary heart disease (CHD) has been recognized as the leading cause of death and disability among postmenopausal women (Rich-Edwards *et al.* 1995). The current dietary advice for adults including postmenopausal women to reduce risk of CHD is the replacement of saturated fat with carbohydrate (Department of Health, 1991). However, there is some evidence that high-carbohydrate diets may adversely influence plasma lipid concentrations (Jeppesen *et al.* 1997). We aimed to investigate the effects of a high-carbohydrate, low-fat diet with emphasis on increasing starch intake on lipid profile in free-living postmenopausal women.

Eight healthy postmenopausal women (aged 56.1 (SD 6.9) years and BMI 25.2 (SD 2.9) kg m^{-2}) participated in the study and undertook a dietary trial based on the current dietary guidelines (50%, 35% and 15% energy from carbohydrate, fat and protein, respectively for 4 weeks) (Department of Health, 1991). The intervention diet was based on the subject's habitual diet with modification and encouragement to include more starchy foods. Dietary intakes were assessed by weighed food record and fasting plasma triacylglycerols (TAG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) were measured and low-density lipoprotein cholesterol (LDL-C) concentrations were estimated at baseline, and at 1 and at 4 weeks after intervention.

Daily energy intake and the proportion of energy from total fat, SFA and MUFA were significantly lower ($P<0.05$) while the proportion of energy from total carbohydrate, starch and sugars were significantly higher ($P<0.05$) following 1 and 4 weeks of dietary intervention. In addition, HDL cholesterol concentration was significantly lower ($P<0.05$) following 4 weeks of dietary intervention (Arefhosseini *et al.* In the Press). The percentage change in TC/HDL-C ratio, LDL-C and TAG concentrations were significantly inversely correlated with the percentage change in energy from starch ($r=-0.75$, $P=0.05$; $r=-0.82$, $P=0.02$ and $r=-0.82$, $P=0.02$, respectively, Spearman's correlation) after 4 weeks of dietary intervention. In addition, there was a significant positive association between change in energy from simple sugar and change in TC/HDL-C ratio ($r=0.74$, $P=0.04$). No statistically significant relationships were found between the percentage change in fasting lipid concentrations and percentage change in dietary intakes of fat.



Our findings indicate a protective effect of complex carbohydrates but an adverse influence of simple sugars on fasting lipid concentrations after the dietary intervention.

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The effect of low- and high-glycaemic-index meals on appetite, satiety and energy intake after 6 d low- or high-glycaemic-index diet. By A. NOROUZY, A.R. LEEDS and P.W. EMERY, *Nutrition, Food and Health Research Centre, King's College London, London SE1 9NN*

Low glycaemic index (GI) meals could help to suppress appetite (Robert, 2000), and there is some evidence of lower energy intake after a low-GI meal compared with a high-GI meal (Ludwig & Majzoub, 1999). The aim of this study was to determine the effect of consuming one low- or high-GI meal on each of six consecutive days on subjective measures of appetite and on voluntary energy and macronutrient intake.

Eleven healthy subjects with normal body weight participated in this cross-over study. Subjects were fed an intermediate GI meal on the previous evening in the metabolic unit and fasted overnight for at least 10 h. On the first day, a low- or high-GI breakfast (310 kcal, GI 45 or 85) was consumed, followed 3.5 h later by an isocaloric low- or high-GI lunch. An *ad-libitum* buffet meal was provided 90 min after lunch. Visual analogue scales (VAS) rating appetite, satiety, feeling of fullness and amount that could be consumed were completed every 30 min from just before breakfast until 5.5 h after lunch. The subjects also kept a weighed food diary throughout this first day. A low- or high-GI lunch was then provided in the metabolic unit for next 5 d. Subjects completed VAS each day at 08.30, 10.30, 12.30, 14.30, 15.30, 18.30 and 22.30 hours, and kept a food diary in household measures. After a 2-week wash-out period the study was repeated with the subjects crossed over to the opposite treatment.

VAS scores for appetite, satiety, fullness feeling and prospective consumption for the different time points were added together to give a single reading for each of the six days. The Table below shows the median (SD) values for summed VAS scores. There was a lower appetite score (P=0.01), higher satiety (P=0.04), higher fullness feeling (P=0.05) and lower prospective consumption (P=0.01) during the low-GI compared with the high-GI period (Wilcoxon test).

	Appetite	Satiety	Fullness feeling	Prospective Consumption
Low GI	1788 (370)	2631(270)	2518(340)	2076(540)
High GI	2263(480)	2180(330)	2337(340)	2451(604)

Mean (SD) nutrient intake values calculated from the food diaries are shown in the Table below. There was a significantly lower energy intake during the low-GI compared with the high-GI period (P=0.007, paired t-test). However, there were no significant differences in individual macronutrient intakes (P>0.05, paired t-test).

	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)	Fibre (g)
Low GI	2930 (328)	324(40)	106(14)	177(19)	46(3)
High GI	3268(565)	338(49)	110(19)	178(13)	47(6)

This study demonstrates that consumption of low-GI foods induced lower appetite and higher satiety compared with high-GI meals over 6 d. This led to a reduction in energy intake but did not alter the balance between the macronutrients. The reduction in intake is potentially useful as part of a weight management programme. These results were achieved by prescription of one low-GI meal for 5 d and two low-GI meals for 1 d. The difference in GI was not extreme (45 *v.* 85), and was produced by altering the staple (e.g. pasta *v.* rice) but not the sauce within the meal.

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Investigation of the action of pancreatic α-amylase on starches from different botanical sources. By R.Y. TAHIR[1], P.R. ELLIS[1], P.J. BUTTERWORTH[1] and L. HARTLEY[2], [1]*Biopolymers Group, School of Health and Life Sciences, King's College London, Franklin-Wilkins Building, London, SE1 9NN and* [2]*RHM Technology Ltd, The Lord Rank Centre, High Wycombe, HP12 3QR.*

Starch is a major component of the human diet. Originally it was thought that starch, whatever its source, is digested at the same rate and to the same extent. However, there is compelling evidence in the literature to indicate that different starch-containing foods with identical amounts of starch elicit different postprandial glycaemic responses. This has led to starch-rich foods being ranked according to their postprandial glucose response, the so-called glycaemic index (GI).

The aim of this study is to investigate the action of pancreatic α-amylase (EC 3.2.1.1) on various native and hydrothermally-treated starches, in order to understand how structural differences in the substrate influence enzyme kinetic parameters. This will help to provide a mechanistic understanding of the differences in GI.

An enzyme kinetic assay, originally developed by Slaughter *et al.* (2001), was modified and used in a series of experiments. Improvements were made to the original enzyme assay procedure, including the use of sodium carbonate as a quenching agent, rather than the heat denaturation process used previously. Additional steps were incorporated into the original method, to allow better temperature control during the enzyme assay. Improvements were also made to the reducing sugar assay, which is used to quantify the release of hydrolysed products (e.g. maltose) from starch. Starch sources from wheat, potato, waxy and non-waxy rice and pea were used as substrates for α-amylase to test the modified method.

Starch source	K_M (g/100ml PBS)*		Relative k_{cat}/K_M †	
	Native	Gelatinised	Native	Gelatinised
Wheat	0.50	0.16	1.00	42.7
Potato	1.37	0.40	0.14	32.6
Non-Waxy Rice	0.72	0.28	3.70	31.3
Waxy Rice	0.69	0.22	4.08	35.1
Pea	1.85	0.29	1.23	58.1

* K_M = substrate concentration at which the velocity is half-maximal; it is an index of substrate accessibility.
† k_{cat}/K_M is a measure of the efficiency of the amylolytic process.

Overall, the results showed that the enzyme assay was reproducible and also because there was a good correlation between the modified assay and the original method (Slaughter *et al.* 2001). The kinetic data indicated that the catalytic efficiency (k_{cat}/K_M) was strongly influenced by the starch source. Moreover, the results were consistent with our previous work showing that the catalytic efficiency of amylase was substantially increased following heat processing of starch. The relative k_{cat}/K_M values for the native starches suggest that the rice starches are a better substrate for α-amylase, whereas pea starch was found to be the most efficiently hydrolysed substrate of all the gelatinised starches.

Recently, we extracted starch from chemically mutated pea seeds using a centrifugation method developed at the John Innes Centre (Bogracheva *et al*. 1995). The starches vary in their physical–chemical structures and properties. The use of such starches should allow a better mechanistic understanding of how starch behaves during digestion.

This research was supported by the BBSRC and RHM Technology Ltd.

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The glycaemic index and glycaemic load of decorticated finger millet. By N.G. MALLESHI[1], H.J. LIGHTOWLER[2] and C.J.K. HENRY[2], [1]*Central Food Technological Research Institute, Mysore 570 013, India and* [2]*Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford OX3 0BP*

Finger millet (*Eleusine coracana*) has long been recognised as being beneficial to people with diabetes (Pathak *et al*. 2000) due to its putative slow carbohydrate release. Finger millet is commonly pulverised without any pre-treatment and the whole meal is made into flatbread or porridge. However, the dark-coloured, highly fibrous seed coat and characteristic odour of finger millet are major limitations in using the flour in the diets of non-traditional consumers. Decorticated finger millet, prepared following a recently developed process at the Central Food Technological Research Institute, Mysore, largely overcomes these limitations. Decorticated finger millet has the characteristics of couscous and may be eaten boiled as a cooked cereal.

The aim of this study was to determine the glycaemic response to decorticated finger millet in normal individuals. Eleven healthy subjects (four male and seven female, mean age 32 (SD 9) years, mean BMI 21.8 (SD 2.7)) were recruited. Decorticated finger millet was presented as a boiled cereal. Finger millet and a standard food (white bread) were each fed as portions providing 50 g available carbohydrate to all subjects in a randomised, repeated-measures design. Each subject repeated the standard food test on three separate occasions and the decorticated finger millet test on two separate occasions, with more than 1 d between tests. A standard or test meal was eaten in the morning after a 10–12 h overnight fast. Blood samples were taken at fasting and at 15, 30, 45, 60, 90 and 120 min postprandially using a multipatient lancing system (Softclix Pro®); blood glucose was measured using an automatic analyser (Accu-Chek® Advantage).

Incremental areas under the blood glucose response curves (AUC), ignoring area beneath the baseline, were calculated geometrically (FAO/WHO, 1998). For each subject, the AUC for decorticated finger millet was expressed as a percentage of the standard response (white bread); the mean of the resulting values was the glycaemic index (GI) of decorticated finger millet. The glycaemic load (GL) of decorticated finger millet was calculated as the amount of available carbohydrate in a typical serving of the food, multiplied by the GI of the food and divided by 100.

	GI (bread = 100)		GI (glucose = 100)		Serving size	Available CHO	GL
	Mean	SE	Mean	SE	(g)	(g/serving)	(per serving)
Decorticated finger millet	89	10	64	7	120	42	27
Millet, boiled*	101	–	71	10	150	36	25
Millet flour porridge[1]	153	14	107	–	–	–	–

*Data from Foster-Powell *et al*. (2002).

The GI of decorticated finger millet was 89 (SE 10) and fell within the intermediate GI food range (77–98, where bread = 100). This value compares favourably to that of boiled millet (Foster-Powell *et al*. 2002). The purpose of calculating the GL was to quantify the overall glycaemic effect of a portion of decorticated finger millet. The GL of the decorticated finger millet was similar to that reported for millet (Foster-Powell *et al*. 2002) and fell within the high GL range (≥20). In conclusion, decorticated finger millet had an intermediate GI value. The GL value suggests that the cereal may not be ideal in the dietary management of diabetes, as previously believed. Further work on the GI of minor cereals from the tropics may be a useful avenue of research.

N.G.M was supported by a Raman Research Fellowship, CSIR (Government of India), New Delhi.

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The relationship between dietary Glycaemic Index and urinary chromium in British adults. By M. HAJI FARAJI[1], A.R. LEEDS[1], J. POWELL[1] and G. FROST[2], [1]*Department of Nutrition and Dietetics, King's College London, 150 Stamford Street, London SE1 9NN and* [2]*Department of Nutrition and Dietetics, Hammersmith Hospital, London W12 0HS.*

Chromium (Cr) is an essential nutrient required for sugar and fat metabolism. Normal dietary intake of Cr for humans in a western population is sub-optimal. Insufficient dietary intake of Cr leads to signs and symptoms that are similar to those observed for diabetes and cardiovascular diseases. Chromium was originally identified as the active component of the 'Glucose tolerance factor', but is now believed to be the specific metal ion of chromodulin. Cr primarily functions by potentiating insulin action, which leads to the normalisation of post-prandial glucose levels. Once chromodulin (containing Cr) has been mobilised, in response to increased glucose metabolism and/or elevated insulin response, it is not retained but is lost in the urine. In humans, typical urinary Cr concentrations vary from 0.24 to1.8 µg Cr/litre in healthy individuals. A low Glycaemic Index (GI) diet can reduce post-prandial glucose levels and help keep blood glucose levels within a near normal range, and may increase insulin sensitivity. Since both GI and Cr have been shown to be involved in reducing risk of the complications associated with diabetes, we anticipated a direct link between dietary GI and Cr excretion. Persistently raised urinary Cr levels (e.g. from a habitual high-GI diet) could deplete body stores of chromium leading ultimately to a chromium-depleted state and increased risk of diabetes.

We investigated the association between dietary Glycaemic Index and urinary Cr, among 2020 British adults who participated in the 1986/87 cross-sectional British Adult Dietary Survey. We measured Cr in forty-eight randomly selected urine samples from 24 hour urine collections made by subjects of the above population, defined as a control group (to ensure that urine Cr levels fell within the normal range), forty-eight subjects ingesting a high-GI diet (≥85) and forty-eight with a low-GI diet (≤78) using Dynamic Reaction Cell- Inductively coupled plasma Mass Spectrometry (DRC-ICPMS).

There was no significant difference in urinary Cr excretion between the low-GI group (0.95±0.80µg/24h) and the high GI group (0.73± 0.32 µg/24 h). However, Cr excretion of the low-GI group per gram of carbohydrate (CHO) ingested (0.007±0.011µg24 h per gram CHO) was significantly higher than the high-GI group (0.003 ± 0.002 µg24 h per gram CHO), (P<0.008).

Mean and standard deviation of nutrients, glycaemic index and glycaemic load in the low and high GI groups

Variable	Group	Mean ± SD	P value	N
Energy intake (kJ/d)	Low GI	7239.3 ± 3170.8		46
	High GI	9065.7 ± 3122.3	0.007	44
Fat intake(g/d)	Low GI	70.5 ± 36		46
	High GI	92.2 ± 32	0.004	44
CHO intake(g/d)	Low GI	190.3 ± 88		46
	High GI	252.6 ± 94	0.002	44
Glycaemic Index	Low GI	73.1* ± 4.3		46
	High GI	88.2** ±2.5	0.0001	44
Glycaemic load†	Low GI	143 ± 71		46
	High GI	221 ± 84	0.0001	44

GI values, against bread = 100 as standard, * GI 51, ** GI 62 against glucose= 100
† Glycaemic load is GI of diet × g CHO of diet

The high urinary Cr seen in the low-GI diet group (expressed per gram CHO) may reflect higher total body concentration, or a higher dietary intake. Further studies on the effect of dietary GI on chromium excretion are indicated.

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A reduction in glucose absorption is observed in the presence of dietary polyphenols using the Caco-2 cell model of human intestinal absorption. By K.L. JOHNSTON, P.A. SHARP, M.N. CLIFFORD and L.M. MORGAN, *Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH*

Polyphenols are a complex group of chemicals that are widely distributed throughout the plant kingdom and thus form an integral part of the human diet. Accumulating evidence has reported that certain classes of dietary polyphenols have the ability to inhibit intestinal glucose uptake. This has been shown using a variety of *in vitro* methods, *in vivo* in rats and recently we have shown a similar effect in humans using different dietary sources of polyphenols (Johnston *et al*. 2002, 2003). The aim of this study, therefore, was to determine the effects of different groups of polyphenols on intestinal glucose absorption using the Caco-2 cell model of human intestinal absorption.

Caco-2 cells were seeded at a density of 1×10^4 cells cm^{-2} into six-well plates and were grown for 17 d. Glucose uptake assays were performed under sodium-dependent or sodium-free conditions using a radiolabelled transport buffer (^{3}H glucose) that contained 1 mM glucose and 100 μM of the test compound or control (mannitol). Uptake was measured over 2 min and was initiated by the addition of control or test solutions. After this time the wells were aspirated and the reaction terminated by the addition of ice-cold PBS. Cells were solubilised overnight in 200 mM NaOH and their ^{3}H glucose content was measured to determine glucose uptake. The following groups of compounds were amongst some of those tested: the dietary glycosides phloridzin, neohesperidin dihydrochalcone, arbutin and rutin, the non-glycosylated dietary phenols ellagic acid, gallic acid, EGCG and ECG, and the model aglycones quercetin, myricetin, apigenin and phloretin.

Glycosides		
	Na^+ dep.	Na^+ free
Rutin	84	100
Arbutin	73	100
Neohespe.	50**	90
Phloridzin	44***	100

Aglycones		
	Na^+ dep.	Na^+ free
Phloretin	100	53**
Quercetin	100	25**
Apigenin	90	61*
Myricetin	70	15***

Non-glycosylated		
	Na^+ dep.	Na^+ free
ECG	12***	36**
EGCG	54**	70**
Gallic	84	100
Ellagic	66*	100

Values are expressed as percentage of control (n=4).
Indicates significant differences from control (P<0.05, **P<0.01, ***P<0.001) by one sample t-test.

Results showed that dietary glycosides were more effective at inhibiting glucose uptake under sodium-dependent conditions with almost no effect under sodium-free conditions, which would suggest an interaction with the sodium-dependent intestinal transporter SGLT1. The reverse, however, was true for the model aglycones which were more effective under sodium-free conditions, which implies an interaction with the facilitated GLUT transporters. The non-glycosylated polyphenols appear to exhibit non-specific inhibition, possibly via steric hindrance, since certain types were effective under both sets of conditions. These data show that different classes of polyphenols reduce intestinal glucose uptake in the Caco-2 cell model via interaction with different transport mechanisms depending on their chemical structures.

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Sedentary lifestyles are associated with being overweight and consumption of savoury snacks in young people (4–18 years). By K.L. RENNIE[1] and S.A. JEBB[1], [1]*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL*

Sedentary activities, such as television viewing, have been implicated in the rise in the prevalence of excess weight in children, but the factors that mediate this relationship are not clear (Crespo, 2001).

Data from the National Diet and Nutrition Survey conducted in 1997(Gregory, 2000), a nationally representative sample of young people aged 4–18 years, were used to examine associations between overweight, inactivity and selected food groups: savoury snacks, fruit biscuits, chocolate confectionery and cakes. A weighed 7 d diet diary and hours spent in sedentary activity, including television and computer games, were collected in 816 boys and 783 girls. Groups were defined as being overweight (OW) or lean (LN) using the age-specific International Obesity Taskforce cut-offs for body mass index, equivalent to a BMI >25 kg/m^2 at age 18 years (Cole, 2000). All data were adjusted for seasonality.

After adjustment for seasonality, time spent on sedentary activity was positively associated with age in both girls and boys (P< 0.001) and with further adjustment for age, inversely associated with socio-economic category in girls (P=0.002) and boys (P=0.04). After adjustment for age and gender, OW spent significantly more hours per week in sedentary activity than LN (18.6 h (SE 0.6) *v.*16.9 h (SE 0.3, P=0.005). The odds ratio (95% CI) for being OW between quartiles of sedentary activities was 1.18 (1.04, 1.33), P=0.008, and was significant in girls (16.0 (0.4) *v.* 18.0 (0.8) h, P=0.02), but not boys (17.8 (0.4) *v.* 19.5 (0.8) h, P=0.06). No associations were found between consumption of the selected food groups and OW.

Greater time spent in sedentary activity was associated with higher savoury snack consumption in girls (P<0.001) and boys (P=0.001) after adjustment for age and height. Those who were sedentary for 5+ h/d consumed more savoury snacks per week than those who were inactive for <2 h/d (mean (SE) girls: 134 g (13 g) *v.* 100 g (4 g), P=0.003; boys: 141 g (14 g) *v.* 102 g (6 g), P=0.004). After adjustment for age and height, weekly fruit consumption was significantly lower in girls who reported high sedentary activity (5+ h/d) than those who reported less inactivity (427.1 g (15 g) *v.* 248.7 g (66 g), P<0.009) and boys who were sedentary 5+ h/d consumed more biscuits per week than those reporting lower inactivity (193.8 g (20 g) *v.* 132.6 g (5.0 g), P=0.003). No associations were found with chocolate confectionery or cakes and sedentary activity.

These data emphasise the importance of sedentary activity as a specific lifestyle factor for the risk of overweight and suggests it may operate by both constraining energy expenditure and increased consumption of energy-dense snack foods.

We acknowledge the Office for National Statistics for depositing an electronic copy of the dataset with the UK Data Archive and for granting permission for these further analyses.

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Food fortification in the UK: impact on calcium intake in young people aged 4–18 years. By C.W. THANE and C. BOLTON-SMITH, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL*

Fortification of food with calcium was introduced in the early 1940s with the mandatory fortification of white flour, to compensate for the increased extraction rate of flour introduced in the UK due to wartime austerities. The continued need for mandatory Ca fortification of flour has been questioned several times, notably with a Government report (DHSS, 1981) that recommended its abolition. However, with respect to Ca, the law has not been repealed.

The impact of fortification on dietary Ca intake was examined in a nationally representative sample from the 1997 National Diet and Nutrition Survey of young people aged 4–18 years (Gregory *et al*. 2000). Ca fortification is either mandatory (e.g. 94–156 mg/100 g added to almost all flours except wholemeal) or voluntary (e.g. some breakfast cereals and soft drinks). From over 6000 foods, around 600 were identified as being subject to Ca fortification, and the amounts of Ca due to fortification in the respective foods estimated using recipe and manufacturers' information.

Mean daily dietary Ca intakes and contributions of fortification were derived from 7 d weighed dietary records for 1420 participants according to gender, age group, occupational social class of head of household, region and season. Adequacy of dietary Ca intake was examined, with and without contributions from fortification, by comparison with the appropriate LRNI and RNI (Department of Health, 1991). The impact on dietary Ca:P intake ratio was also examined.

Overall, fortification contributed 14% (range 0.2–41%) of mean dietary Ca intake, of which 7% was derived from white bread. Apart from a significant rise with age in the percentage contribution of Ca fortification from cereals (including bread) to dietary Ca intake, few socio-demographic differences existed. The impact of excluding current Ca fortification on dietary Ca intake is shown below.

Fortification state of foods	Ca intake as % of RNI	% with Ca intake <RNI	% with 'low' Ca intake (<LRNI)	Ca:P intake ratio
Including Ca fortification	113 (111, 116)	44 (42, 47)	7 (6, 9)	0.71 (0.70, 0.72)
Excluding Ca fortification	99 (97, 101)	57 (55, 60)	13 (11, 15)	0.62 (0.61, 0.63)
P value	<0.0001*	<0.0001†	<0.0001†	<0.0001*

For each variable, n=1420. Values are arithmetic means and percentages (each with 95% CI) for continuous and discrete variables respectively. All tests were one-tailed: *Paired *t*-test, †McNemar's test. Statistically significant differences between measures of Ca intake/adequacy remained for each of the socio-demographic variables listed above.

Boys and girls aged 11–18 years reported the highest prevalence of Ca intakes below the LRNI, with significant increases (boys 8% to 17%, girls 18% to 27%, each P<0.0001), after excluding the contribution from fortification. Results presented above were not altered significantly after excluding likely under-reporters (energy intake:estimated BMR <1.2).

Given the importance of adequate Ca intake for normal bone development during childhood, and particularly during adolescence and young adulthood, current Ca fortification of foods appears desirable and further voluntary fortification has the potential to bring further benefits.

This work was funded by the Union of EU Soft Drinks Associations (UNESDA). We also acknowledge the UK Data Archive for granting permission for these further analyses and the Office for National Statistics for depositing an electronic copy of the dataset, which bears no responsibility for this further analysis or its interpretation.

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FAST: Food Assessment in Schools Tool. By A.J. ADAMSON[1], J.M. GRIFFITHS[1], L.E. CARLIN[1], K.L. BARTON[3], W.L WRIEDEN[3], J.N.S. MATTHEWS[2] and J.C. MATHERS[1], [1]*Human Nutrition Research Centre,* [2]*School of Maths and Statistics, University of Newcastle, Newcastle NE1 4LP and* [3]*Centre for Public Health Nutrition Research, University of Dundee, DD1 9SY*

Measuring food intake in young children is particularly problematic; issues of literacy, writing skills, limited food recognition skills, memory constraints and concentration span are of particular concern. No tool existed that offered the facility of non-specialist assessment of the diets of young children. The objective was to produce a concise, simple tool that could be used by non-specialists to assess the diets of large groups of primary-school children with a particular focus on those under the age of 7 years. This tool was required by the Department of Health specifically to assess the impact of the National School Fruit Scheme on the diet of children aged 3–7 years. The method was given the acronym FAST (Food Assessment in Schools Tool).

Key principles in the development of the tool were that the assessment should not place any burden on the school and that children could not be expected to record any information themselves. A prospective dietary assessment method was designed which incorporates elements of both the food diary and the food frequency methods. No interviews or estimations of portion size were required in data collection. The development of FAST drew extensively on the data sets of the relevant National Diet and Nutrition Surveys (NDNS) (Gregory *et al.* 1995,; Gregory & Lowe, 2000). A plot of energy intake recorded by NDNS for children aged 3–7 years against time was used to determine six discrete timeslots for each day. Within each of the timeslots the foods which were most frequently consumed by children aged 3–7 years were determined, and these foods (or composites of them) were included in simple tick lists printed within each timeslot. An additional space was provided to record items not appearing in the simple tick lists. Intake was recorded over 4 d (including three schooldays). Paid parent lay-observers recorded intake during the schoolday. Parents and other carers completed the records at all other times. Age- and sex-specific portion sizes for all foods consumed were ascertained from the NDNS database. Nutrient compositions of foods included in the tick lists were calculated, with weighting within composites according to frequency of consumption by children aged 3–7 years, derived from the NDNS. The validity of FAST was measured by comparison with 4 d weighed dietary intakes (WFI) collected in a crossover design from a subsample of participating children (n=70) and then manually converted to FAST. Method comparison analysis included simple correlation, assessment of bias and limits of agreement.

Consent was received for 192 children (45%); 186 (97%) returned completed diaries of which 182 (95%) were used in the analysis. The tool was well accepted by the schools, teachers, parents and children. No problems were encountered during data collection. Lay-observers were competent to the task after brief training (2 h) and found the experience rewarding. Schools reported that FAST had created little or no disruption and also that children had enjoyed participating.

The method was principally intended to assess fruit and vegetable intake (F&V). For fruit, mean intakes of 2.1 and 2.0 portions/d (limits of agreement −0.6 to 0.5) and for F&V together 3.4 and 3.2 portions/d (limits of agreement −1.3 to 0.8 portions) were reported by WFI and FAST, respectively. The Table shows the results for weight of fruit and F&V together.

	Weighed food intake		**FAST**		*P* value of difference	Limits of agreement for class of 30
	Mean (g)	SD	Mean (g)	SD		
Fruit	184	134	189	140	0.54	−19.5 to 29.5
Fruit and vegetables	250	249	249	151	0.82	−30.8 to 26.5

FAST offers a novel approach to dietary assessment for young children. It has the potential to provide a robust measure of the impact of the National School Fruit Scheme.

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A comparison of children's and adult's ability to estimate portion size using food photographs. By E. FOSTER[1], M. NELSON[2] and A.J. ADAMSON[1], [1]*Human Nutrition Research Centre, University of Newcastle NE1 7RU and* [2]*Department of Nutrition and Dietetics, Kings College, London SE1 8WA*

Assessing nutrient intake at the individual level requires a determination of portion size for each food consumed. Portions can be weighed, but weighing may influence dietary intake as, either consciously or subconsciously, the respondent reduces or alters their intake to avoid having to weigh the food (Macdiarmid & Blundell, 1997). Where children are the subjects of dietary investigations, weighing foods consumed outside of the home and away from the parents poses additional problems. A number of methods have been developed to assist subjects in providing an estimate of portion size as an alternative to weighing foods, including the use of food photographs (Nelson *et al*. 1997).

This study examines children's ability to estimate food portion sizes using two methods: (1) the MAFF photographic atlas of food portion sizes, based on adult portion sizes (Nelson *et al.* 1997), and (2) an atlas developed for use with children and based on children's portion sizes (Children Assessing Food Size study (CAFS)). For comparison, data from a study examining adults' portion size perception and conceptualization using food photographs is also presented (Nelson *et al*. 1996).

Children aged 4–14 years were asked to estimate the portion size of foods they had either consumed or been shown the previous day. The ten foods included in this analysis, varying according to shape, texture, etc., featured in both the adult study and the present study. Errors in the assessment of portion size were assessed as percentage error (weight of food in photograph selected − actual weight)*100/actual weight) and as a ratio (weight of food in photograph selected/actual weight).

Subjects	Tool	No. of estimates	Mean % error	95% CI of mean % error	Geometric mean ratio	95% CI of geometric mean	*P**
Adults	Adult atlas	196	12.3	2.7–21.8	0.995	0.914–1.062	–
Children	Adult atlas	872	54.5	50.0–59.1	1.419	1.381–1.458	0.000
Children	Child atlas	350	11.2	2.3–20.1	0.888	0.825–0.955	0.870

**P* values are based on unpaired *t*-tests comparing geometric mean ratios between (a) adults using adult atlas and children using adult atlas and (b) adults using adult atlas and children using child atlas.

The geometric mean ratio for adults is not significantly different from 1 (0.995, CI 0.914–1.062).Children, however, greatly overestimated food portion size using the adult food photograph atlas (Nelson *et al.* 1997), (1.419, CI 1.381–1.458). In contrast, children using the child atlas marginally underestimated portion size (0.888, CI 0.825–0.955). The mean error in children's estimates of portion size using an atlas based on children's portion sizes was not statistically significantly different from that of adults. This highlights the need for portion sizes presented in a photographic atlas of food to be appropriate to the target population.

The work on children's portion sizes (CAFS study) is part of a project currently funded by the Food Standards Agency (N08019) exploring tools to assist in portion size estimation by children. The work on adults' portion sizes was funded by MAFF (2B015).

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How accurate are standard children's food portion sizes for estimation of dietary intake of children of different ages? By K.L. BARTON[1], W.L. WRIEDEN[1], P.J. LONGBOTTOM[2] A.J. ADAMSON[3] and S.A. OGSTON[1], [1]*Faculty of Medicine, University of Dundee, Ninewells Medical School, Dundee, DD1 9SY,* [2]*Arbroath Infirmary, Arbroath, DD11 2AT and* [3]*Human Nutrition Research Centre, University of Newcastle upon Tyne, Newcastle NE1 4LP*

Standard food portion sizes (MAFF, 1993) are useful for estimating the dietary intakes of groups where weighed intakes are not available, are impracticable or are too expensive. Although not advised for use in assessing individual diets, they are useful for pooled data of normal healthy individuals. To date there has been no equivalent source of typical children's food portion sizes.

The aim of the present study was to produce and test a set of typical food portion weights for children aged 1–3, 4–6, 7–10, 11–14 and 15–18 years, corresponding to the UK dietary reference values (Department of Health, 1991).

Food portion data from 3374 children aged 1½–4½ and 4–18 years, reported in the National Diet and Nutrition Surveys (NDNS) (Gregory *et al*. 1995; Gregory & Lowe, 2000) were examined and foods eaten by ≥1% of all children were established. These foods were grouped by similar type and composition, and a list was compiled of grouped foods, eaten by ≥2% of all children, which were allocated a new code and food name in order to ease future calculations. Foods that had more than one serving size (e.g. milk on cereal and as a drink) were allocated separate codes depending on the mode of use. The mean weight for each subject's consumption of each of the grouped foods was calculated and the data were transferred to SPSS for further analysis. The data were split by age group and thereafter statistical tests and calculations were carried out to obtain the mean, standard deviation, median, and smoothed means by regression methods. Portion size was calculated for all foods consumed by ≥2% of all children (foods consumed by 2–9.5% were recorded as estimates only). A database was constructed in Microsoft Access to compare the use of the calculated portion sizes against actual weights recorded in food diaries collected for previous studies (Lietz *et al*. 2002; Longbottom *et al*. 2002; Payne & Belton, 1992). Food weights from fifty diaries in each of the age groups 1–3, 4–6 and 11–14 years were entered into the database. The resulting tables were then linked to tables containing data on the calculated portion sizes and nutrient data (from the NDNS nutrient databank), for the most commonly eaten food in each of the grouped foods. This enabled the creation of a file containing the average daily energy and nutrient intake (for nine nutrients) for each subject's diary using the calculated portion weights and the actual weights. Data were exported to SPSS and paired *t*-tests were carried out for each nutrient. If no statistically significant difference was apparent then Bland-Altman plots were carried out to assess level of agreement.

Overall 119 foods were consumed by 10% or more of children and a further 135 by 2–9.5%. The mean energy and nutrient values calculated using the mean, median, and smoothed mean portion weights were all significantly correlated with those calculated using the actual weights. Paired *t*-tests on the data showed that the nutrient data from median portion weights for the 1–3 years age group, median and smoothed mean by linear regression for the 4–6 years age group and mean and smoothed mean by linear regression for the 11–14 years age group gave no significant differences from those calculated using actual weights. The final list of typical children's portion sizes was based on median portion weights for the 1–3 and 4–6 years age groups and smoothed mean (by linear regression) portion weights for the 7–10, 11–14 and 15–18 years age groups.

These calculated portions sizes will be useful in assessing the diets of groups of children. In addition they can be used as a guide for researchers devising dietary assessment tools for children.

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The development of a dietary assessment tool for school children aged 3 to 7 years. By L. FREAR, D.C. GREENWOOD and J.E. CADE, *Nutrition Epidemiology Group, Nuffield Institute for Health, 71–75 Clarendon Road, Leeds LS2 9PL*

Children have low intakes of fruit and vegetables amounting to around 2–3 servings per day and one in five children eat no fruit at all in a week (Gregory *et al*. 2000). The NHS Plan (2000) stated a commitment to entitling every child aged 4–6 years to a free piece of fruit each schoolday. Younger children in nursery classes attached to infant schools will also receive fruit. Assessing the impact of such initiatives on the diet of children is important, but difficult. We therefore aimed to develop a concise, simple tool for use by non-specialists to assess diet in primary-school children.

A 24 h food tick list was developed for the project. It was designed to be completed prospectively over the course of one day. The section for foods eaten at home was completed by the parent, the children completed the section for foods eaten at break-time with classroom support and the use of pictures, and a dinner helper completed the section for foods eaten at lunch.

Two classes from each of six primary schools with a wide range of socio-economic and ethnic backgrounds in Leeds were selected to take part in the validation of the questionnaire. The comparison method was a 24 h semi-weighed food diary obtained for the same day as the tick list. This was completed by the study team for foods eaten at school and by the parent for foods eaten at home.

The parents of 234 children out of 375 contacted (62%) gave consent for their child to take part in the study. Of these, 180 (77%) returned completed packs of information. The sample consisted of 56% boys and 44% girls, with a mean age of 4.8 years. About 10% of children lived in households with one adult. On the day of recording 27% of boys and 23% of girls reported not eating any fruit. 36% of boys and 24% of girls did not eat any vegetables. The mean intake of fruit and vegetables was 2.1 and 1.6 items per day, respectively, with fruit juice, beans and pulses counted only once. Correlations comparing the tick list with the diary for specific fruits and vegetables gave coefficients from 0.44 to as high as 0.89. The tick list was also able to generate nutrient values which compared well to the 1 d food diary, correlations ranged from 0.41 for carbohydrate and protein to 0.68 for β-carotenes. The tool was repeated in a subsample of children. Mean values of nutrients were consistently reproduced and the ability of the tool to detect changes in diet was good (correlation=0.72 for items of fruit and vegetables).

Parent and teacher evaluation of the tick list was very positive. Parents felt that the tick list was easy and quick to complete, with only, 2% reporting it was difficult and only 1% of children not liking it. Teachers found the project caused very little disruption to normal classroom activity, reporting that that the tool had potential as a learning experience for the children in a number of ways. The majority of the children enjoyed taking part.

By including a comprehensive generic list of food types we have avoided the costs associated with coding diaries, and avoided regular updating of the tick list as shopping habits change. In this validation study the tick list has been used successfully for rapid collection of food and nutrient information from children aged 3–7 years from diverse social and ethnic backgrounds. The tool has performed better than many food frequency questionnaires in comparison to a food diary, and has performed better than the research team expected in that we have been able to accurately assess, not only food intake, but also nutrients in a traditionally difficult population to study.

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A nutrition education intervention to increase the fruit and vegetable intake of denture wearers. By J. BRADBURY, J.M. THOMASON, N.J.A. JEPSON, A.W.G. WALLS, P.F. ALLEN and P.J. MOYNIHAN, *School of Dental Sciences, University of Newcastle, Newcastle upon Tyne NE2 4BW*

In the UK, 58% of adults ≥75 years are edentulous (total tooth loss) (Kelly *et al.* 2000). Complete denture wearers report that their compromised chewing ability affects their food choice (Steele *et al.* 1998), and there is some evidence to support a lower intake of fruit and vegetables when compared with dentate individuals (Johansson *et al.* 1994; Joshipura *et al.* 1996). However, improvement in chewing ability seldom results in a change in dietary intake (Moynihan & Bradbury, 2001). This randomised controlled nutrition education intervention study aimed to increase the fruit and vegetable intake of patients receiving replacement complete dentures.

Patients attending Newcastle Dental Hospital for replacement complete dentures completed a 3 d estimated food diary pre-treatment. Patients with a fruit and vegetable intake <500 g/d were randomly assigned to either intervention or control groups. Perceived chewing ability was assessed via a questionnaire. The intervention group participated in two individual counselling sessions and received a tailored, personalised written package. Diet was assessed using a second 3 d diary 6 weeks post-insertion of replacement dentures and completion of the intervention programme.

Of the patients, 65% reported improved chewing ability following provision of replacement dentures. The intervention group increased their fruit and vegetable intake by 209 g/d, compared with the control group's increase of 25 g/d, a difference which was significant (P=0.001). The percentage of intervention group participants who consumed some fruit during the 3 d recording period was increased (80% *v.* 97%), compared with the control group which remained stable (32% *v.* 31%). Significantly more of the intervention group drank some juice post- compared with pre-intervention (20% *v.* 43%, P=0.027). The percentage of control group participants drinking juice also increased (4% *v.* 25%), but this did not reach significance (P=0.078). The percentage consuming the recommended intake of ≥400 g/d fruit and vegetables increased from 17% to 57% for the intervention group, and from 11% to 21% for the control group.

	Intervention group (g/d)				Control group (g/d)			
	Pre-intervention		*Post-intervention*		*Pre-intervention*		*Post-intervention*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fruit (exc. juices)	90	68	191	149	78	70	78	74
Vegetables	157	73	224	109	168	83	175	91
Total fruit & veg. (inc. juices)	269	109	478	256	256	115	281	151

An improvement in perceived chewing ability was insufficient to provoke an increased intake of fruit and vegetables. However, a relatively brief intervention programme, applied at the time of receiving replacement dentures, can substantially increase the fruit and vegetable intake of complete denture wearers.

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The nutrition knowledge of older adults living in sheltered housing accommodation. By C.E. WOOD[1], R. PRASAD[1], A.J. ADAMSON[2], R. STACY[3], J.C. MATHERS[2] and P.J. MOYNIHAN[1], [1]*School of Dental Sciences, University of Newcastle upon Tyne NE2 4BW,* [2]*Human Nutrition Research Centre, Wellcome Research Laboratories, RVI, Queen Victoria Road, Newcastle upon Tyne NE1 4LP and* [3]*Department of Primary Health Care, Medical School Framlington Place, Newcastle upon Tyne NE2 4HH*

The number of older adults in the UK population is increasing dramatically. Approximately 20% of the UK population are now aged 60 years and above, yet health and quality of life in older age are not improving. Diet is an important determinant of health in older age and lower socio-economic groups, in particular, have been reported to have a poor diet, high in saturated fat and salt and low in fruits, vegetables and fibre (Finch *et al*. 1998). Knowledge is a factor underlying behaviour and a lack of nutrition knowledge forms an important barrier to people consuming a healthier diet (Buttriss, 1997).

As part of a larger nutrition intervention trial of older adults, the aim of the present study was to obtain baseline information on the nutrition knowledge of 288 older adults living in sheltered housing accommodation in socially deprived areas of Tyne and Wear. Subjects were asked to complete a questionnaire on food and health. The questionnaire was based on a previously validated nutrition knowledge questionnaire (Parmenter & Wardle, 1999) with additional questions relevant to the intervention. From a list of options, participants were asked to: (1) select the correct dietary recommendations; (2) correctly identify nutrient sources; (3) select the healthiest meal option; and (4) identify the associations between diet and diseases.

Final analysis included 177 completed questionnaires. The mean age of respondents was 75.8 years (SD 8.0) ranging from 61 to 90 years, and 147 were female. The Balance of Good Health plate model (Health Education Authority, 1994) was correctly identified by 12.4% of respondents. From a list of ten foods, only 6.2% of subjects were able to correctly state which foods to eat more or less of, in order to meet dietary recommendations. Over one-third of subjects knew the recommendation to consume at least five portions of fruit and vegetables per day and 39% knew that a reduction in intake of saturated fat is recommended. The majority of subjects knew that fruits and vegetables are a good source of fibre; however, 65% also thought fish and approximately 40% thought meat and poultry to be high in fibre. Only 15% could identify oily fish from non-oily fish. The proportion of subjects who were able to correctly identify the healthiest choice from a list of meal options is shown in the Table. When looking at the association between diet and disease, although 61% were aware of the association between dietary fat and cardiovascular disease and obesity, only 8% knew of the associations between fruit and vegetable intake and cardiovascular disease, cancer and bowel disorders.

Meal options	% correct
Healthier sandwich (choice of two)	46
Low fat high fibre meal (choice of four)	70
Healthier filled potato meal (choice of two)	72
Meat meal lowest in fat (choice of four)	53

The data show that older adults had a good knowledge of the need to increase dietary intakes of fruits and vegetables but did not know why this increase is recommended. Conversely, participants had a good knowledge of the association between fat intake and coronary heart disease and obesity but were not able to correctly identify low-fat items or sources of oily fish. Older adults had insufficient knowledge to instigate dietary change with respect to fat intake. More information on the health benefits associated with increased fruit and vegetable consumption is required. Knowledge of both diet–disease associations and how to achieve dietary goals in terms of foods may be important in facilitating dietary change older adults.

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Determinants of adequate daily energy intake (energy intake − energy expenditure) on expeditions. By K.M. APPLETON, *Department of Experimental Psychology, University of Bristol, 8 Woodlands Road, Bristol BS8 1TN*

Energy requirements on expeditions are frequently inadequately matched by concurrent energy intake, predominantly due to a combination of unusually high levels of energy expenditure and the often compromised nature of expedition food and the conditions in which it is consumed (Friedl & Hoyt, 1997; Stroud, 1998). High levels of energy expenditure on expeditions are often necessary. The compromised nature of expedition food is similarly often unavoidable (Stroud, 1998). The conditions in which food is consumed, however, are more adjustable (Friedl & Hoyt, 1997). This project investigated the determinants of adequate daily energy intake (energy intake − energy expenditure) on expeditions, with specific emphasis on variables that are adjustable in the expedition environment.

Twelve adolescents took part in the study, all were lean and of average fitness. Energy intake, energy expenditure and various potential determinants of energy intake were measured concurrently, using adapted self-report food diaries, completed per eating occasion (e.g. de Castro, 1999). Energy intake was measured by recording all foods and fluids consumed. Energy expenditure was measured by recording details of all activities undertaken. Potential determinants of energy intake included: subjective ratings of hunger, thirst, happiness, alertness, energy and temperature, prior to eating; subjective ratings of pleasantness, tastiness, sweetness, savouriness, heat, coldness, familiarity and satisfaction, of the food consumed; and details of the eating situation: day of the expedition, type of meal consumed, location, number of others present, and number of others consuming. At the end of each day, participants also recorded total number of eating occasions and total time spent consuming. Diaries were completed for 28 consecutive days, during BSES Expedition Arctic Norway 2002. Throughout this period, aspects of energy expenditure and external eating situation were highly variable; aspects of internal situation and food consumed remained natural.

Following checking for under-reporting using metabolic equivalent equations, eight complete sets of food diaries were analysed. After discarding some variables due to high inter-correlations, adequate daily energy intake (energy intake − energy expenditure) was significantly predicted by regression equation: R=0.716, R^2=0.513, adj. R^2=0.464, $F(17,171)$=10.59, P<0.01, using predictors: location (P<0.01), number of eating occasions (P<0.01), perceived happiness (P<0.01), perceived pleasantness (P<0.01), and time spent consuming (P=0.04). Greater daily energy intake was significantly associated with eating at camp >rest >moving, a greater number of eating occasions, greater perceived happiness prior to consuming, greater perceived pleasantness of the food consumed, and a greater time spent consuming. Weight loss was not assessed due to technical difficulties.

Associations between energy intake and location have been found previously in expedition and non-expedition environments. Energy intake is suggested to be greater in environments conducive to eating (Meiselman *et al*. 1988; de Castro, 1999), and on expedition, conducive to cooking (Meiselman *et al*. 1988). Associations between energy intake, number of eating occasions and time spent consuming are also well known in expedition and non-expedition environments (Popper *et al*. 1989; de Castro, 1999). Indeed, number of eating occasions and time allowed for eating are suggested to be among the most important determinants of energy intake in military fields (Popper *et al*. 1989). Effects of perceived happiness and perceived pleasantness on energy intake have also been previously reported (de Castro, 1999; Meiselman *et al*. 1988). No associations were found between adequate daily energy intake and any of the other variables measured. These results suggest that these variables are not important determinants of adequate daily energy intake in the expedition environment. Many of the variables of lesser importance in this study, however, were also those of lesser variance. Caution should also be exercised when considering the results of this study, due to the number of participants involved, the expedition environment used, and the method of data collection.

In summary, these data suggest that guidelines related to adequate energy intake on expeditions should focus on allowing consumption in situations conducive to eating and cooking, allowing an adequate number of eating occasions, allowing adequate time for consuming, ensuring morale remains high, and providing foods that are perceived as pleasant.

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Homocysteine and B-vitamin intake are associated with alcohol consumption. By J.V. WOODSIDE[1], P.M.L. SKIDMORE[1], J.W.G. YARNELL[1], I.S. YOUNG[1], D.L. HARMON[2], A.S. WHITFIELD[3], K.F. GEY[4] and A. EVANS[1], [1]*School of Clinical Medicine, Queen's University Belfast, Belfast BT12 6BJ,* [2]*Department of Pathology, University College Dublin, Ireland,* [3]*Department of Pharmacology, University of Pennsylvania, USA and* [4]*Department of Biochemistry, University of Berne, Switzerland*

Epidemiological studies have shown that a low or moderate consumption of alcohol has a protective effect against cardiovascular disease (CVD). The relationship between alcohol consumption and cardiovascular mortality is a U-shaped curve, with the lowest mortality at an alcohol consumption of 2–4 units (16–32 g) of alcohol per day. Homocysteine (tHcy) is recognised as a CVD risk factor. tHcy is elevated in patients with chronic alcoholism and falls following alcohol withdrawal; therefore alcohol may actually have a deleterious effect on tHcy levels. tHcy is controlled through a series of pathways which are dependent on B-vitamins, particularly folic acid. A common genetic variant of the methylenetetrahydrofolate reductase (MTHFR) gene has been associated with an increase in tHcy levels, particularly in those who have low folate status.

In a population with a high prevalence of cardiovascular disease, we screened a group of working men in Belfast aged 30–49 years (n=765) for plasma homocysteine (Ubbink *et al.* 1991), and serum folate, cobalamin (radioassay, ICN Pharmaceuticals), and pyridoxal 5-phosphate concentrations (Reynolds & Brain, 1992). Subjects were also screened for the C677T thermolabile MTHFR polymorphism (Frosst *et al*. 1995). Dietary intake of the B-group vitamins was assessed by food frequency questionnaire, which was also used to assess alcohol intake.

Those who consumed alcohol most frequently (more than four times per week) had higher intakes of folate, vitamin B_{12} and vitamin B_6 than those either consuming no alcohol or alcohol three or fewer times per week (P<0.001, <0.001 and <0.01, respectively). There was no association between frequency of alcohol consumption and serum levels of folate, cobalamin and pyridoxal 5-phosphate. Those consuming alcohol three or fewer times per week had lower homocysteine levels (Mean (SD) 6.92 (1.37)) than those consuming alcohol more than four times per week (7.91 (1.48)) or those not consuming alcohol (7.82 (1.51), μmol/l, P<0.001, one-way ANOVA). When the daily intake of alcohol was examined, dietary intake of B_{12} (r=0.203, P<0.001) and folate (r=0.362, P<0.001) were associated with daily alcohol intake (g) (Pearson correlation coefficient). When subjects were classified into abstainers, those consuming ≤32 g/d (moderate drinkers) and those consuming >32 g/d (heavy drinkers), intake of folate, vitamin B_{12} and vitamin B_6 increased as the amount of alcohol consumed daily increased, while moderate drinkers had lower levels of homocysteine (Mean (SD) 7.01 (1.41) than heavy drinkers (7.64 (1.35)) or abstainers (7.95 (1.52), μmol/l, P<0.01, one-way ANOVA). Daily alcohol intake did not vary by MTHFR genotype, despite thermolabile homozygotes having lower folate status and high homocysteine concentrations than homozygous normals.

This study shows that increased alcohol consumption (whether assessed as frequency of consumption or daily intake) is associated with increased dietary intake of B-group vitamins, but that homocysteine is lower in moderate drinkers than in heavy drinkers or abstainers.

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Effect of nausea and vomiting in pregnancy on food intake in early and late pregnancy. By B. AL-RASASI[1], J.COAD[3] and J. MORGAN[2], [1]*PO Box 1020, Ruwi, Postal Code 112, Sultanate of Oman,* [2]*School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7GX and* [3]*Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North, New Zealand*

The aim of this study was to assess the effect of nausea and vomiting of pregnancy (NVP) on the intake of macronutrients in both the first and third trimester on a sample of fifty-two women.

The women kept 7 d food records, once in the first trimester and once again in the third trimester. Forty-three diaries of intakes in the first trimester and fifty-one diaries in the third trimester were returned and the women were categorized as either having or not having NVP by means of a questionnaire. Dietary data were analysed using Win Diets, and statistical analysis was carried out using SPSS (version 10.0).

As mentioned in a previous abstract (Al Rasasi *et al*. 2002), although macronutrient intake was lower in women with NVP than in those without NVP in the first trimester, these differences failed to reach significance at the 5% level. The Table illustrates that in the third trimester, once NVP had resolved, energy intake adjusted for maternal weight was lower in women with NVP than in those without NVP (P=0.041).

Nutrient	First trimester (n=43)	Third trimester (n=51)
NVP		
Energy (kJ/kg) (SD)	114 (32)	110 (24)
Fat (g/kg) (SD)	1.07 (0.39)	0.98 (0.26)
Protein (g/kg) (SD)	1.00 (0.29)	1.02 (0.22)
Carbohydrates (g/kg) (SD)	3.63 (0.99)	3.55 (0.84)
No NVP		
Energy (kJ/kg) (SD)	129 (30)	123 (30)
Fat (g/kg) (SD)	1.26 (0.34)	1.16 (0.35)
Protein (g/kg) (SD)	1.12 (0.28)	1.11 (0.30)
Carbohydrates (g/kg) (SD)	3.94 (0.96)	3.81 (0.89)

It was found that this difference was due to a reduction in fat intake, which was significantly lower in women with NVP than in women without NVP (P=0.032). These differences failed to reach significance for intakes in the first trimester.

We speculate that women with NVP reduce their intake of fat, as fatty foods have been reported to exacerbate symptoms of NVP. We have shown previously that women with NVP reported that meats, ethnic and spicy foods and fried foods, all of which have a high fat content, were common aversions (Al-Rasasi *et al*. 2001). It is interesting to note that this low-fat intake continues into the third trimester. It could be that the women have either become accustomed to eating a low-fat diet, or that they are continuing to consume a diet low in fat, for fear of NVP reoccurring.

The effect these differences in intakes on birth outcome could not be established, as the target sample size needed to achieve 80% power, based on birth outcomes, was not achieved, due to the time frame of the study.

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Stability over time of dietary patterns in the UK Women's Cohort Study. By D.C. GREENWOOD[1,2], M.S. GILTHORPE[1], C. GOLDING[2] and J.E. CADE[2], [1]*Biostatistics Unit, University of Leeds, 24 Hyde Terrace, Leeds LS2 9LN and* [2]*Nutrition Epidemiology Group, Nuffield Institute for Health, 71–75 Clarendon Road, Leeds LS2 9PL*

Nutrition epidemiology is increasingly focusing on whole diets and lifestyles rather than single nutrients, to aid investigation of complex diet–disease relationships and facilitate public health promotion. One example is the so-called 'Mediterranean Diet'. It is well known that actual diet changes from day to day, and from season to season, and that even well-designed cohort studies are therefore subject to bias caused by measurement error. Whilst nutrient intake varies greatly, it is less well known how stable consumers' general dietary patterns are in the long term.

The UK Women's Cohort Study is a large national cohort of women being studied with the aim of investigating potential associations between diet and cancer. It recruited over 35 000 women between 1995 and 1998. Sampling was stratified to give equal proportions of vegetarians, fish eaters and meat eaters. Each woman returned a 218-item food frequency questionnaire covering their diet over the previous year. A sample of 2200 of these women were mailed a second food frequency questionnaire 5 years later, with 1918 returned. This gave a response rate of 87%.

Cluster analysis was used to identify eating patterns in the cohort on the basis of their reported diet. Seven clusters were identified, characterised by type of food and dietary diversity (Greenwood *et al*. 2000): (1) Monotonous low quantity omnivores, (2) Health conscious, (3) Traditional meat, chips and pudding eaters, (4) Higher diversity traditional omnivores, (5) Conservative omnivores, (6) Low-diversity vegetarians, (7) High-diversity vegetarians. Groups differed significantly in their socio-demographic profiles and nutrient intakes. The participants were then re-classified, using the same cluster definitions, based on their reported diet 5 years later.

n=1918	Cluster:	**1**	**2**	**3**	**4**	**5**	**6**	**7**
% in cluster at baseline		12%	8%	10%	9%	16%	24%	23%
% in cluster 5 years later		12%	9%	9%	8%	18%	21%	23%
% moving out		33%	51%	47%	53%	50%	46%	40%
% moving in		35%	60%	41%	52%	57%	39%	39%

It was found that 1055 (55%) of the women maintained the same dietary pattern (κ=0.5, suggesting moderate stability). The most stable pattern was for the monotonous low quantity omnivore (67%), with 22% changing to the similar pattern of meat, chips and pudding eaters, and the conservative omnivores. The two vegetarian clusters were the next most stable with 54% and 60% pattern maintenance for low- and high-diversity vegetarians, respectively. However, 19% of low-diversity vegetarians moved to the high-diversity group and 18% of the high-diversity group moved to the low. Such changes indicate people on the boundaries of similar groups, and highlight a high degree of stability in dietary patterns over the 5-year period. The most unstable grouping appeared to be cluster 2, the 'health conscious' omnivores.

In contrast to this apparent stability of dietary patterns, when intake of total energy, fat, vitamin C, and percentage energy from fat were categorised into seven equal-sized groups, the κ statistics were much lower, ranging from 0.18 to 0.21, suggesting poorer stability. Around 80% of women in central categories of nutrient intake changed to other categories, whilst the extremes were more stable, with around 50% changing group.

Many studies in nutrition epidemiology measure diet at one time point and assume that this reflects long-term diet. Using groupings of dietary patterns should not only avoid complex modelling of interactions between nutrients, but also reduce bias caused by such measurement error.

The UK Women's Cohort Study is funded by the World Cancer Research Fund. Thanks to the data entry team, Claire Calvert and Alyson Greenhalgh, for baseline data collection support, and James Thomas for database management.

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Socio-economic and geographical differences in the consumption of fresh fruit and vegetables in the diets of families in Scotland and England in the 1930s: the Boyd Orr cohort. BY C. FROBISHER[1], M. MAYNARD[2], P. EMMETT[1], G. DAVEY SMITH[3], S. FRANKEL[3], A. NESS[1] and D. GUNNELL[3], [1]*Institute of Child Health, University of Bristol, 24 Tyndall Avenue, Bristol BS8 1TQ,* [2]*MRC Social and Public Health Sciences Unit, Suite 1, 3 - 5 Islington High Street, London N1 9LJ,* [3]*Department of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Bristol BS8 2PR*

Lower socio-economic groups have been found to have a greater incidence of cardiovascular diseases (CVD) (Davey Smith *et al*. 1997), and in the UK, Scotland has one of the highest levels of CVD (British Heart Foundation, 2003). Availability of fresh food may contribute to socioeconomic and geographical differences in CVD mortality. The extent of variation in fresh fruit and vegetable consumption within Scotland and England according to social class was studied in the families who took part in the dietary assessments of Boyd Orr cohort members (1937-1939) – previous analysis of which has demonstrated social class differences in CVD (Frankel *et al*. 1999). The Boyd Orr cohort also called the Carnegie survey of family diet and health was started in 1937 to investigate the diet and health of families generally from deprived areas. We hypothesised that in Scotland, socio-economic variation in the proportion of families consuming fresh produce would be greater than in England. Complete 7-day inventories obtained from 1339 families were used to calculate the proportion of families consuming fresh fruit and vegetables (excluding potatoes) overall, and by socio-economic group.

Nearly all the families both in England (97.2%) and Scotland (96.7%) included fresh vegetables in their diets during the survey week. Hence there was little socio-economic variation between the two regions in whether or not fresh vegetables were consumed. However there was considerable variation in the proportion of families consuming fresh fruit. Overall, significantly more families from England had included fresh fruit in their diets than those in Scotland. This was true for each of the socio-economic groups. Within both England and Scotland, there was a similar decrease in the proportion of families consuming fresh fruit from the most affluent to the unemployed (p = 0.007 and p = 0.000, respectively).

	% of families in England (n = 853)		**% of families in Scotland (n = 486)**	
	Consuming fresh fruit	**Not consuming fresh fruit**	**Consuming fresh fruit**	**Not consuming fresh fruit**
Overall (n = 1339)	71.6%	28.4%	57.6%	42.4%
				p<0.001
By socio-economic group				
I and II (n = 123)	87.7%	12.3%	72.4%	27.6%
III (n = 265)	84.8%	15.2%	66.7%	33.3%
IV (n = 300)	68.7%	31.3%	50.3%	49.7%
V (n = 193)	66.2%	33.8%	56.4%	43.6%
Unemployed (n = 343)	65.1%	34.9%	49.5%	50.5%
				p<0.001

Socio-economic groups: I & II: professional & intermediate, III: skilled worker, IV: semi-skilled worker, V: unskilled worker, unemployed; based on male head of household. Some subjects could not be classified into a socio-economic group and are not included as a separate group here.

These findings may contribute to the differences in CVD rate between England and Scotland, and those between socio-economic groups. The geographical differences in CVD rate may become less pronounced in the future since in the Dietary and Nutritional Survey of British Adults, conducted in the UK in 1987, there was no difference between England and Scotland in the proportion of people eating fresh fruit (Gregory *et al.* 1990).

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Degree of adherence to dietary recommendations of African Americans and Latinos in the USA. By S. SHARMA, S.P. MURPHY, L. WILKENS and L. KOLONEL, *Epidemiology Section, Cancer Etiology Program, Cancer Research Center of Hawaii, 1236 Lauhala Street, Honolulu, Hawaii, HI 961813, USA*

Examining differences in dietary patterns between ethnic groups may provide insight into causes of differing rates of chronic disease such as cancer and cardiovascular disease and provide guidance for updating dietary recommendations and developing interventions. The Food Guide Pyramid (FGP) is a general guide designed to help Americans make healthful food choices and to reduce the risk of chronic disease (USDA, 1992). The objective of the FGP is to translate dietary standards and recommendations into useful education tools. The Pyramid recommends a range of daily servings based on age and caloric intake from five major groups: grains, vegetables, fruits, dairy and meat. There is little information available on whether ethnic minority groups in the USA adhere to the FGP. We present data on degree of adherence to the FGP for African Americans (AA) and Latinos (L) from the Multiethnic Cohort (MEC) which collected comprehensive dietary data primarily from 215 000 Japanese Americans, Native Hawaiians and Caucasians in Hawaii, and AA and L in Los Angeles. AA and L aged 45–75 years in 1993–6 completed a mailed self-administered quantitative food frequency questionnaire (FFQ) (Kolonel *et al*. 2000). Here we examine adherence to the FGP recommendations among the AA, Latinos born in Mexico (LM) and Latinos born in the USA (LU). Each individual's pyramid servings intakes were computed by summing the servings across the food items. A person was defined as not adhering to the FGP if the number of servings consumed was less than that recommended for their energy intake category.

% not adhering to the following food groups	African American men (*n* 11 772)	African American women (*n* 20 130)	Latinos Mexico-born men (*n* 10 180)	Latinos Mexico-born women (*n* 10 903)	Latinos US-born men (*n* 10 613)	Latinos US-born women (*n* 11 255)
Grain	66	70	46	51	51	55
Vegetables	57	49	38	32	51	45
Fruit	54	42	46	34	53	43
Dairy	85	86	65	65	74	76
Meat and alternatives	50	60	30	50	47	61

For all ethnic groups, more men than women adhered to the recommendations for grain and the meat group; women were more likely to adhere to the recommendations for fruits and vegetables. The dairy recommendations were the least likely of the food groups to be met by all ethnic and sex groups. AA men and women were the least likely to meet the recommendations for grain, vegetables and dairy foods compared with LM and LU. For all food groups, for men and women, LM had a greater percentage who did not meet the recommendations compared with LU. In this large cohort we have shown that a high percentage of all the ethnic groups do not adhere to the recommendations in the FGP, and particularly to the dairy group recommendations. We are using these data to examine degree of adherence to the recommendations and its relationship to cancer and fatal cardiovascular disease outcomes.

We are grateful to the American Heart Association of Hawaii, the United States Department of Agriculture, and the National Cancer Institute for funding this research.

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Fatty acid composition of human milk from the Thessaloniki region in Greece: comparison with dietary habits and food intakes. By D.J. TRIANTAFILLOU and H. KATSIKAS, *Department of Nutrition, TEI of Thessaloniki, Greece*

Human milk fatty acid profile is influenced by many factors, among which dietary habits and nutritional intakes are the most important (Rocqelin *et al*. 1998; Francois *et al*. 1998). In this study the fatty acid profile of human milk from women living in the Thessaloniki region is presented. A correlation with nutritional intakes is also attempted.

Samples from forty-three women breast-feeding for 4–7 months, whose anthropometric status and dietary intakes are presented in Table1, were collected during 2001 and 2002.

Table 1.Anthropometric status and dietary intake of Greek mothers (mean ± s.d.)

Age	31 ± 4.5
BMI	23.5±3.9
Energy MJ/day (kcal/d)	10.28±2.62 (2457±625)
Protein (%energy)	15±3.3
Fat (%energy)	47.1±5.9
Carbohydrate (%energy)	37.3±5.8

Fat was extracted and gas chromatographic analysis performed using a CP-Sil 88 capillary column. The results showed that the total saturated fatty acids content was 43.08% (SD 5.36), monounsaturated fatty acids content was 40.43% (SD 4.37) and polyunsaturated fatty acids content was 16.03% (SD 3.74). The saturated fatty acids content appears to be high compared with results of studies from other countries (Agostoni *et al*. 2000; Xiang *et al*. 1999). Palmitic (16:0) and stearic (18:0) acids were the main contributors, with 21.27% (SD 3.12) and 7.44% (SD 2.35), respectively. From the class of monounsaturated fatty acids, oleic acid (18:1) was found to be 38.81% (SD 5.16). This value is one of the highest that has been reported in other countries and is similar to that of Italy. This finding can be explained by the use of olive oil as the main fat source in both these countries (Agostoni *et al*. 2000). The polyunsaturated fatty acids content was found to be among the lowest reported. Linoleic (18:2*n*-6) acid contributed 13.7% (SD 3.29), while linolenic (18:3*n*-3) and docohexaenoic (22:6*n*-6) acids were present as 0.56% (SD 0.35) and 0.26% (SD 0.15), respectively. Traces of *trans*-isomers were detected in the form of linoleic (18:2*n*-6) acid.

Statistically significant ($P<0.05$) differences were found for the total saturated fatty acid content, which was higher in women with a BMI >25 and for those women who frequently consumed butter. Docohexaenoic (22:6*n*-6) acid was elevated in those women who often consumed fish, while there were no statistically significant differences for consumption of eggs, meat and margarine.

If these data are confirmed by similar studies in different regions of Greece, nutritional guidelines should be given to breast-feeding Greek women in order to improve the milk fatty acid profile.

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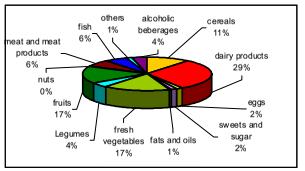
Development of a questionnaire and a software program of dietetic advice designed for menopausal–perimenopausal women: application in Spain. By M. BASAGOITI, N. ÚBEDA, G. VARELA MOREIRAS and E. ALONSO-APERTE *Facultad de Ciencias Experimentales y de la Salud, Universidad San Pablo-CEU, Crta. Boadilla del Monte Km. 5.3, 28668 Boadilla del Monte, Madrid, Spain*

Menopause can be defined by the cessation of the female menstrual cycle. Many hormonal changes take place during the menopause, such as an important reduction in oestrogen production. As a consequence of this reduction, postmenopausal women are at higher risk of heart disease, osteoporosis and obesity. In Spain, menopause generally takes place when women are between 45 and 51 years old.

The main objective of this research project is the development and application of a food frequency questionnaire and a software program of dietetic advice specifically addressed to perimenopausal and menopausal Spanish women. This software, according to data obtained from the questionnaire, allows individual dietetic counselling. It is the first program of such characteristics developed in Spain. Women joining the study come from all communities, and from urban, semi-urban and rural environments. The next step was to determine the general patterns of food and nutrient intake of the studied population, compared with the food intakes of the Spanish population as a whole, during the year 2002, and also compared with a model of healthy diet pattern. Other aims of the study were to determine food intakes for different regions of Spain (North, South, Central, East and Canary Islands), and to evaluate possible differences between menopausal and perimenopausal women. Other objectives are to evaluate the influence of social demographic factors (home), as well as lifestyle factors (physical activity, smoking, fortified food and vitamin or mineral supplement intakes) in the food intakes of menopausal women. The nutritional assessment was completed with anthropometric (body mass index) and some biochemical markers (glucose, cholesterol and triacylglycerol).

In total, 941 women were evaluated; 751 of them were already diagnosed as menopausal. The observed food intake pattern is shown in the Figure (percentage of total food intake as wet matter (g)).



No differences were found from the general Spanish population's feeding habits, except for dairy products, whose consumption was statistically higher in the study group.

The average energy intake was higher than recommended for 50–59-year-old Spanish women. As a consequence, we observed an high prevalence of obesity (61%). Furthermore, there was none of the nutrient intakes were below recommendations, and protein and cholesterol intakes were high.

Energy (kJ)	Protein (g)	Carbohydrate (g)	Fat (g)	Fibre (g)	Calcium (mg)	Iron (mg)	Iodine (μg)	Magnesium (mg)	Zinc (mg)	Sodium (mg)	Potassium (mg)
10630	124	399	54	40	1408	22	511	482	15	2185	4900
Vit B_1 (mg)	Vit B_2 (mg)	Niacin (mg)	Vit B_6 (mg)	Folic acid (μg)	Vit B_{12} (μg)	Vit C (mg)	Vit A (μg)	Retinol (μg)	Carotenoid (μg)	Vit D (μg)	Vit E (mg)
2.0	2.5	42.5	2.3	297.7	7.9	218.3	1798.3	270.7	9068.8	5.2	3.8
SFA (g)	MUFA (g)	PUFA (g)	Chol (mg)								
30.9	17.6	6.8	371.3								

We found marked differences between subjects from different geographical areas: In northern Spain we found the highest consumption of legumes, cereals and cereal derivatives and a very low ingestion of fish. The highest intake of fish was recorded in the southern regions, while the highest intake of legumes was found in the east of the country. The Canary Islands had the highest meat consumption. There was no difference between the eating patterns of the menopausal and perimenopausal women. Differences seem to be due to regional eating styles.

Our study group showed more interest in a healthy diet than those reported in previous research studies in our country. This result could be explained by a bias in sampling, as participants were all volunteers.

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High saturated and low unsaturated dietary fat intakes and high prevalence of risk factors of coronary heart disease among urban middle-aged office workers in Sri Lanka. By C.P. ALAHAKOON[1] and K.D.R.R. SILVA[2], [1]*University of Kelaniya, Sri Lanka and* [2]*Department of Applied Nutrition, Wayamba University of Sri Lanka, Kuliyapitiya, Sri Lanka*

The dietary patterns of urban Sri Lankans are changing rapidly as a result of a nutrition transition and rapid urbanization. Association of diet with cardiovascular risk factors has been poorly studied in Sri Lanka. A cross-sectional study was conducted to assess the dietary fat intakes and prevalence of risk factors of CHD among urban middle-aged office workers in Sri Lanka.

A sample of 240 apparently healthy volunteers (127 males, 113 females) with a mean age of 39 (SD 7) years employed in the state and private sector was studied. All participants were permanent or temporary residents in urban areas, non-vegetarian and non-smokers who engaged in low/moderate physical activities. They had monthly income (Sri Lankan rupees) of between 10 000 and 30 000. Three-day diet diaries were used to assess nutrient intake. Dietary intakes were compared to those recommended by the World Health Organization (FAO/WHO/UNU, 1985). Anthropometry included measurements of height, weight, waist (W) and hip (H) circumferences. Fasting plasma total cholesterol (TC), HDL-cholesterol (HDL-C), triacylglycerol (TAG) and glucose were measured using enzymic methods and LDL-cholesterol (LDL-C) was calculated using the formula. BMI cut-offs for Asians were used to classify obesity and overweight (WHO/FAO, 2003). This study was approved by the ethics committee of the University of Kelaniya.

Table 1

Dietary intake/d	Men (*n* 127)		Women (*n* 113)	
	Mean	SD	Mean	SD
Energy (MJ)	9.0‡	0.7	8.9†	0.9
Protein (g)	55‡	12	59‡	14
Carbohydrate (g)	324‡	46	319†	50
Total fat (g)	73‡	18	72†	18
SFA (g)	36†	8	35†	9
MUFA (g)	19‡	9	19‡	9
PUFA (g)	6‡	3	6‡	30
Cholesterol (mg)	292	113	285	102
% energy fat	30.8	7.0	30.6	6.9
% energy SFA	15.0†	3.0	15.0†	3.5
% energy MUFA	7.9‡	3.5	7.8‡	3.4
% energy PUFA	2.6‡	1.2	2.6‡	1.2

‡ Significantly lower than WHO recommendation $P<0.0001$; † Significantly higher than WHO recommendation $P<0.0001$

Table 2

	Men (*n* 127)			Women (*n* 113)		
	Mean	SD	%[a]	Mean	SD	%[a]
BMI (kg m^{-2})	25.4	3.1	78.7[b]	25.2	3.0	81.5[b]
W (cm)	87.8	7.8	–	84.3	8.0	–
W:H	0.91	0.06	55.9	0.86	0.06	62.8
	Men (*n* 105)			Women (*n* 89)		
TAG (mmol/l)	1.49	0.58	9.5	1.32	0.52	7.9
TC (mmol/l)	5.60	0.93	66.7	5.19	1.08	46.1
LDL-C (mmol/l)	3.74	0.79	70.5	3.35	0.93	49.4
HDL-C (mmol/l)	1.29	0.33	2.9	1.27	0.14	0.0
TC:HDL-C	4.42	0.69	79.0	4.10	0.76	59.6
Glucose (mmol/l)	5.50	1.36	14.6	4.99	1.07	6.7

[a] Unfavourable % of subjects
[b] Obese (>25 kgm^{-2}) + overweight (23-25 kgm^{-2})

Both men and women had significantly higher mean intakes of SFA and percentage energy from SFA than the recommended levels (see Table 1; $P<0.0001$). The MUFA and PUFA intakes and percentage energy from MUFA and PUFA were significantly ($P<0.0001$) lower than the recommended levels. High contribution of fat and SFA may be due to consumption of foods of animal origin, cheaper high-fat ready-to-eat foods away from home. The prevalence of overweight (men 18.1%, women 20.4%), obesity (men 60.6%, women 61.1%), central obesity (women: W:H >0.85; men: >0.90) and biochemical risk factors was high in the group studied (see Table 2). Saturated fat intakes showed significant ($P<0.05$) positive correlations with anthropometric and biochemical risk factors except with W:H and HDL-C (data not shown).

The findings indicate that pro-atherogenic fat intakes and other coronary risk factors are common in urban middle-aged, middle-income office workers. The present study supports the view that increased fat intake and sedentary lifestyle in developing countries alter the fat and energy metabolism and increase the risk of cardiovascular disease.

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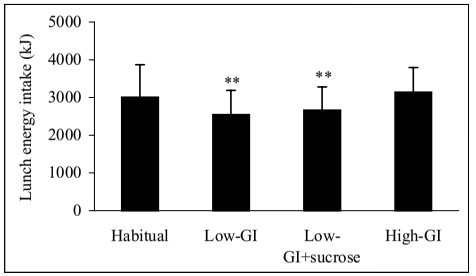
The effect of altered glycaemic index breakfasts on subsequent food intake and satiety in children aged 9–12 years. By J.M. WARREN and C.J.K. HENRY, *Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford OX3 0BP*

Currently there is much interest in the potential of low-glycaemic index (GI) foods in the management of obesity (Ludwig, 2002; Pi-Sunyer, 2002). It has been hypothesised that low-GI foods may benefit weight regulation in two ways: (1) by promoting satiety; (2) by promoting fat oxidation at the expense of carbohydrate oxidation (Brand-Miller *et al.* 2002). Single-day studies in adults have shown lowered satiety, increased hunger or higher voluntary food intake after consumption of high-GI compared with low-GI meals. Studies of the effect of GI in children are limited. Uniquely, this study investigated the effect of consuming breakfasts of varying GI on appetite and food intake in a group of normal and overweight preadolescent children. Food intake was then measured at a buffet style lunch using covert and detailed observational recordings, which enabled an accurate quantification of nutrient intake.

Children aged 9–12 years (*n* 38) were recruited from a school in Oxford which already ran a breakfast club. The study was a three-way cross-over study where each subject acted as his/her own control. Children were divided into groups and a rolling programme devised whereby, week by week, each group would randomly receive one of the three test breakfasts for three consecutive days. The test breakfasts were: (1) low-GI; (2) low-GI with sucrose added to 10% energy; (3) high-GI. In addition, there was a control day where children consumed their usual breakfast at home followed by the buffet-style lunch at school. Test breakfasts were designed to match the energy and macronutrient content of a normal breakfast. Satiation post-breakfast and satiety pre-lunch were assessed using 5-point rating scales.

Lunch energy intake was significantly lower ($P<0.01$) after the low-GI and the low-GI with added sucrose breakfasts compared with after the high-GI breakfast or habitual breakfast eaten at home. This result was independent of sex or weight.



Lunch intake was significantly lower than after high-GI breakfast: $^{**}P<0.01$.

There were no significant differences in satiation (i.e. feelings of fullness) post-breakfast, but satiety ratings pre-lunch were lower (i.e. hunger was greater) on two of the three experimental days after the high-GI breakfasts compared with the other test breakfasts.

This study is consistent with the growing body of evidence that low-GI diets may reduce food intake and have a role to play in weight control and obesity management. The challenge is to study the long-term impact of GI on daily food intake and body weight regulation.

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Temporal changes in anthropometric measurements, dietary intake measures and health status in the frail elderly living in residential care. By W.S. LESLIE[1], L. MCCOMBIE[2], M.E.J. LEAN[1] and C.R. HANKEY[1], [1]*University of Glasgow Division of Human Nutrition, Division of Developmental Medicine, Glasgow, G31 2ER* [2]*Counterweight Project, Glasgow Royal Infirmary, Glasgow, G31 2ER*

Nutrition-related health problems increase with age. In the elderly, under-nutrition, rather than over-nutrition, is still the main cause for concern (DoH, 1992) and is associated with increased morbidity and mortality (Potter *et al.* 1998). The purpose of this study was to establish the feasibility of a dietary intake study in a frail elderly population by describing the changes in anthropometric, dietary intake, and health (acute illness, death) measures observed over 12 weeks.

Thirty-five subjects (fourteen male, twenty-one female; mean age 91 (range 84–100) years), living in a residential care home, were invited to participate in the study. One male resident refused to participate. Thirty-four residents had their weight and height measured and habitual dietary intake assessed using 3 d weighed intake food records. All measurements and weighed intakes were completed by a trained nutritionist and were repeated 12 weeks later in a random subsample of 50% (*n*=18) of the study population.

In both men and women, recorded mean energy intake was lower than the current estimated average requirement (DoH, 1991). Mean BMI was 22.2 (range 14.5–34.4) kg/m^2. Six residents had a BMI <18.5 kg/m^2 and three of these residents required assistance with feeding. Use of supplemental feeding was low, with only two residents with BMI <18.5 kg/m^2 prescribed nutritional supplements.

Measurement	Baseline Mean (SD) *n*=34	Week 12 Mean (SD) *n*=12	Differences Mean (SD)	Significance of difference *P*
Body weight (kg)	54.9 (10.7)	53.1 (10.9)	−1.6 (2.7)	0.1
Energy intake (kJ/d)	6257 (702)	5184 (1231)	−1073 (1065)	0.093
Fat intake (g)	63.7 (12.8)	49.5 (15.2)	−14.2 (10.4)	0.006
Carbohydrate intake (g)	192.7 (18.1)	162.5 (35.2)	−30.3 (39.2)	0.933
Protein intake (g)	46.7 (12.8)	43.5 (17.3)	−3.2 (8.7)	0.23
NSP intake (g)	7.4 (2.2)	6.7 (2.9)	−0.69 (2.0)	0.27

At week 12, six of the total resident population were unavailable for follow up (three deaths and three acute illness). Two residents unavailable for follow up had a BMI <18.5 kg/m^2 at baseline. Energy intake had fallen significantly in women (−1096 (SD 1062) kJ, $P=0.02$) but not in men (−1004 (SD 1204) kJ, $P=0.2$), while percentage energy from fat fell in the population as a whole (−3% (SD 3.1), $P=0.02$). Weight change, although not statistically significant, was clinically evident in the majority of residents.

Attrition rates due to acute illness and death were high, so although no firm conclusions can be drawn from the dietary data, there does appear to be a trend towards detrimental changes in food intake over a 12-week period. These findings confirm the frailty of this elderly population, and provide direction for those planning research interventions in this field.

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The effect of eating environment on energy intake in the elderly. By M.R.D. GIBBONS[1] and C.J.K. HENRY[2], [1]*Leatherhead Food International, Randalls Road, Leatherhead, Surrey KT22 7RY and* [2]*Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford OX3 0BP*

It is widely recognised that poor nutrition in the elderly can have a negative impact on morbidity, functional capacities and mortality (Marcus & Berry, 1998). Conversely, improving nutritional status in free-living elderly people can reduce susceptibility to disease, risk of hospitalisation and improve quality of life (Mowe & Bohmer, 1996). If simple affordable changes in the eating environment can have a positive effect on energy intake in the elderly, this may have important health implications. The aim of this study was to investigate the effect of eating environment on energy intake in the elderly.

Identical meals were prepared and served to forty-nine elderly people (mean age 74.3 (SD 7.7) years) in two randomly ordered different eating environments. The Oxford Brookes Restaurant was the setting for the improved eating environment. The restaurant is a state-of-the-art facility with the appearance of a five-star restaurant including silver service. In contrast, the staff canteen provided the standard eating environment, which was less grand, simpler in décor, with limited waiter service. Subjects consumed a total of two meals – one in each environment. Each subject selected a two-course meal from a preset menu, which was designed to be appealing to the elderly age group. Hunger ratings of each subject were determined before each meal using a 100-mm anchored scale. Meals were made from a standard recipe and portion sizes were carefully controlled at each meal session. In addition, at least two plates from each meal choice were weighed prior to service and all plates were photographed as a secondary check. After each meal, food waste, where appropriate, was accurately measured using electronic scales. Nutrient consumption was calculated using food tables (McCance & Widdowson, 1991). Repeat measurements were taken of height, weight and grip strength in all subjects.

Total energy (kJ) consumption by subjects

				Eating environment				
		Age (years)		Improved (kJ)		Standard (kJ)		
Sex	*n*	Mean	SD	Mean	SD	Mean	SD	P
Both	49	74.3	7.7	4894	613	4536	620	***
Male	13	76.7	8.1	4853	604	4408	613	**
Female	36	73.5	7.4	4909	625	4583	620	***

$^{**}P<0.01$, $^{***}P<0.001$

When the association between hunger ratings and food intake was evaluated, there was no difference (P=0.485) in ratings measured before the meals consumed in the improved and standard environment. There was a 358 kJ difference in energy intake between the improved and the standard environments (P<0.001). Both male and female subjects also consumed significantly more energy in the improved environment. However, between genders, female subjects consumed more energy than male subjects in both environments. Twenty-one underweight and normal subjects (BMI <25) participated in this study and they consumed significantly more in the improved environment (320 kJ, P<0.001) than in the standard environment. Similarly, the twenty-eight overweight and obese individuals (BMI >24.9) also consumed significantly more in the improved environment (386 kJ, P<0.001). Eating environment has a significant effect on energy intake of elderly subjects. Enhancing eating environment may be a useful intervention strategy to improve food intake in this vulnerable population.

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