

Proceedings of the Nutrition Society

Abstracts of Original Communications

A Scientific Meeting was held at the University of Ulster, Coleraine, Northern Ireland, 16–19 July 2007, when the following papers were presented.

All abstracts are prepared as camera-ready material.

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Is the C677T methylenetetrahydrofolate reductase (MTHFR) polymorphism a risk factor for osteoporosis? A pilot study. By C.R. WHITTLE¹, J.J. STRAIN¹, M. WARD¹, A.M. MOLLOY², J.M. SCOTT², A.S. McCANDLESS¹ and H. McNULTY¹, ¹Northern Ireland Centre for Food and Health, University of Ulster, Coleraine BT52 1SA, UK and ²Department of Immunology and Biochemistry, Trinity College, Dublin, Republic of Ireland

Recent evidence indicates an association between the common C677T polymorphism in MTHFR and bone mineral density (BMD). To date research in this area has been contradictory; some studies show that individuals who are homozygous for this polymorphism (TT genotype) have significantly lower BMD, others have observed no association and one has suggested that the polymorphism may be protective against osteoporosis¹. Elevated homocysteine (Hcy) is the typical phenotype observed in those with the TT genotype and several studies show that higher Hcy is associated with a lower BMD (particularly in women)² or a higher rate of osteoporotic fracture³. The aim of this pilot study was to investigate associations between the MTHFR C677T polymorphism and BMD. An original cohort of healthy post-menopausal women (*n* 350) were pre-screened to select those with the TT genotype and age-matched controls (i.e. heterozygous CT and wild-type CC genotypes) and BMD was measured by dual-energy X-ray absorptiometry. Smokers were removed, as they were not evenly represented in the two genotype groups.

	MTHFR genotype				P†
	TT (<i>n</i> 32)		CC/CT (<i>n</i> 21)		
	Mean	SD	Mean	SD	
Age (years)	58.3	6.3	58.2	7.2	0.95
BMI (kg/m ²)	26.5	4.8	26.0	3.2	0.72
BMD (g/cm ²): Lumbar spine	1.126	0.112	1.067	0.122	0.08
Total hip	0.951	0.116	0.952	0.101	0.96
Total body	1.127	0.075	1.117	0.074	0.61
T-score*: Lumbar spine	-0.5	0.9	-0.9	1.04	0.10
Total hip	-0.4	1.0	-0.4	0.9	0.99
Total body	0.0	0.9	-0.1	0.9	0.62

* WHO definition of osteoporosis and osteopenia for white women: normal, T-score ≥ -1.0 SD; osteopenia, T-score > -1.0 SD and < -2.5 SD; osteoporosis, T-score ≤ -2.5 SD⁴.

† Values were compared using an independent sample *t* test.

The different genotype groups did not differ in terms of age or BMI. No significant difference was observed between the TT and the CC/CT genotype control group in BMD or in T-scores. In conclusion this pilot study provides no evidence that the C677T polymorphism in MTHFR is a risk factor for osteoporosis. However, the results will be re-examined in relation to Hcy and the related B-vitamins in a larger cohort.

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- World Health Organisation (1994) *WHO Technical Report Series* no. 843. Geneva: WHO.

LIPGENE Dietary Intervention: cohort details and study design. By A.C. TIERNEY¹, J.A. LOVEGROVE², H. LOVDAL GULSETH³, C. DEFOORT⁴, E. BLAAK⁵, C. MARIN⁶, L. PARTYKA⁷, B. KARLSTRÖM⁸, B. VESSBY⁸, A. DEMBINSKA-KIEC⁷, J. LÓPEZ MIRANDA⁶, W. SARIS⁵, D. LAIRON⁴, C.A. DREVON³, C.M. WILLIAMS², M.J. GIBNEY⁹ and H.M. ROCHE¹, ¹Nutrigenomics Research Group, School of Medicine, Trinity College Dublin, Republic of Ireland, ²Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, University of Reading, UK, ³Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Norway, ⁴INSERM 476, Nutrition Humaine et lipides, INRA 1260, Univ Méditerranée Aix-Marseille 2, Marseille, France, ⁵Maastricht University, The Netherlands, ⁶Lipid and Atherosclerosis Unit, School of Medicine, University of Cordoba, Spain, ⁷Department of Clinical Biochemistry, Jagiellonian University Medical College, Krakow, Poland, ⁸Department of Public Health & Caring Sciences/Clinical Nutrition and Metabolism, Uppsala University, Sweden and ⁹Food & Health, University College Dublin, Republic of Ireland

The metabolic syndrome is associated with increased risk of type 2 diabetes mellitus and CVD¹. Insulin resistance is the key metabolic perturbation of the metabolic syndrome². Although diet is not considered a risk factor, there is little doubt that metabolic stressors including energy-dense high-fat diets promote obesity, insulin resistance and the metabolic syndrome.

LIPGENE is an EU Integrated Project entitled 'Diet, genomics and the metabolic syndrome: an integrated nutrition, agri-food, social and economic analysis' that is funded under the Framework 6 Food Safety and Quality Programme (2003). The LIPGENE Human Dietary Intervention Study was designed to determine the relative efficacy of reducing dietary SFA consumption, by altering the quality of dietary fat or reducing the quantity of dietary fat, on multiple metabolic and molecular risk factors of the metabolic syndrome. LIPGENE also explored whether common genetic polymorphisms associated with the metabolic syndrome determine an individual's response to dietary fat modification.

Over 15 500 subjects were screened to recruit 486 subjects with the metabolic syndrome who initiated the study. Free-living subjects with the metabolic syndrome received one of four dietary treatments for 12 weeks: (A) high-fat (38% energy from fat) SFA-rich diet; (B) high-fat (38% energy from fat), MUFA-rich diet; (C) isoenergetic low-fat (28% energy from fat) high-complex-carbohydrate diet; (D) isoenergetic low-fat (28% energy from fat) high-complex-carbohydrate diet, with 1.24 g long-chain *n*-3 PUFA/d. The clinical characteristics indicative of the metabolic syndrome in the four dietary groups are:

	A (<i>n</i> 121)		B (<i>n</i> 126)		C (<i>n</i> 119)		D (<i>n</i> 120)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Metabolic syndrome criteria:								
Glucose (mmol/l)	5.60	0.07	5.55	0.07	5.48	0.07	5.51	0.07
TAG (mmol/l)	1.94	0.08	1.70	0.06	1.84	0.08	1.77	0.06
HDL-cholesterol (mmol/l)	1.29	0.03	1.30	0.03	1.32	0.03	1.30	0.03
Systolic blood pressure (mmHg)	143.5	1.20	142.3	1.51	141.6	1.51	143.8	1.58
Diastolic blood pressure (mmHg)	89.2	0.84	88.7	0.97	89.2	0.91	90.0	1.11
Waist girth (cm)	104.9	0.91	106.2	1.01	107.7	1.10	107.0	0.98

With the clinical observations (intravenous glucose tolerance test, lipoprotein analysis, cytokines, adhesion molecules, coagulation factors and isoprostane levels) and dietary assessments (3 d weighed-food intake pre-, mid- and post-intervention) carried out throughout the 12 week intervention study, LIPGENE will provide important information in relation to dietary fatty acid modification, genetic determinants of dietary responsiveness and molecular markers of insulin sensitivity.

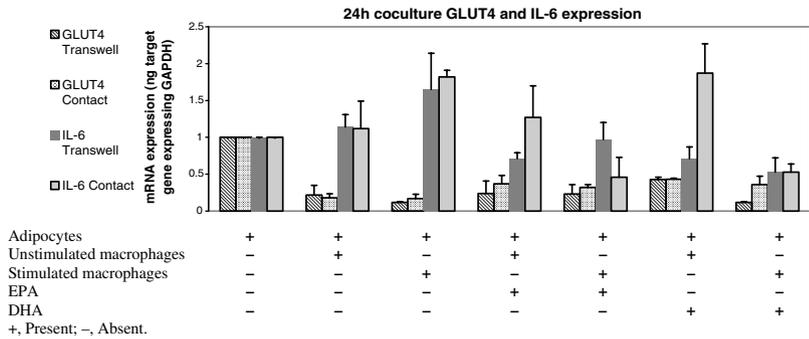
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Co-culture of adipocytes with macrophages promotes a pro-inflammatory state and dampens insulin responsiveness in adipocytes that is attenuated by long-chain n-3 PUFA. By E. OLIVER, C. PHILLIPS, S. TOOMEY and H.M. ROCHE, *Nutrigenomics Research Group, Department of Clinical Medicine, Institute of Molecular Medicine, St James's Hospital, Dublin 8, Republic of Ireland*

Obesity is the key aetiological factor that predisposes individuals to insulin resistance (IR) the hallmark of the metabolic syndrome and type 2 diabetes. Recent studies have shown that obese adipose tissue is characterised by increased infiltration of macrophages, suggesting that they are an important source of inflammation and may underlie obesity-induced IR^{1,2}. The molecular basis of the interaction between adipocytes and macrophages in adipose tissue is unclear. The present study attempts to elucidate the molecular mechanisms whereby adipocytes and macrophages communicate by investigating the effect of these interactions on molecular markers of insulin sensitivity and inflammation in adipocytes by using a co-culture system of both cell types, as a model of obesity-induced IR.

A co-culture of adipocytes (3T3-L1) and macrophages (J774) was performed in two different systems. In the contact system differentiated adipocytes were cultured in six-well plates at 37 °C after which time unstimulated macrophages and macrophages stimulated with 0.1 mg LPS/ml for 24 h were added to the adipocytes for 24 hours. In the transwell system inserts facilitate exchange of soluble molecules between the upper (J774) and lower compartments (3T3-L1). After incubation for 24 h the adipocytes in the lower compartment were harvested. Total RNA was extracted from cultured cells and quantitative real-time PCR was performed. Levels of mRNA were normalised to those of glyceraldehyde-3-phosphate dehydrogenase. Cultured cell supernatants were analysed by ELISA.

The presence of macrophages significantly reduced adipocyte glucose transporter 4 (GLUT4) and insulin receptor substrate 1 mRNA, classical markers of insulin sensitivity. In contrast, adipocyte interleukin 6 (IL-6), tumour necrosis factor α (TNF α) and toll-like receptor 4 (TLR4) mRNA levels were up-regulated by the presence of macrophages. Also cell supernatant TNF α , IL-6 and IL-1 β concentrations were increased but adiponectin levels were reduced on exposure to the macrophages in both the contact and transwell systems. Interestingly, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) modify these effects within the adipocytes, reducing the expression of pro-inflammatory mediators (TNF- α , IL-6 and IL-1 β) and increasing markers of insulin sensitivity (GLUT4 and IRS-1).



These results suggest that obesity is associated with the generation of macrophage-derived soluble factors that promote a pro-inflammatory insulin-resistant adipocyte function. The work shows that pre-culturing macrophages with EPA and DHA can attenuate this effect. Further work is required to further elucidate the molecular interactions between adipocytes, macrophages and dietary fatty acids.

E. O. is a recipient of the PhD in Molecular Medicine and the HRB PhD Training Site Programme at Trinity College Dublin, Republic of Ireland. This work was supported by the European Commission, Framework Programme 6 (LIPGENE): contract number FOOD-CT-2003-505944.

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The impact of the apoE genotype on fasting and postprandial TAG. By A.M. MINIHAINE¹, E. OLANO-MARTIN¹, R. GILL-GARRISON², A.M. VALDES², K. GRIMALDI², J.A. LOVEGROVE¹ and K.G. JACKSON¹, ¹Hugh Sinclair Unit of Human Nutrition, Department of Food Biosciences, University of Reading, Reading RG6 6AP, UK and ²Sciona Inc, 1401 Walnut St, #203, Boulder, CO 80302, USA

ApoE plays a role in many stages of lipoprotein metabolism, including TAG-rich lipoprotein (TRL) hydrolysis and the receptor-mediated removal of TRL remnants by the liver. The *APOE* gene, located on chromosome 19, is known to be highly polymorphic with fifty-four individual single-nucleotide polymorphisms (SNP) identified to date. The most widely studied of these is the apoE ϵ genotype, with carriers of the $\epsilon 4$ allele known to be at a 40–50% increased risk of CVD¹. However, the molecular aetiology of this increased risk is poorly understood. In the current study the impact of apoE genotype on fasting and postprandial TAG response is investigated.

Healthy UK men (n 153) and women (n 109), mean age 53 (SE 1.0) years, BMI 26.2 (SE 0.2) kg/m², fasting total cholesterol 5.76 (SE 0.06) mmol/l and TAG 1.64 (SD 0.05) mmol/l, underwent postprandial assessment, whereby following a standard breakfast (time 0h, 49 g fat) and lunch (time 5.5 h, 29 g fat) blood samples were taken at regular intervals for ≥ 8 h post breakfast. DNA was isolated from the buffy-coat layer and allelic discrimination of the apoE2, E3 and E4 alleles was achieved using TaqMan PCR technology with SNP genotyping kits (Applied Biosystems, Warrington, UK). Fasting lipids and postprandial TAG were quantified using commercially-available kits (Instrumentation Laboratories, Warrington, UK).

For the purpose of the present paper, only the common E2/E3 (n 42), E3/E3 (n 142) and E3/E4 (n 63) genotypes will be considered. For fasting TAG 11% and 23% higher levels were evident in E2/E3 and E3/E4 individuals relative to the wild-type E3/E3 genotype ($P=0.001$), with apoE genotype explaining 7.2% of the fasting TAG levels in a stepwise linear regression model ($P=0.000$). Furthermore, a significant age \times genotype interaction was evident ($P=0.007$), with the impact of genotype more evident in the 20–50 years age-group relative to >50 years. As shown in the Fig., a significant impact of apoE genotype on the postprandial area-under-the-curve (AUC) was observed ($P=0.032$) with a near significant effect on the incremental AUC ($P=0.075$).

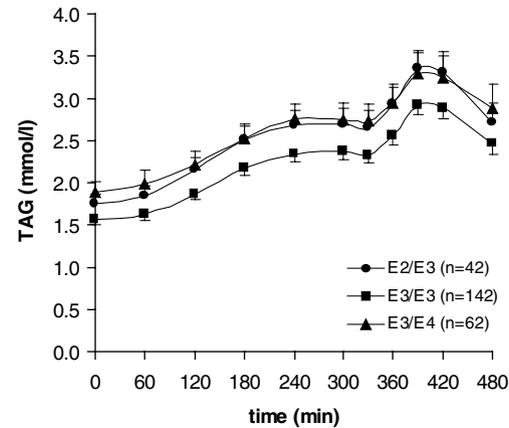


Fig. Postprandial TAG response according to apoE genotype AUC, $P=0.032$.

The present study provides evidence that the impact of apoE genotype on fasting and postprandial TAG is likely to contribute to the apoE genotype–CVD associations. It is speculated that the higher differential affinities of the apoE isoforms for the hepatic LDL receptor, together with an impact of genotype on apoE-lipoprotein distribution, is in large part responsible for the observed effects. Further investigations into the molecular basis of this genotype–disease association are merited.

1. Song Y, Stampfer MJ & Liu S (2004) *Ann Intern Med* **141**, 137–147.

Plasma sterols as markers of cholesterol absorption and synthesis: inter-relationships with plasma lipids and apoE genotype. By N.L. HARMAN¹, M. POURFARZAM², R.D.G. NEELY² and B.A. GRIFFIN¹, ¹School of Biomedical and Molecular Science, University of Surrey, Guildford, GU2 7XH, UK and ²Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, UK

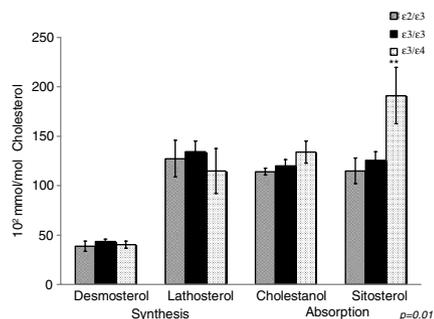
The plasma lipid response to changes in dietary fat and cholesterol is highly variable between individuals. This variation may be explained, in part, by differences in apoE genotype and the rates of cholesterol synthesis and absorption in the gut. ApoE genotype has been putatively linked with cholesterol homeostasis, with carriers of the ε4 allele showing increased absorption of cholesterol and and plasma sterols to assess the potential utility of the latter as biomarkers of cholesterol synthesis and absorption in dietary studies.

Total plasma cholesterol, LDL, HDL-cholesterol and TAG was determined in forty-four normal healthy volunteers. Plasma sterols were measured by GC–MS (desmosterol, lathosterol, cholestanol and sitosterol). ApoE genotype was determined by restriction isotyping.

The distribution of apoE genotype was similar to that previously described², with ε3 homozygotes being the most frequent (ε2/ ε3, *n* 7; ε3/ ε3, *n* 30; ε3/ ε4, *n* 7). There was no difference in plasma lipids, desmosterol, lathosterol or cholestanol between apoE genotypes but carriers of the ε4 allele had a significantly higher concentration of plasma sitosterol than non-carriers. In the total group plasma TAG was inversely associated with plasma cholestanol

(*r* −0.36, *P*<0.05) and sitosterol (*r* −0.31, *P*<0.05), positively associated with desmosterol (*r* 0.38, *P*<0.05) and weakly with lathosterol (*P*=0.06). There were significant associations between cholestanol and both desmosterol; (*r* −0.42) and lathosterol (*r* −0.74, *P*<0.01), and between sitosterol and lathosterol (*r* −0.38, *P*<0.05).

The higher concentration of plasma sitosterol in ε4 carriers is consistent with elevated cholesterol absorption in the apoE4 genotype. The association of plasma TAG with markers of cholesterol absorption might reflect the inverse relationship between cholesterol absorption and synthesis (VLDL production). The lack of differences in plasma lipids and sterols between apoE genotype may be explained by the under-representation of the rarer ε4 and ε2 alleles in this small group. Nevertheless, the present study provides evidence for the use of plasma sterols (sitosterol and cholestanol) and cholesterol precursors (desmosterol and lathosterol) as markers of cholesterol absorption and synthesis.



The association among vitamin D status, body composition and glucose metabolism in apparently-healthy normal-weight and overweight women. By M.S. BARNES, A. MULLEE, M.P. BONHAM, P.J. ROBSON, J.J. STRAIN and J.M.W. WALLACE, Northern Ireland Centre for Food & Health (NICHE), University of Ulster, Coleraine BT52 1SA, UK

Epidemiological studies have reported lower vitamin D status (25-hydroxyvitamin D (25(OH)D concentrations) in obese individuals compared with non-obese individuals¹. As vitamin D status is inversely correlated with glucose and insulin concentrations², vitamin D insufficiency may partially explain the link between obesity, insulin resistance (IR) and diabetes. The aim of the present study was to assess the associations among vitamin D status, body composition and glucose metabolism. Overweight (*n* 20) and normal-weight (*n* 32) apparently-healthy women aged 18–40 years were recruited and sampled within a 4-week period in early summer. Anthropometric measurements were made and percentage fat mass (%FM) was measured using the BodPod[®] (Life Measurement Inc., Concord, CA, USA) body composition analyser. Vitamin D status was measured using the IDS OTEIA vitamin D kit (Boldon, UK). Glucose and insulin concentrations were measured in fasting blood samples and the homeostatic model assessment (HOMA) of IR (HOMA-IR) and β-cell function (HOMA-β) were calculated³.

	Normal weight (<i>n</i> 32; BMI 18.0–24.9 kg/m ²)		Overweight (<i>n</i> 20; BMI ≥25.0 kg/m ²)	
	Mean	SD	Mean	SD
Height (m)	1.65	0.07	1.64	0.06
Weight (kg)***	60.3	7.0	75.5	10.7
BMI (kg/m ²)***	21.9	1.7	27.8	3.1
WC (cm)***	73.4	6.6	87.6	7.6
WTHR***	0.44	0.04	0.53	0.05
%FM***	26.3	6.6	36.0	4.5
Glucose (mmol/l)	4.93	0.28	5.16	0.67
Insulin (mU/l)	12.1	4.21	12.6	4.78
HOMA-IR	2.64	0.91	2.94	1.37
HOMA-β	175.8	76.9	167.9	84.2
25(OH)D (nmol/l)	62.8	26.1	68.8	24.4

WC, waist circumference; WTHR, waist:height ratio. Mean values were significantly different between groups (independent samples *t* test): ****P*<0.001.

Vitamin D status was adequate in the current population group as defined by 25(OH)D concentrations >50 nmol/l. Despite significant differences in BMI, abdominal fat and %FM between the lean and overweight groups, there was no significant difference in vitamin D status, glucose, insulin or IR between the two groups. Furthermore, vitamin D status was not a significant predictor of glucose metabolism. BMI, WTHR and %FM were significant predictors of fasting glucose concentrations; WC was a significant predictor of fasting insulin concentrations, whilst BMI, WC and WTHR were significant predictors of HOMA-β.

In conclusion body composition was not a significant determinant of vitamin D status in lean and overweight individuals with adequate vitamin D status. It is speculated that previous reports of associations may have been driven by the inclusion of morbidly-obese individuals in the study group or that such associations may only be evident when vitamin D status is low.

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 2. Chiu KC, Chu A, Go VL *et al.* (2004) *Am J Clin Nutr* **79**, 820–825.
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TQ1 1. Sarkkinen E, Korhonen M, Erkkila A, Ebeling T & Uusitupa M (1998) *Am J Clin Nutr* **68**, 1215–1222.
 2. Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE & Stoehla BC (2002) *Am J Epidemiol* **155**, 487–495.

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Selenium status influences the effect of folate, vitamin B₁₂ and pyridoxal 5'-phosphate on homocysteine concentrations. By B. BEKAERT¹, M.L. COOPER¹, F. GREEN¹, H. McNULTY², K. PENTIEVA², J. SCOTT², A. MOLLOY³ and M.P. RAYMAN¹, ¹School of Biomedical and Molecular Sciences, University of Surrey, GU2 7XH, UK, ²School of Biomedical Sciences, University of Ulster, Coleraine, UK, ³School of Biochemistry and Immunology, Trinity College, Dublin, Republic of Ireland

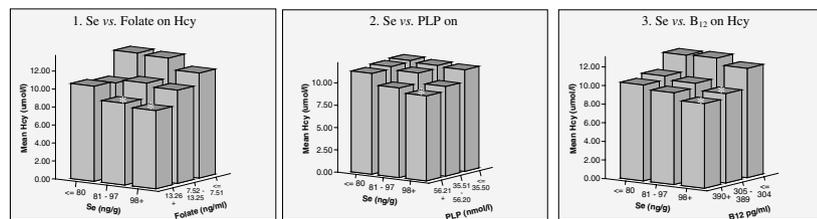
Elevated homocysteine (Hcy) concentrations have been associated with a plethora of complex diseases including CVD, neurodegenerative diseases, osteoporosis and cancer. A prerequisite for dealing efficiently with Hcy is an adequate supply of the B-vitamins folate, vitamin B₁₂, vitamin B₆ and riboflavin that are involved in its metabolism. Concentrations of these vitamins are inversely related to Hcy concentrations and have been used in an attempt to lower Hcy in a number of studies. Se has recently been implicated in reducing Hcy in elderly Spanish¹ and Silesian² populations, although in New Zealand an intervention using 200 µg Se-enriched yeast/d was not found to change Hcy concentrations³. In rats the opposite effect was observed; Se deficiency decreased Hcy⁴.

The interaction between five dietary factors has been investigated in a study that was ancillary to the UK PRECISE (PREvention of Cancer by Intervention with SElenium) Pilot Study. In this double-blind placebo-controlled intervention trial 501 healthy UK volunteers aged 60–74 years (from the West Midlands, Cleveland and Suffolk) were randomly allocated to receive 100, 200 or 300 µg Se high-Se yeast or a placebo yeast/d. Blood was collected at baseline and 6 months, enabling the measurement of plasma Se, Hcy, folate, vitamin B₁₂ and pyridoxal 5'-phosphate (PLP) at these time points.

Plasma Se at baseline and after 6 months of Se supplementation

Treatment	Baseline (ng/g)	Follow-up after 6 months (ng/g)	P
Placebo	89.98	91.36	0.738
100 µg	87.85	144.49	<0.001
200 µg	89.31	191.16	<0.001
300 µg	91.33	226.71	<0.001

Females had higher folate and lower Hcy concentrations than males ($P < 0.05$ in both cases). At baseline Se was inversely correlated with Hcy, but when stratified for gender the effect only remained significant in males ($P < 0.001$). Before supplementation, Hcy concentration in males was significantly lower in the highest than in the lowest Se tertile ($P < 0.05$). The effect of folate, PLP and vitamin B₁₂ on Hcy was dependent on Se at baseline (see Figure). Except for Se, none of the variables changed significantly after 6 months of treatment.



Interactions between Se, folate, PLP and B₁₂ on Hcy concentrations

Research supported by Cancer Research UK.

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- Klapcinska B, Szmigiel H, Ratajczak R, Szybinski Z & Zachwieja Z (2005) *Biol Trace Elem Res* **108**, 1–15.
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Dietary enrichment with almond (*Prunus amygdalis*) supplementation: effects on CVD risk biomarkers. By K. CHOUDHURY and H.R. GRIFFITHS, *Aston University, Aston Triangle, Birmingham B4 7ET, UK*

Epidemiological evidence suggests that diets rich in fruits, vegetables and pulses reduce the risk of CVD. The Physicians Health Study has demonstrated reduction of CHD death with regular nut consumption¹. One major modifiable risk factor for CHD is an unhealthy diet. Thus, an almond-enrichment study has been undertaken to examine the benefit of almonds (*Prunus amygdalis*) in healthy individuals either with or without significant risk of vascular disease. Almonds contain various macronutrients (low SFA content, absence of cholesterol and high MUFA content) and micronutrients, including vitamin E, polyphenols and arginine, which afford vascular benefit. The effects of almond consumption (25 g/d for 4 weeks followed by 50 g/d for 4 weeks) were evaluated in three non-smoking subject groups: healthy male volunteers between the ages of 18 and 35 years ($n = 15$); men at risk of heart disease between the ages of 18 and 35 years ($n = 12$); mature men and women >50 years of age ($n = 18$). A fourth control group ($n = 14$) were followed over 8 weeks without dietary almond enrichment as a treatment control. None of the subjects withdrew from the study and 90% completed the study. The interim results of the study showed that in the three active groups there was little evidence for a change in total cholesterol, LDL-cholesterol or HDL-cholesterol. In the mature group there was a trend towards increasing HDL-cholesterol. The mature and 'at-risk' groups also showed a significant changes in systolic blood pressure ($P < 0.05$) during almond consumption. The healthy group showed a decrease in diastolic blood pressure ($P < 0.05$). The 'at-risk' group showed a significant increase ($P < 0.05$) in flow-mediated dilation after 8 weeks of almond consumption. Data analysis is ongoing, with completion of the study in November 2007. The beneficial effects of almond consumption on flow-mediated dilation and blood pressure may be attributed to the high content in almonds of arginine, which serves as a precursor to the vasodilatory molecule, NO.

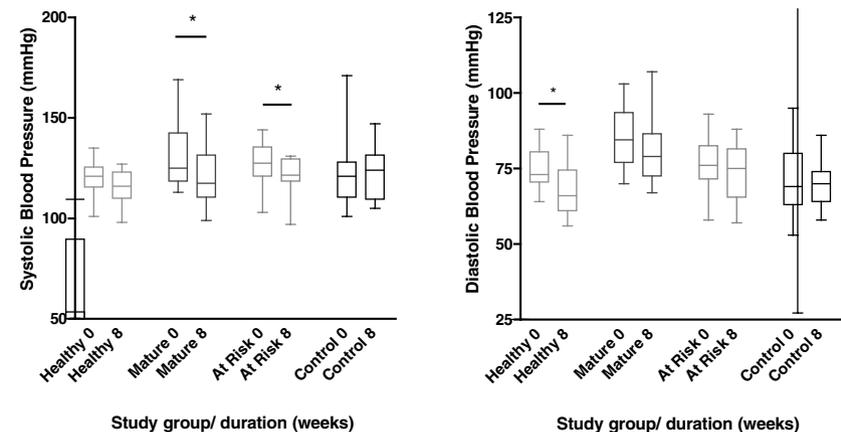


Fig. 1. Systolic and Diastolic Blood Pressure levels at time points 0 and 8 weeks before and after intake of almonds (25 g/d over 4 weeks and 50 g/d over 4 weeks). Box and whisker plots (median, range, upper and lower quartiles).

- Albert CM, Gaziano JM, Willett WC & Manson JE (2002) *Arch Intern Med* **162**, 1382–1387.

Plasma selenium, zinc, copper and glutathione peroxidase concentrations in older subjects during both acute illness and recovery. By S.E. FORSTER¹, S.E. GARIBALLA² and H.J. POWERS¹, ¹Human Nutrition Unit The School of Medicine, University of Sheffield, Sheffield S10 2RX, UK and ²The University of United Arab Emirates, Department of Internal Medicine, Faculty of Medicine and Health Sciences, United Arab Emirates

Se, Zn and Cu are essential trace minerals. Plasma Se and Zn concentrations have been found to be low in older subjects^{1,2}.

The results presented are part of a large randomised placebo-controlled intervention trial, detailed methods for which have been described elsewhere³. Subjects (*n* 445) aged ≥65 years who were recovering from an acute illness were randomised to receive a food supplement or a placebo for 6 weeks. The food supplement contained 1881 kJ (450 kcal) and vitamins and minerals, including 75 µg Se, 1.2 mg Cu and 9.5 mg Zn in a daily dose. The placebo contained the same flavours and colourings as the supplement and 60 kcal energy. Blood samples were taken at baseline, 6 weeks and 6 months, and plasma was analysed for Se, Zn and Cu concentrations using inductively-coupled plasma spectroscopy–MS (HP 4500; Agilent, Cheadle, Cheshire, UK). Glutathione peroxidase activity was determined with a Ransel kit (Randox Laboratories Ltd, Crumlin, Co. Antrim, UK) using a Cobas Bioautoanalyser (Roche Products, Welwyn Garden City, Herts., UK).

At baseline mean plasma Se, Zn and Cu concentrations (µmol/l) and glutathione peroxidase activities (U/l) were 0.86 (SD 0.3), 9.5 (SD 2.3), 16.8 (SD 3.6) and 170.2 (SD 51.8) respectively. Plasma Se concentrations were low compared with those found for younger adults². Plasma Zn concentrations were significantly lower than those found in other studies for this age-group⁴, both initially when the participants were acutely ill and after 6 months. Adjusted 6-week plasma concentrations, were significantly higher than baseline values for Se (*P*<0.01) and Zn (*P*<0.01). Adjusted 6-week plasma concentrations were significantly lower than adjusted 6-month values for Se (*P*=0.015), Zn (*P*=0.047) and Cu (*P*<0.01). No effect of supplementation was observed.

Factors influencing plasma selenium, zinc, copper and glutathione peroxidase (GSHP_x) during acute illness were examined using a regression model.

Factor	Selenium Regression Coefficient (95% CI)	Zinc Regression Coefficient (95% CI)	Copper Regression Coefficient (95% CI)	GSHP _x Regression Coefficient (95% CI)
Age	-0.085 (-0.730 to 0.109)	-0.121 (-5.476 to -0.266)*	-0.003 (-4.405 to 4.197)	-0.121 (-1.99 to -0.025)*
Chronic disease	-0.053 (-3.25 to 1.24)	-0.75 (-23.304 to 4.560)	-0.010 (-15.298 to 13.077)	-0.082 (-9.258 to 1.831)
Drugs	0.048 (-0.822 to 1.95)	0.094 (-1.419 to 15.767)	-0.011 (-6.843 to 5.675)	0.032 (-2.496 to 4.264)
BMI	0.013 (-0.538 to 0.638)	0.107 (0.015 to 7.597)*	0.71 (-0.199 to 0.698)	-0.048 (-2.017 to 0.845)
C-reactive protein mg/L	-0.147 (-0.095 to -0.008)*	-0.191 (-0.708 to -0.165)**	-0.104 (-11.734 to 1.300)	0.206 (0.061 to 0.270)**
Albumin g/L	0.153 (0.136 to 1.408)*	0.211 (2.967 to 10.861)**	-0.013 (-25.534 to 20.470)	0.150 (0.206 to 3.290)*

Plasma Se and Zn are negative acute-phase reactants and it is likely that the low concentrations at baseline are at least partly related to illness. However, illness does not explain the low concentrations found at 6 months.

The results of this large study show that in older subjects plasma concentrations of Se and Zn are low, and acute illness affects these concentrations. The functional significance of this effect should be investigated.

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Stable-isotope studies to measure phyloquinone kinetics and bioavailability. By K.S. JONES, L.J.C. BLUCK, L.Y. WANG and W.A. COWARD, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Rd, Cambridge CB1 9NL, UK*

In Western diets phyloquinone is the primary dietary form of vitamin K¹. The biochemical function of phyloquinone is as a cofactor in the γ -carboxylation of glutamic acid. Evidence is accumulating for a role of phyloquinone beyond its established role in blood clotting. Studies have shown a beneficial effect of higher vitamin K status and intake on markers of bone health and, furthermore, there is evidence implicating vitamin K as being protective against vascular calcification. Partly in recognition of this evidence recommended dietary intakes for phyloquinone have recently been increased in the USA to 90 µg/d and 120 µg/d for women and men respectively². The current guideline for phyloquinone intake in the UK is 1 µg/kg body weight per d³. Average daily intake is about 70 µg/d; however, almost 60% of adults have intakes below the guideline level⁴. In order to set appropriate recommended intakes data are required on phyloquinone absorption and metabolism. Stable isotopes provide a valuable tool for such an investigation.

A method has been developed that reliably and accurately measures the isotopic enrichment of plasma phyloquinone using GC–MS⁵. In the first study two stable-isotope species and compartmental modelling were used to measure the absorption, uptake and disposal kinetics of phyloquinone in ten volunteers. Isotopic data were fitted to a two-compartment model with all input and output from the sampled (plasma) pool, and exchange between the sampled pool and a remote compartment. Half-times for the disappearance of phyloquinone were calculated as 0.2 and 2.7 h. More recently, the methodology has been applied to the measurement of phyloquinone bioavailability from meals. Three test meals were formulated based on dietary clusters identified in dietary-pattern analysis of the 2001 National Diet and Nutrition Survey. To aid identification the three clusters were labelled ‘convenience’, ‘cosmopolitan’ and ‘animal-oriented’⁶. Twelve subjects were studied in a three-way cross-over. Immediately preceding consumption of a test-meal the subjects took a capsule containing 20 µg ¹³C-labelled phyloquinone. Each test meal was designed to contain 40 µg phyloquinone and meals were balanced for percentage energy from fat, protein and carbohydrate and the amount of dietary fibre. Blood samples were taken over the following 8 h, and both total concentration and isotopic enrichment of phyloquinone were measured.

Appearance of labelled phyloquinone in plasma was used to assess the effect of the gut milieu created by the meal on absorption of phyloquinone. Mean tracer area-under-the-curve measurements (nmol/l-h) were 0.88 (SD 0.42), 1.30 (SD 0.49) and 1.13 (SD 0.60) for the convenience, cosmopolitan and animal-oriented meals respectively. The effect of food matrix on phyloquinone absorption was assessed by comparison of regressions between tracer and tracee, adjusted for the size of the dose. More phyloquinone was found to be absorbed relative to the tracer from the convenience meal than from either the cosmopolitan or animal-oriented meals (slopes of regressions were 1.88 (SD 0.81), 0.59 (SD 0.32) and 0.44 (SD 0.40) respectively).

These studies have demonstrated the application of stable-isotope-based methodologies to the absorption and metabolism of phyloquinone. The reported methods and data may be useful for the future consideration of recommended dietary intakes in the UK and elsewhere.

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Potential mechanisms for the chemoprotective effect of cruciferous vegetables in colo-rectal cancer: a clinical trial of indole-3-carbinol. By D.R. McGRATH, H.R. FRYDOONFAR, J.J. HUNT, C.J. DUNKLEY and A.D. SPIGELMAN, *Discipline of Surgical Science, University of Newcastle, Callaghan, NSW 2308, Australia*

Despite the well-recognised protective effect against various human cancers, including colo-rectal, of cruciferous vegetables little is known of how this effect is conferred. It is thought that some phytochemicals only found in these vegetables confer the protection. These compounds include the glucosinolates, of which indole-3-carbinol is one. They are known to induce carcinogen-metabolising enzymes such as the phase 2 enzymes of the glutathione transferase (GST) family. The other effects in human subjects are not well documented. The aim of the present study was to assess the effect of indole-3-carbinol on GST enzymes and the effect of treated human serum on two colon-cancer cell lines in a similar fashion to previous laboratory work¹.

A double-blind placebo-controlled human volunteer study was carried out. All patients were given 400 mg indole-3-carbinol daily for 3 months, followed by placebo. Serum samples were tested for GSTM1 genotype by PCR. Serum GST levels were assessed using ELISA and western blot methodologies. Serum was exposed to HT29 and HCT116 colon-cancer cells.

Forty-nine volunteers completed the study. GSTM1 genotypes were obtained for all but two volunteers. A slightly-greater proportion of volunteers were GSTM1-positive, in keeping with the general population. GST was detected in all patients. Total GST level was not affected by indole-3-carbinol dosing *v.* placebo (1.73 µg/ml *v.* 1.70 µg/ml; $P=0.67$). Although not significant, GSTM1 genotype affected the serum GST level in response to indole-3-carbinol ($P=0.49$). There was a significant decrease in proliferation of both cancer cell lines when exposed to serum during the indole-3-carbinol treatment period *v.* baseline ($P=0.03$ and $P=0.002$ respectively; Table 1). This difference was not seen when compared with the placebo period.

Difference in colon cancer cell proliferation following exposure to volunteer serum			
Cell Line	Blood Sample	Mean Difference*	P value
HT29	I3C1 <i>v.</i> Blood 0	-0.062	0.03
	I3C1 <i>v.</i> Placebo	0.007	0.57
	I3C1 <i>v.</i> Blood 0	-0.096	0.002
HCT116	I3C1 <i>v.</i> Placebo	0.014	0.46
	I3C2 <i>v.</i> Blood 0	-0.107	0.002
	I3C2 <i>v.</i> Placebo	0.010	0.63

I3C1 – Blood sample after one month treatment; I3C2 – Blood sample after 3 months treatment; Blood 0 – Baseline blood sample.
* – Mean Difference of UV intensity (as an expression of cell proliferation).

Indole-3-carbinol does not alter the total serum GST level during prolonged dosing. Serum from volunteers during the indole-3-carbinol dosing period caused inhibition of proliferation in colon cancer cells *v.* baseline.

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Energy intake and appetite responses of adolescent girls following a netball-specific exercise protocol. By P.L.S. RUMBOLD and C.J. DODD, *Nutrition, Exercise and Appetite Research Group, School of Psychology and Sport Sciences, Northumbria University, Wynne Jones Centre, Ellison Place, Newcastle upon Tyne NE1 8ST, UK*

Previous work has indicated a lack of exercise-induced elevation in energy intake (EI) following laboratory-based cycling¹. Children generally choose not to participate in structured exercise, however; their behaviour tending to be sporadic². The present study is the first to impose intermittent netball-based exercise and explore subsequent EI and appetite responses of young subjects.

In order to replicate the physiological demands of a netball match, a sport-specific exercise protocol (NSEP) was adapted from an existing netball-specific fitness test³. Appetite and EI responses were investigated in nine young female netball players aged 12 (sd 0.8) years following the NSEP. The 47 min protocol was compared with a sedentary (SED) condition using a repeated measures design. Heart-rate data were collected on both occasions. Immediately after both conditions participants were provided with an identical *ad libitum* meal, which was of homogenous macronutrient composition (carbohydrate–fat–protein, 53%:35%:12%). Food items consisted of ham and lettuce white bread sandwiches, chocolate cake and yoghurt, served in excess. EI was measured via observation and food was covertly weighed pre- and post-meals. Hunger, fullness and prospective consumption were assessed using visual analogue scales, administered immediately after NSEP and SED and every 10 min thereafter for 60 min.

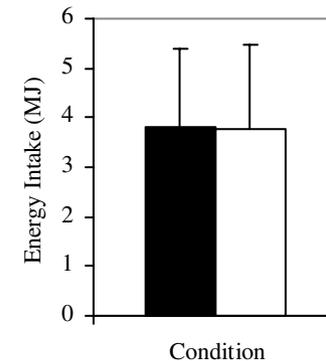


Fig. EI after SED (■) and NSEP (□) conditions. Values are means and standard deviations represented by vertical bars.

Heart rate (beats per min) was significantly different between NSEP and SED conditions (165.2 (sd 23.64) and 105.8 (sd 19.27) respectively; $P<0.001$). There were no differences for any appetite variables, either between conditions or over time ($P>0.05$). EI values (MJ) were not significantly different between conditions (NSEP 3.79 (sd 1.68) and SED 3.82 (sd 1.59) respectively; $P>0.05$). A single bout of netball-specific exercise resulted in no immediate EI increase in 12–14-year-old girls.

This lack of immediate elevation in exercise-induced EI supports existing literature using a laboratory-based cycling protocol¹. Investigation is required over a longer period to elucidate the apparent delay in EI compensation of young netball players.

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Influence of milk ingestion on the hormonal and immune responses to prolonged exercise. By P. WATSON, J. VIANA, S. LESER, T. KIRK and M. GLEESON, *School of Sport and Exercise Sciences, Loughborough University, Loughborough, Leicestershire LE11 3TU, UK*

Repeated bouts of strenuous exercise have been shown to result in a marked stress response and a suppression of the immune system, consequently increasing an individual's susceptibility to infection. The effectiveness of low-fat milk in attenuating the exercise-induced stress response was compared with that of a commercially-available sports drink.

Five endurance trained males (age 23 (SD 6) y; height 1.80 (SD 0.10)m; body mass 70.5 (SD 17.5)kg, peak oxygen uptake 62.7 (SD 6.3)ml/kg/min) were recruited. The study took place over two separate weeks, during which the subjects completed their normal training regimen. During (400ml/h) and after (500ml) training volunteers were instructed to ingest either skimmed milk or a commercially-available 6.4% (w/v) carbohydrate sports drink (CHO). Subjects entered the laboratory on days 1 and 7 of each experimental week and completed 2h 30min of cycle exercise at 65% $V_{O_{2max}}$ to allow the immunoendocrine response to exercise to be monitored. Only water was available for ingestion during these laboratory-based exercise tests.

The volume of training undertaken by the subjects was 12.4 (SD 5)h/wk, in the form of 5 (SD 1) sessions/wk. Blood leucocyte ($P=0.015$), neutrophil ($P=0.016$), lymphocyte ($P=0.017$) and monocyte ($P=0.027$) counts all increased following exercise before and after both CHO- and milk-ingestion weeks, but the drink ingested did not influence these responses (Table). This response was accompanied by a fall in the functional capacity of the immune cells to respond to a challenge, with a reduction in bacterially-stimulated elastase release per neutrophil ($P=0.262$). Mean plasma IL-6 concentrations at rest were 0.26 (SD 0.37)pg/ml. Circulating IL-6 increased during exercise to 1.94 (SD 1.28), 1.42 (SD 0.93), 3.84 (SD 3.11) and 2.52 (SD 1.82)pg/ml in the CHO1, CHO7, Milk1 and Milk7 trials respectively ($P=0.171$). There was no change in mean plasma cortisol concentrations following exercise ($P=0.757$), but there was a marked individual variation observed in this response, with some individuals displaying an increase of ≤ 4 -fold. Neither milk nor sports-drink ingestion altered this response ($P=0.552$).

Changes in immune cell counts ($\times 10^9$ cells/l) during the experimental trials

		Pre1	Post1	Pre7	Post7
Neutrophil	CHO	2.7 \pm 0.7	9.0 \pm 3.6*	2.5 \pm 0.6	7.0 \pm 2.1*
	Milk	2.8 \pm 0.9	8.9 \pm 3.8*	2.8 \pm 1.0	7.7 \pm 3.4*
Lymphocyte	CHO	2.0 \pm 0.5	2.9 \pm 0.6*	2.0 \pm 0.6	2.6 \pm 0.6*
	Milk	2.0 \pm 0.5	3.0 \pm 0.6*	2.0 \pm 0.7	2.7 \pm 0.6*
Neu:Lym	CHO	1.3 \pm 0.3	3.2 \pm 1.4*	1.3 \pm 0.3	3.0 \pm 1.4*
	Milk	1.4 \pm 0.5	3.1 \pm 1.3*	1.5 \pm 0.6	2.8 \pm 1.0*

* Denotes a significant difference from the rest.

The present data fail to support a significant benefit to the immune system of ingesting commercially-available sports drinks or skimmed milk during training, but it is important to note that the subjects' dietary CHO intake was not monitored during the study.

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No association between plasma concentrations of n-3 long-chain PUFA and self-reported depressed mood in a non-clinical population. By K.M. APPLETON¹, R.C. HAYWARD², D. GUNNELL³, D. KESSLER⁴, A.R. NESS⁵, T.J. PETERS⁴ and P.J. ROGERS², ¹*School of Psychology, Queen's University, Belfast, 18-30 Malone Road, Belfast BT9 5BP, UK,* ²*Dept of Experimental Psychology, University of Bristol, 8 Woodland Road, Bristol BS8 1TN, UK,* ³*Dept of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Bristol BS8 2PR, UK,* ⁴*Unit of Primary Health Care, Dept of Community Based Medicine, University of Bristol, 25 Belgrave Road, Bristol BS8 2AA, UK and* ⁵*Dept of Oral and Dental Science, University of Bristol, Lower Maudlin Street, Bristol BS1 2LY, UK*

Lower levels of n-3 long-chain PUFA (n3FA) and lower n3FA:n6 long-chain PUFA (n6FA) balance have previously been observed in the plasma and erythrocyte cell membranes of individuals diagnosed with a major depressive disorder compared with matched non-depressed controls^{1,2}. Associations between n3FA intake and depressed mood have also been reported in non-clinical populations³, yet analyses of n3FA status are rarely available in these populations. The present analysis investigated associations between n3FA status and depressed mood in a non-clinical population.

The analysis was conducted on data collected as part of a large randomized controlled trial investigating the impact of n3FA supplementation on depressed mood and cognitive function. On entry into the trial plasma concentrations of n3FA, self-reported depressed mood and various demographic variables were collected for 191 individuals from the non-clinical population. Plasma concentrations of a range of fatty acids were assessed from a fasting venous blood sample using GC. Depressed mood was assessed using the short form of the depression, anxiety and stress scales (DASS)⁴ and the Beck depression inventory (BDI)⁵.

Mean concentrations of n3FA (5.79 (SD 2.19) mg/100 mg total fatty acids) were similar to those found in non-depressed and depressed individuals in previous studies. Plasma concentrations of n3FA ranged from 2.07 to 9.33mg/100 mg total fatty acids, DASS depressed mood scores were between 0 and 30 and BDI depressed mood scores were between 0 and 40. Regression analyses showed no associations between depressed mood assessed using the DASS or the BDI and plasma concentrations of total n3FA or individual n3FA (18:3n-3, 20:5n-3, 22:5n-3 or 22:6n-3; largest β 0.16, $P=0.09$). Furthermore, no associations were found between depressed mood (DASS or BDI) and plasma concentrations of n3FA:n6FA balance, total n6FA or individual n6FA (18:2n-6, 20:2n-6, 20:3n-6 or 20:4n-6; largest β -0.09, $P=0.24$). No associations were found when demographics were added into the regression models, although associations between depressed mood and gender and age were found (smallest β 0.17, $P=0.02$).

These findings demonstrate no association between depressed mood and plasma concentrations of n3FA or n3FA:n6FA balance in a non-clinical population. They stand in contrast to those that have been previously reported in studies conducted on clinical populations^{1,2}. The absence of an association between depressed mood and n3FA intake in non-clinical populations has been found previously in epidemiological studies⁶ and in several randomized controlled trials⁷. The results of the present analysis therefore provide further evidence that n3FA status may be unrelated to depressed mood in the non-clinical population.

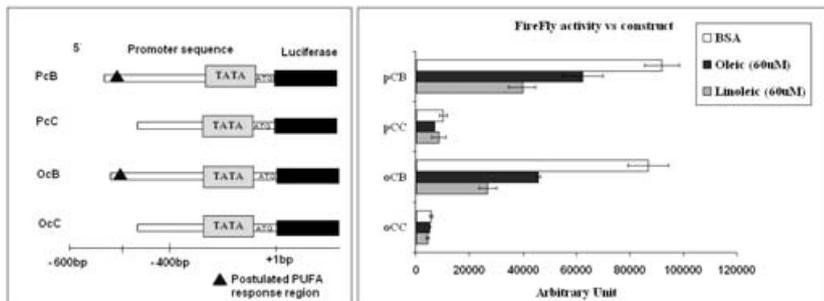
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Effects of fatty acids on porcine and ovine stearoyl-CoA desaturase (SCD) promoter activities in McArdle cells. By R.M. ZULKIFLI, J.M. BRAMELD, T. PARR and A.M. SALTER, *Division of Nutritional Sciences, School of Biosciences, University of Nottingham LE12 5RD, UK*

Dietary fat, particularly PUFA, not only act as a source of energy, but also interact with genes regulating their expression. SCD is the enzyme responsible for incorporating an unsaturated (*cis*) bond at the Δ -9 position of fatty acids, converting stearic and palmitic acids to oleic and palmitoleic acids respectively. Mouse SCD1 gene expression has been shown to be repressed by PUFA but not oleic acid¹. In contrast, a recent report suggests that bovine SCD expression is unaffected by PUFA but markedly down regulated by oleic acids². Until now, very little work has been done in porcine and ovine; no promoter nucleotide sequence for ovine SCD was available on any database.

The aims of the study were to (1) isolate and clone the promoter region for the porcine and ovine SCD (pSCD and oSCD) genes, (2) investigate the effects of PUFA (linoleic acid) on pSCD and oSCD promoter activities and (3) identify the region responsible for any effects. Approximately 600 bp sequences upstream from the first coding sequence of the pSCD and oSCD genes were isolated and cloned into PGL3 vectors containing the firefly (*Photinus pyralis*) luciferase reporter gene (Promega, Southampton, UK). pSCD and oSCD were found to have 87% and 96% similarity with the bovine SCD promoter. To investigate the regulatory response region, 5' end deletions of these sequences were carried out as shown below. The constructs were transfected into the rat hepatoma cell line, McArdle RH-7777, using Fugene-6 (Roche Diagnostics Ltd, Burgess Hill, West Sussex, UK), and promoter activities measured using a luciferase assay kit (Promega, Southampton, UK) and luminescence plate reader.



Treatment with either oleic acid or linoleic acid (both at 60 μ M) decreased the PcB and OcB promoter activities, with linoleic acid being the more potent ($p < 0.001$). There were no effects of either fatty acid on PcC or OcC promoter activities. Further studies showed that PcB and OcB promoter activities were down-regulated ($p < 0.001$) by treatment with cholesterol (10 μ g/ml cholesterol and 1 μ g/ml 25-hydroxycholesterol), but there was no effect of cholesterol on PcC or OcC promoter activities (data not shown). This suggests that the postulated PUFA-response regions (-550 bp to -480 bp) of the porcine and ovine SCD promoters are vital for down-regulation by both fatty acids and also cholesterol.

In conclusion, transcription of both porcine and ovine SCD gene promoters was down-regulated by both linoleic and oleic acids, which is different from previous studies on bovine SCD², but similar to human SCD³. These apparent species differences in responsiveness cannot be accounted for by differences in the sequence structure of the putative PUFA-response region as the region is highly conserved.

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Alcohol consumption and depressed mood: different associations in Northern Ireland and France. By K.M. APPLETON¹, J.V. WOODSIDE², J.W.G. YARNELL², D. ARVEILER³, B. HAAS³, P. AMOUYEL⁴, M. MONTAYE⁴, J. FERRIERES⁵, J.B. RUIDAVETS⁵, P. DUCIMETIERE⁶, A. BINGHAM⁶ and A. EVANS², ¹School of Psychology, Queen's University Belfast, 18–30 Malone Road, Belfast BT9 5BP, UK, ²School of Medicine and Dentistry, Queen's University Belfast BT12 6BJ, UK, ³The Strasbourg MONICA Project, Strasbourg, Department of Epidemiology and Public Health – EA1801, France, ⁴The Lille Monica Project, INSERM U744, Lille, France, ⁵The Toulouse MONICA Project, INSERM U558, Toulouse, France and ⁶The Coordinating Center, INSERM U780, Hôpital Paul Brousse, Villejuif, France

Previous research suggests that increased alcohol consumption is associated with increased risk of a number of poor health outcomes, including depressed mood (for example, see Alati *et al.*¹). Cross-cultural work, however, has suggested different patterns of alcohol consumption in different populations². The present analysis investigates associations between depressed mood and alcohol consumption in two countries of different alcohol-consumption patterns, Northern Ireland and France.

The analysis was conducted on data collected as part of the Prospective Epidemiological Study of Myocardial Infarction (PRIME), a 5-year cohort study investigating the predictors of myocardial infarction in 9758 men aged 50–59 years from Northern Ireland (26%) and France (74%)³. At the start of the study (1991–4) data on alcohol consumption was collected using an FFQ and data on depressed mood was collected using a self-report questionnaire based on the Welsh Pure Depression sub-scale of the Minnesota multiphasic personality inventory⁴. Data on demographics and various lifestyle characteristics were also collected.

In Northern Ireland depressed mood was not associated with total alcohol consumption (linear β 0.05, $P=0.11$; non-linear β -0.02, $P=0.43$), but was negatively associated with wine consumption (linear β -0.10, $P < 0.01$) and positively associated with beer consumption (linear β 0.10, $P < 0.01$; non-linear β -0.07, $P=0.04$). On addition of demographic and lifestyle characteristics, however, these associations attenuated, resulting in no independent associations between depressed mood and alcohol intake. Associations were found between depressed mood and various demographic and lifestyle characteristics, as expected. In France depressed mood was negatively associated with total alcohol consumption (linear β -0.10, $P < 0.01$; non-linear β 0.15, $P=0.04$) and with wine consumption (linear β -0.12, $P < 0.01$; non-linear β 0.14, $P < 0.01$) and positively associated with aperitif consumption (non-linear β 0.06, $P < 0.01$). On addition of all demographic and lifestyle characteristics, all independent associations remained (wine consumption: linear β -0.10, $P < 0.01$; non-linear β 0.13, $P < 0.01$; aperitif consumption: non-linear β 0.06, $P < 0.01$). Associations were also found between depressed mood and various demographic and lifestyle characteristics as expected.

Different associations between depressed mood and alcohol consumption are found in the two countries. In Northern Ireland any association between depressed mood and alcohol consumption is accounted for entirely by other factors associated with depressed mood. In France, however, the relationship between depressed mood and alcohol consumption remains independent of these factors. This relationship may be a result of other lifestyle factors that have not been accounted for, but may also be a genuine association as a result of the chemical or psychological effects of alcohol consumption. Protective health benefits have been suggested as a result of the chemical composition of some alcoholic drinks, such as red wine⁵. Health benefits have also been associated with the chemical and psychological effects of some consumption patterns, such as consumption of one glass of alcoholic drink per d with food⁵. Type of alcoholic drink and consumption pattern, thus, may explain different associations between depressed mood and alcohol consumption in different countries.

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Nutritional and physical well being: a role for the mental health services. By M. BYRNE¹, B. ELLAHI¹, S. HIGGINS² and D. HOWLIN³, ¹*Department of Biological Sciences, University of Chester, Parkgate Road, Chester CH1 4BJ, UK*, ²*Health Promotion Office HSE South, Health Promotion Office, Dean Street, Kilkenny, Republic of Ireland* and ³*Health Promotion Office, HSE South, Wexford Local Health Office, Georges Street, Wexford, Republic of Ireland*

Medication has a pivotal role to play in maintaining mental wellness but is also implicated in the development of other conditions such as obesity^{1,2,3}. Wexford and Enniscorthy Mental Health Day Centres are governed by the Health Service Executive (HSE) South. The aim of the present study was to inform service plan development by assessing the requirement for establishing a specific nutrition-based service for service users with mental health difficulties. The research examined the nutritional intakes of service users and lifestyle factors contributing to this. Additionally, the nutritional knowledge of other healthcare professionals involved in service provision was explored.

The first phase of the research involved collecting data for service users of the mental health day centres. This utilised a one-to-one interview using a 24 h recall and collecting anthropometric data (BMI, waist circumference, percentage body fat). A questionnaire derived from three validated questionnaires^{4,5,6} was used to assess physical inactivity, food-consumption patterns and dietary knowledge. The second phase utilised semi-structured interviews with community mental health nurses involved in service provision. Third-person vignettes developed from data emerging from the first phase were used to stimulate the discussion. Ethical approval for the study was gained from the Ethics Committee, HSE South Eastern Area, Waterford.

Fifty-four service users were recruited via random sampling, having given informed consent. Participants were interviewed while attending Wexford town and Enniscorthy clinics for routine appointments. Four data collections were spoilt. Data was complete for 18 males and 32 females. Ten nurses were interviewed on a normal working day in these same centres.

SPSS version 13 (SPSS, Chicago IL, USA) was used to analyse the non-normally-distributed data (because of the small sample size) from service users. Of the study group 46% (*n* 23) were overweight, with 38% (*n* 19) classified as obese. Mean waist circumference (cm) was 105 (SD 10.16) for males and 95 (SD 13.35) for females. Current smokers accounted for 58% (*n* 29). Inactivity among the study group was a major concern. The percentage of the study group with fibre intakes classified as low, medium and high was 34 (*n* 17), 42 (*n* 21) and 24 (*n* 12) respectively. Consumption of five portions of fruit and vegetables daily was only achieved by 2%. Knowledge of the benefits of increasing consumption of fruit and vegetables and portion size was poor for all participants. There was a significant difference in the level of fruit and vegetables consumed relative to assessed knowledge (*P*<0.03). The 24 h recalls were analysed using NetWISP version 2 (Tinuviel Software, Llanfechell, Anglesey, UK) for macro- and micronutrients. Total energy intake is below the reference nutrient intake (RNI) for 80% (*n* 40) of the sample. Underreporting was suspected as it is common among overweight subjects. The mean energy intake was 7773.7 (SD 3181.2) kJ/d. For Ca and vitamin C 44% (*n* 22) were below the RNI. Males consumed significantly more protein (*P*<0.004) and Fe (*P*<0.001) than females.

Themes generated from the semi-structured interviews with nurses indicate that they could identify the health and nutritional issues for the client in the vignette and could suggest pathways of intervention to assess and improve service users' health. This included education about healthy food choices and drugs including side effects. Enhancing motivation and self esteem of clients to facilitate adoption of change and integration with others in the community are broader public health issues that need to be addressed. Food and health programmes are essential for increasing knowledge, awareness and cooking skills and can be used for users of mental health services as a priority. Nutrition and Dietetics departments can deliver this agenda and should work in partnership with community mental health nurses to improve the well being of service users, particularly in relation to nutritional health and obesity.

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Dietary determinants of micronutrient intake in Irish children aged 5–12 years. By C. CRONIN, E.M. HANNON and A. FLYNN, *Department of Food and Nutritional Science, University College Cork, Cork, Republic of Ireland*

The present study was carried out in order to identify the dietary determinants of differences in the intake of a number of critical micronutrients between low and high consumers.

The National Children's Food Survey (NCFS) was carried out between April 2003 and April 2004 to establish a database of habitual food and drink consumption in a representative sample of Irish children aged 5–12 years. A 7 d weighed food record was used to collect food intake data from 594 children (293 boys, 301 girls). Analysis of dietary intake data was carried out using WISP© (Tinuviel Software, Llanfechell, Anglesey, UK), which contains data from *McCance and Widdowson's The Composition of Foods 6th Edition*¹. The study focused on micronutrients for which a significant prevalence of inadequacy has been shown in children aged 5–12 years². Respondents were categorized into tertiles of intake of micronutrient stratified by gender and age. For each micronutrient, the food groups that account for the difference in intake between high (top third) and low (bottom third) consumers were identified.

Nutrient	Low (<i>n</i> 196)	High (<i>n</i> 197)	Intake difference (high–low consumers)	% Contribution of food groups to the difference between high and low consumers
Ca (mg)	572	1181	609	Milk and yoghurt (65), breakfast cereals (12), cheeses (9), bread and rolls (8)
Fe (mg)	6.4	12.8	6.4	Breakfast cereals (59), bread and rolls (7), nutritional supplements (13), meat and meat products (6)
Riboflavin (mg)	1.1	2.8	1.7	Milk and yoghurt (44), breakfast cereals (26)
Vitamin D (µg)	0.7	4.7	4.0	Nutritional supplements (55), breakfast cereals (14), milk and yoghurt (10)
Vitamin A (µg)	282	1248	966	Vegetable and vegetable dishes (43), nutritional supplements (24), meat and meat products (14), milk and yoghurt (5)
Folate (µg)	144	324	181	Breakfast cereals (30), milk and yoghurt (17), nutritional supplements (15)

The frequency of intake and quantity consumed per eating occasion for key foods largely explain the differences between low and high consumers of Ca (milk and yoghurt), Fe (breakfast cereals), riboflavin (milk and yoghurt and breakfast cereals) and folate (breakfast cereals). For vitamin A, the difference between low and high consumers is largely a result of the frequency of consumption of vegetables and vegetable dishes, while for vitamin D consumption of vitamin D-containing supplements is the main factor.

Other food groups are significant contributors to nutrient intake but do not distinguish between high and low consumers (e.g. the contribution of potatoes and potato products to folate intake). The study will help in the development of food-based dietary guidelines for children.

This project was funded by the Irish Government under the National Development Plan 2000–2006.

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Promoting diet related change: An evaluation of a Community Cooks scheme. By R. GREGG and B. ELLAHI, *University of Chester, Department of Biological Sciences, Parkgate Road, Chester, CH1 4BJ*

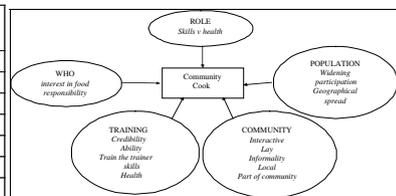
Community food-based initiatives have gained popularity as a method to direct health promotion efforts particularly for low-income populations^(1,2). The Community Cook Scheme (CCS) was developed to improve nutrition-related health of low-income groups in Knowsley in Liverpool. The key feature of this scheme is its utilisation of local individuals *from* the community recruited to deliver services *within* that community for improved health outcomes. The CCS aims to influence the dietary habits of local people through nutrition education and by improving food-related skills. The research aimed to evaluate the extent to which the CCS was meeting these aims and to examine the role of the Community Cook in facilitating this.

A multi-method approach using observation and audit, structured questionnaires, interviews and focus groups with adult beneficiaries and CCS employees was utilized. This enabled the examination of a range of project activities in relation to impact at an awareness raising level and importantly at an intended or actual behaviour change level. The questionnaire was design to measure outcomes specific to CCS and was informed by the Stages of Change model⁽³⁾. Process evaluation involved observation of activities and individual interviews with Community Cooks and project leader. For the evaluation of outcomes, participants were opportunistically recruited from CCS activities to complete questionnaires and for follow up via focus group interviews.

Results show an increased awareness of what constitutes a healthy diet and healthy cooking practices for more than three quarters of participants ($P<0.01$) as a result of participation in the scheme. Change or intended change in terms of improving the healthiness of the diet and specifically for decreasing salt intake and increasing fruit and vegetable intake was observed (Table).

The use of qualitative methods enabled the exploration into the characteristics and role of a Community Cook (Figure). The importance of the peer educator role undertaken by the CCS for overcoming or addressing common barriers to change was noted. This included cooking skills, food access and self-confidence in addition to knowledge of a healthy diet. Lack of confidence to make positive diet-related change and the role of the scheme in building confidence is an interesting outcome of this research.

Due to attending the Community Cooks scheme what changes have you made to your diet?	Yes, all the time (%)	Yes, sometimes (%)	Yes, rarely (%)	No, but thinking about change (%)	No intention to change (%)	Not applicable (%)	Missing data (%)
Grilling instead of frying*	44	25	3	0	8	17	3
Buying low-fat alternatives*	50	28	0	0	3	17	3
Reducing the salt added during cooking*	53	17	3	3	3	19	3
Reducing salt added at the table*	50	19	3	3	6	17	3
Increasing my fruit intake*	67	19	0	3	3	6	3
Increasing my vegetable intake*	61	19	3	3	3	8	3
Reducing my intake of sweet foods	25	19	11	17	14	11	3



* Statistically significant result $p<0.01$

The evaluation has demonstrated diet related changes as a direct result of the CCS and hence the importance community food-based initiatives and peer-educators for over coming barriers and facilitating change or intention to change for individuals in the most deprived areas of Knowsley. As a result of this evaluation this service has been mainstreamed within the Knowsley Primary Care Trust.

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Is food choice of 9–10-year-old children in Liverpool related to physical fitness? By A.F. HACKETT¹, L.M. BODDY^{1,3}, B. JOHNSON² and G. STRATTON³, ¹*Liverpool John Moores University, IM Marsh Campus, Barkhill Road, Liverpool L17 6BD, UK*, ²*Liverpool PCT, Abercromby Health Centre, Grove Street, Liverpool L7 7HG, UK* and ³*Research Institute for Sports and Exercise Sciences Liverpool John Moores University, Henry Cotton Campus, Liverpool L3 2ET, UK*

SportsLinx is an annual project that assesses the diet, fitness and nutritional status of an entire cohort of 9–10-year-old children in Liverpool¹. The eating habits of these children leave a lot to be desired². In addition, the prevalence of overweight and obesity in Liverpool children is high³ and fitness has deteriorated⁴. Whether, and how, eating habits are related to fitness is unclear.

A food-intake questionnaire, evaluated for validity and reliability⁵, was used to record the intake of a list of key foods including nineteen foods that children would normally be encouraged to eat more of, or more often, such as fruit and vegetables and twenty-five foods that children would normally be encouraged to eat less of, or less often, such as sweets and chips. Fitness was assessed by a variety of tests¹, four only are reported here: 10 × 5 m sprint (10 × 5), flexibility (flex), handgrip (HG) and shuttle run (MST). Data were collected between 2005 and 2006, but fitness data and dietary data were not collected on the same day. Mean fitness test results were calculated for girls and boys who claimed to have eaten (Y) or not eaten (N) some selected meals and foods. These were evaluated using an unpaired *t* test (Table).

Fitness test...	Boys (n=1532)								Girls (n=1579)							
	10 × 5 (s)		Flex (cm)		HG (kg)		MST (no.)		10 × 5 (s)		Flex (cm)		HG (kg)		MST (no.)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Breakfast: Y	22.8	0.08	15.4	0.19	15.4	0.12	42.1*	0.75	23.8	0.08	18.1	0.20	14.3	0.11	29.3	0.56
N	23.7	0.40	15.8	0.63	15.6	0.36	36.7	2.50	24.1	0.23	18.2	0.58	14.6	0.35	26.2	1.18
Sweets: Y	22.9	0.12	15.6	0.26	15.6	0.16	42.2	0.99	23.8	0.11	18.0	0.26	14.3	0.15	28.4	0.74
N	22.9	0.11	15.3	0.26	15.3	0.17	40.8	1.06	23.9	0.11	18.2	0.28	14.4	0.15	29.8	0.77
Chocolates: Y	23.1*	0.12	15.4	0.25	15.4	0.16	41.5	0.95	23.9	0.11	18.1	0.26	14.1*	0.14	29.2	0.73
N	22.7	0.11	15.6	0.27	15.5	0.17	41.9	1.11	23.8	0.12	18.2	0.27	14.6	0.16	28.9	0.78
Take-away: Y	23.3*	0.17	14.8*	0.32	15.4	0.21	39.7*	1.32	23.9	0.16	18.2	0.37	14.2	0.20	28.9	1.03
N	22.7	0.09	15.7	0.22	15.5	0.14	42.4	0.86	23.8	0.09	18.1	0.22	14.4	0.13	29.0	0.62
Fruit: Y	22.8	0.09	15.4	0.20	15.6*	0.12	42.0	0.79	23.8**	0.08	18.2	0.20	14.4*	0.11	29.5*	0.58
N	23.1	0.19	15.6	0.50	14.9	0.30	39.0	1.76	24.6	0.24	17.6	0.55	13.7	0.30	26.2	1.20
Vegetables: Y	22.8	0.14	15.5	0.28	15.7*	0.18	44.1**	1.08	23.8	0.11	18.9**	0.27	14.4	0.15	29.5	0.76
N	22.9	0.10	15.4	0.17	15.3	0.09	39.7	0.99	24.0	0.11	18.0	0.26	14.4	0.16	28.7	0.76
Chips: Y	23.1**	0.12	15.4	0.27	15.4	0.17	40.4	1.04	23.9	0.13	18.0	0.29	14.2	0.17	28.8	0.85
N	22.6	0.11	15.4	0.25	15.5	0.16	43.1	1.03	23.8	0.10	18.1	0.26	14.5	0.14	29.4	0.71

Mean values were significantly different from those for N within gender for the same food choice: * $P<0.05$, ** $P<0.01$.

Some associations between fitness and food choice were found; notably for take-away meals (boys) and fruits (girls) and vegetables (boys and girls). All significant differences showed an association between more-desirable food choice and better fitness-test result. This is evidence that better food choice is associated with a higher level of fitness, which presumably reflects increased physical activity, but the nature of this association remains to be evaluated.

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Contribution of ready-to-eat breakfast cereals to nutrient intakes in Irish children aged 5–12 years. By E.M. HANNON and A. FLYNN, *Department of Food and Nutritional Science, University College Cork, Cork, Republic of Ireland*

The objective of the present study was to estimate the contribution of ready-to-eat breakfast cereals (RTEBC) to macro- and micronutrient intakes in Irish children aged 5–12 years, using data from the National Children's Food Survey (NCFS). The NCFS was carried out between April 2003 and April 2004 to establish a database of habitual food and drink consumption in a representative sample of Irish children aged 5–12 years. A 7 d weighed-food record was used to collect food-intake data (including supplements) from 594 children (293 boys, 301 girls). Analysis of dietary intake data was carried out using WISP© (Tinuviel Software, Llanfechell, Anglesey, UK), which contains data from *McCance and Widdowson's The Composition of Foods, 6th Edition*¹.

Approximately 95% of boys and 91% of girls were RTEBC consumers and the average daily intake in consumers was 39 g (boys) and 27 g (girls).

	Contribution of RTEBC					
	Boys 5–12 years (n 293)			Girls 5–12 years (n 301)		
	Contribution		% total	Contribution		% total
Mean	sd	Mean		sd		
Energy	0.59 MJ	0.42 MJ	7.8	0.39 MJ	0.31 MJ	5.8
Macronutrients						
Protein	2.9 g	2.4 g	4.9	1.9 g	1.6 g	3.5
Total fat	1.0 g	1.7 g	1.5	0.6 g	0.7 g	1.0
Saturated fat	0.3 g	0.5 g	1.1	0.2 g	0.2 g	0.8
Carbohydrate	30.9 g	21.4 g	12.4	20.5 g	16.6 g	9.4
Total sugars	8.1 g	8.4 g	7.3	5.7 g	5.9 g	6.0
Starch	22.7 g	15.7 g	17.6	14.8 g	12.2 g	13.0
Micronutrients						
Fe	3.7 mg	2.9 mg	33.3	2.4 mg	2.2 mg	26.6
Folate	64.9 µg	50.2 µg	26.5	44.8 µg	39.6 µg	21.1
Thiamin	0.4 mg	0.3 mg	26.4	0.3 mg	0.2 mg	20.7
Riboflavin	0.5 mg	0.4 mg	24.2	0.3 mg	0.3 mg	19.1
Vitamin B ₆	0.5 mg	0.5 mg	23.7	0.4 mg	0.3 mg	19.8
Niacin	6.0 mg	4.4 mg	19.4	4.0 mg	3.4 mg	14.9

In relation to their percentage contribution to mean daily intakes (MDI) of energy, RTEBC contribute lower percentages of MDI of protein, total fat and saturated fat, higher percentages of MDI of total carbohydrate and starch and the same percentage of MDI of total sugars. RTEBC contribute significant percentages of MDI (including supplements) of Fe, folate, thiamin, riboflavin, vitamin B₆ and niacin (boys 19–33%, girls 15–27%).

The study was funded by Kelloggs.

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A systematic review of the effect of nutrition, diet and dietary change on learning, behaviour and performance of school-aged children. By F.C. HILLER¹, L.J. ELLS¹, C.D. SUMMERBELL¹, J. SHUCKSMITH¹, H. CRAWLEY², L.S. HARBIGE³, J. HAMILTON-SHIELD⁴ and A. WIGGINS⁵, ¹School of Health and Social Care, University of Teesside, Middlesbrough TS1 3BA, UK, ²School of Life Sciences, Kingston University, Kingston-upon-Thames KT1 1LQ, UK, ³School of Science, University of Greenwich, Kent ME4 4TB, UK, ⁴Institute of Child Health, University of Bristol, Bristol, BS8 1TH, UK and ⁵CEM Centre, Durham University, Durham DH1 3UZ, UK

The Department for Education and Skills and the Food Standards Agency are committed to promoting healthier schools and lifestyles among schoolchildren, through improving the quality of school meals and national nutritional standards¹. Whilst this commitment is primarily made on health grounds^{2,3}, there is considerable interest in how good nutrition may also impact on behaviour, learning and performance. However, the current evidence base to support clear associations in this area is confusing and lacks cohesion. This systematic review aims to investigate the effects of nutrition, diet and dietary change on learning, behaviour and performance in school-aged children (4–18 years) from the UK and other developed countries.

MEDLINE, CINAHL, Psycinfo, BEI, ERIC, Australian ERIC, SSCI, ASSIA, International Bibliography of the Social Sciences, Sociological Abstracts, SPECTRE and ZETOC were searched. All peer-reviewed randomised case or cluster controlled trials undertaken in children from developed countries providing an exposure or intervention focusing on nutrition, diet or dietary change (achievable by diet alone) and at least one of the following outcomes were investigated: educational performance; behaviour; motivation.

Twenty-nine studies were included. Fifteen studies examined the effect of breakfast, of which ten identified an association between breakfast provision and some small cognitive and behavioural improvements. Six studies examined the effect of short-term exposure to sugar intake in populations of predominantly sufferers of attention-deficit hyperactivity disorder. No dramatic detrimental effects on educational or behavioural outcomes were observed. Five studies investigated the effect of fish oil supplementation in a population with symptoms of neurodevelopmental disorders; however, the findings were mixed and therefore inconclusive. Two studies examined the effect of vitamin and mineral supplementation: one showed a significant positive effect on IQ in a small sub sample; the other found no effect. The final study examined 'good diet' in the first year of school, but lacked sufficient detail and quality to inform the evidence base. Several studies lacked quality in research methodology and reporting (particularly those investigating breakfast consumption). Many studies failed to account for important confounders, such as habitual dietary intake, physical activity levels, locality and family context, whilst two-thirds of the studies were carried out in primary-aged children and over half took place over a short duration (<1 month).

Findings suggest there is insufficient evidence to identify any effect of nutrition, diet and dietary change on learning, education or performance of school-aged children from the developed world. Further research is required that must be of high quality, representative of all populations, undertaken for longer durations and use universal standardised measures of educational attainment. Challenges in terms of interpreting the results of such studies within the context of confounders such as family and community context, poverty, disease and the rate of individual maturation and neurodevelopment will remain.

This research was funded by the Food Standards Agency. The full report is available at the FSA website: <http://www.food.gov.uk/multimedia/pdfs/systemreview.pdf#page=1>

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Investigation into factors influencing dietary intake and physical activity in children aged 10–11 years. By T. FINNERTY, N. RANDELL, S. ROWBOTTOM, C. VOGELE, J. DABINETT, S. REEVES and Y. JEANES, *School of Human and Life Sciences, Roehampton University, Holybourne Avenue, London SW15 4JD, UK*

The role of dietary over-consumption and lack of physical activity in the aetiology of childhood obesity remains unclear¹. There are very few studies investigating the combined effects of physical activity, dietary intake and peer influence on such behaviours. The present study aims to determine what factors influence the dietary intake and physical activity of children.

Children from three primary schools in south-west London, who ranged from mid-high socio-economic backgrounds, were recruited during July 2006. Children were asked to complete a 3 day food and activity diary and a questionnaire, as well as to wear a pedometer (Yamax Digiwalker SW-200). Weight, height and triceps-skinfold measurements were taken. Overweight and obesity were determined using the paediatric BMI cut-offs proposed by the International Obesity Task Force².

A total of 119 children (fifty-four males and sixty-five females) participated (mean age 10 (sd 0.4) years), however not all children completed all aspects of the study due to drop out or absenteeism. Out of 119 children, 35 males and 51 females completed the food and activity diary. Eighty-eight percent were normal weight, 10% were overweight and 2% were obese; mean BMI z score was 0.035 (range -4.22–1.99). The average number of steps taken per d was 12491 (sd 4763). The boys took significantly more steps per d than the girls (13 713 (sd 4924) v. 11 481 (sd 4417); $P=0.016$). Children who reported that they were told they were good at sports had a greater number of steps per d than those who reported that they were told they were not very good at sports. In boys there was a significant positive correlation between BMI z score and energy intake ($r\ 0.45$; $P<0.01$) and fat intake ($r\ 0.34$; $P=0.04$). In girls there was a significant negative correlation between the number of steps taken and energy intake ($r\ -0.27$; $P<0.01$). Triceps-skinfold measurement strongly correlated with BMI z score in males ($r\ 0.7$; $P<0.01$) and moderately in females ($r\ 0.5$; $P<0.01$). Encouragement from family and friends to refrain from unhealthy foods did not influence the energy or fat intake, neither did the reported consumption of unhealthy food by friends and family influence dietary intake.

The BMI z score for boys aged 10–11 years was positively related to energy and fat intake, which is in agreement with the findings reported by Ricketts³. No association was found between number of steps per d and triceps skinfold or BMI, which is contrary to the findings of Forshee *et al.*⁴, who reported that participation in physical activity has a negative association with BMI; analysis of the activity diaries may provide an explanation. Interestingly, peer or family did not appear to influence nutrient intake; however, further analysis will involve analysis of the food groups eaten and number of 'unhealthy foods' within the diet. Food habits established in childhood have previously been reported to be maintained into adulthood⁵ and therefore investigating the influences of peers and family in childhood should be explored and will help inform future intervention studies.

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Palmitate induces insulin resistance in monocytes and increases expression of the integrin CD11b
By D. GAO, H.R. GRIFFITHS and C.J. BAILEY, *School of Life and Health Sciences, Aston University, Birmingham B4 7ET, UK*

Obesity and insulin resistance are important risk factors for atherosclerosis, and elevated level of plasma NEFA is a common feature in individuals with obesity and insulin resistance¹. Palmitate, one of the most abundant non-esterified SFA in plasma, has been reported to induce insulin resistance in adipose tissues and skeletal muscles² and to cause an increased inflammatory response in monocytes³. The present study investigated whether palmitate can induce insulin resistance in monocytes and its effect on monocyte adhesion molecular expression (CD11b). Insulin resistance was measured by *in vitro* uptake of insulin-stimulated ³H-labelled 2-deoxy-D-glucose into THP-1 cells, cell surface CD11b expression was measured by flow cytometry. The data showed that palmitate-induced insulin resistance in THP-1 monocytes was concentration and time dependent (Figure 1). The insulin-stimulated glucose uptake was significantly decreased in cells treated with 300 μ M-palmitate compared with control cells ($P<0.001$) and was observed within 6 h, but was not a result of palmitate toxicity. There was no significant increase in caspase 3 activation ($P>0.05$). Treatment with 300 μ M-palmitate for 24 h also caused a significant increase in surface CD11b expression in both U937 and THP-1 monocytic cell lines and human primary monocytes compared with the control ($P<0.001$). Both these effects were inhibited by co-incubation with Fumonisin B1, an inhibitor of *de novo* ceramide synthesis. In conclusion, these data show that palmitate, at physiological concentrations, can cause insulin resistance in monocytes and increase monocyte surface integrin CD11b expression, which is in part the result of the synthesis of ceramide.

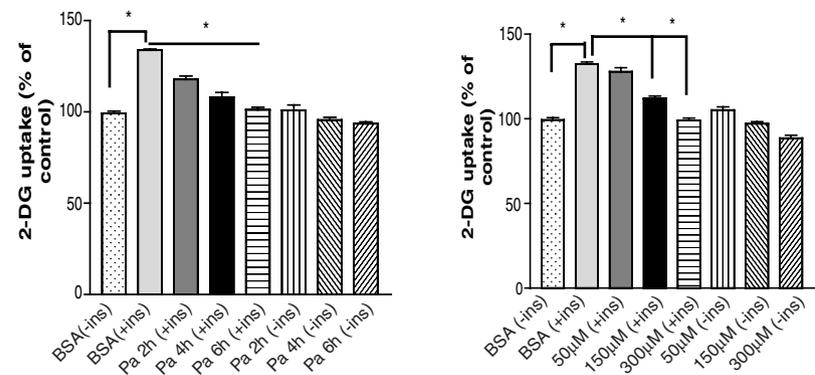


Fig. 1. Time- and dose-dependent induction of insulin resistance in THP-1 cells by palmitate.

THP-1 cells (10^6 /ml) were seeded into 24-well plates and incubated with 10^{-6} M insulin for 12 h with (a) 300 μ M palmitate (Pa) loaded onto albumin (BSA) was added for the final 2 h, 4 h, and 6 h of incubation or (b) varying concentrations of Pa for the final 6 h. Data are the means and SEMs from 4 independent experiments. * $P<0.001$ compared to control cells.

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Trans-10, cis-12 conjugated linoleic acid isomer increases transcellular calcium transport without affecting TRPV6 and Calbindin D9k expression in human Caco-2 cells. By E.F. MURPHY¹, K.D. CASHMAN^{1,2} and R.J. WOOD³, ¹Department of Food and Nutritional Sciences and ²Department of Medicine, University College, Cork, Republic of Ireland and ³Mineral Bioavailability Laboratory, Jean Mayer USDA Human Nutrition Research Centre on Aging at Tufts University, Boston, MA 02111, USA

Conjugated linoleic acid (CLA) is predominantly found in milk and meat of ruminant animals as a mixture of CLA isomers. It has previously been shown that the *trans*-10, *cis*-12 CLA isomer increases Ca transport across Caco-2 cell monolayers, a cell-culture model of intestinal absorption¹. In the current study the hypothesis that the positive effect of *trans*-10, *cis*-12 CLA on Ca transport is a result of changes in TRPV6/CaT1 (the transient receptor potential channel, subfamily V, member 6, also known as calcium transporter-1) and calbindin D9k expression, two putative molecular mediators of transcellular Ca transport, was tested.

Caco-2 cells were grown for 14 d in media containing (l) 80 µmol linoleic acid, *cis*-9, *trans*-11 CLA isomer or *trans*-10, *cis*-12 CLA isomer, or ethanol vehicle. During the last 2 d cells were treated with 100 nmol 1,25-dihydroxyvitamin D or vehicle/l to test for a possible vitamin D–lipid interaction.

As expected, treatment with 1,25-dihydroxyvitamin D increased total transepithelial (34%; $P < 0.0001$) and transcellular (43%; $P < 0.001$) Ca transport and TRPV6/CaT1 and calbindin D9k mRNA expression (3.4-fold and 4.6-fold respectively; $P < 0.0001$). Among the fatty acids tested, only the *trans*-10, *cis*-12 CLA isomer increased total transepithelial (24%; $P < 0.001$) or transcellular (25%; $P < 0.001$) Ca transport. There was no evidence of a vitamin D–lipid treatment interaction on Ca transport.

1,25 (OH) ₂ D ₃ , ... Lipid treatment... n	Untreated				Treated				Pooled SEM
	Ethanol	LA	c9, t11	t10, c12	Ethanol	LA	c9, t11	t10, c12	
Ca transport (nmol/well/min)*:									
Total	1.084	1.097	1.068	1.408	1.366	1.608	1.682	1.873	0.073
Transcellular	0.900	0.944	0.861	1.183	1.177	1.458	1.477	1.665	0.067
Paracellular**	0.165	0.137	0.187	0.215	0.162	0.129	0.183	0.198	0.091

* Values represent back-transformed data to geometric mean.

LA, linoleic acid; c9, t11, *cis*-9, *trans*-11 CLA; t10, c11, *trans*-10, *cis*-12 CLA; 1, 25 (OH)₂ D₃, 1, 25 dihydroxyvitamin D.

** Statistical significance was assessed by means of two-way ANOVA with variation attributed to 1,25 (OH)₂ D₃ and fatty acid treatment: the effect of fatty acid treatment was statistically significant ($P < 0.001$) for all three transport variables; the effect of 1,25 (OH)₂ D₃ treatment was statistically significant for all variables ($P < 0.0001$), except paracellular transport; there was no significant interaction between fatty acid and 1,25 (OH)₂ D₃ treatment.

In contrast to the apparent mechanism of action of 1, 25-dihydroxyvitamin D on transcellular Ca transport, the *trans*-10, *cis*-12 CLA isomer did not increase TRPV6 or calbindin D9k mRNA expression.

In conclusion, additional work will be required to establish the specific molecular mechanism by which *trans*-10, *cis*-12 CLA enhances transcellular Ca transport.

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Nutrient-sensitive interactions between NF-κB and PPARγ. By C. REYNOLDS¹, E. DRAPER², C. CIPOLLETTA¹, C.E. LOSCHER² and H.M. ROCHE¹, ¹Nutrigenomics Research Group, Department of Clinical Medicine, Institute of Molecular Medicine, St James's Hospital, Dublin 8, Republic of Ireland and ²Immunomodulation Research Group, School of Biotechnology, Dublin City University, Republic of Ireland

Dendritic cells (DC) play a key role in the initiation of the inflammatory response, directing the adaptive immune response and determining the nature of the T-helper-cell response to inflammatory stimuli. Long-chain *n*-3 PUFA EPA and DHA have the potential to modulate the immune response. However, little is known about the ability of EPA and DHA to modify the interaction between PPARγ and NF-κB.

The present study investigated the effects of EPA and DHA on expression of PPARγ and on components of the NF-κB pathway in DC isolated from BALB/c mice. Bone marrow-derived immature DC were cultured in RPMI 1640 medium supplemented with fetal calf serum and granulocyte-macrophage colony-stimulating factor for 7 d. DC were treated with dimethyl sulfoxide (vehicle control), EPA (25 µM) or DHA (25 µM) during the length of the culture period. On day 7 DC were stimulated with lipopolysaccharide (LPS; 100 ng/ml) for either 2 h or 5 h. At the end of the incubation period nuclear and cytosolic protein was extracted using the TransAm Nuclear Extraction Kit (Active Motif, Rixensart, Belgium) and levels of NF-κB, PPARγ, α subunit of inhibitor of κB kinase and inhibitory subunit α of NF-κB were measured by western blot. PPARγ and NF-κB TransAm™ kits (Active Motif) were used to assess the ability of PPARγ and NF-κB to bind target DNA sequences. In order to understand the interaction between PPARγ and NF-κB within the cell, DC cultured under similar conditions were immunostained for PPARγ and NF-κB, and their nuclear and cytoplasmic expression were quantified using a confocal microscope (Improvision Software, Coventry, UK).

Western blot and TransAm™ analysis showed that EPA and DHA supplementation was associated with significant down-regulation of components of the NF-κB pathway and a decreased affinity to bind DNA. Treatment with EPA and with DHA also led to an increase in levels of PPARγ and an increase in DNA-binding affinity.

Confocal microscopy confirmed that pre-LPS stimulation nuclear and cytoplasmic NF-κB expression was down regulated in both DHA- and EPA-treated cells. In LPS-stimulated DC nuclear NF-κB localisation was markedly reduced by DHA.

The present work shows important nutrient-sensitive interactions between NF-κB and PPARγ in DC, wherein DHA and EPA mediate their anti-inflammatory effects in PPARγ-dependent and -independent mechanisms. Given the important role of fatty acids and DC in innate immunity this observation requires further investigation.

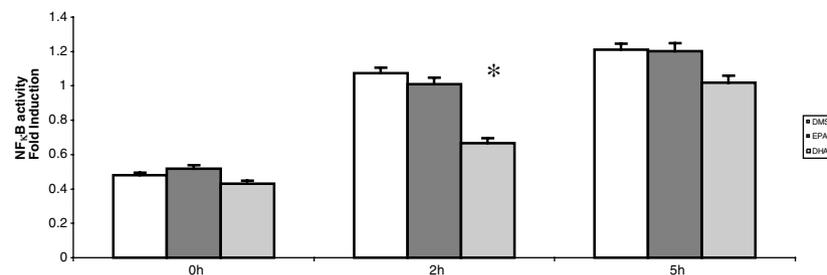


Fig. EPA and DHA modulate NF-κB activity. DC were treated for 7 days with either EPA (25 µM), DHA (25 µM) or DMSO vehicle control and then stimulated with LPS (100 ng/ml) for 0–5 h with the 0h group acting as the non-LPS-stimulated cells. Cells were harvested and nuclear and cytosolic fractions were extracted. NF-κB activation was measured in nuclear fractions by an NF-κB binding assay. Results are expressed as a fold change (±SD) relative to control (DMSO) levels for 4 replicates. *, $P < 0.01$, ANOVA, comparing DMSO to either EPA or DHA at each time point.

Fatty acid effects on endothelial inflammatory gene expression: impact of the approach. By D.I. SHAW¹, W.L. HALL² and C.M. WILLIAMS¹, ¹*School of Food Biosciences, University of Reading, Reading RG6 6AP, UK and* ²*Nutritional Sciences Division, School of Biomedical and Health Sciences, King's College London, London SE1 9NH, UK*

Endothelial dysfunction may be related to adverse effects of some dietary fatty acids (FA) on endothelial function. Although *in vitro* studies have failed to show consistent findings, this may reflect the diverse experimental protocols employed and the limited range of FA and end points studied. The aim of the present study was to investigate the effect of dietary FA type (SFA, MUFA and *n*-6 and *n*-3 PUFA), concentration, incubation time and cell stimulation state on a broad spectrum of inflammatory gene expression in endothelial cells.

Using human umbilical vein endothelial cells with (10 ng TNF α /ml) and without stimulation the effects of arachidonic, DHA, EPA, linoleic, oleic and palmitic acids (10–100 μ M) on the gene expression of a number of inflammatory genes and transcription factors were assessed by quantitative real-time RT-PCR. The relative gene expression ratios were calculated using the Pfaffl equation¹.

Individual FA differentially affect endothelial inflammatory gene expression in a gene-specific manner. Importantly, however, cell stimulation state and FA incubation time can significantly influence reported effects. The effect of FA incubation on vascular cell adhesion molecule-1 (VCAM-1) and E-selectin relative gene expression was significantly greater in stimulated endothelial cells compared with unstimulated cells ($P < 0.001$ and $P < 0.01$ respectively). In addition, VCAM-1 and monocyte chemoattractant protein 1 (MCP-1) gene expression was significantly up regulated after FA incubation for 24 h compared with 6 h. In contrast, E-selectin gene expression was significantly up regulated at 6 h FA incubation compared with 24 h (Fig. 1).

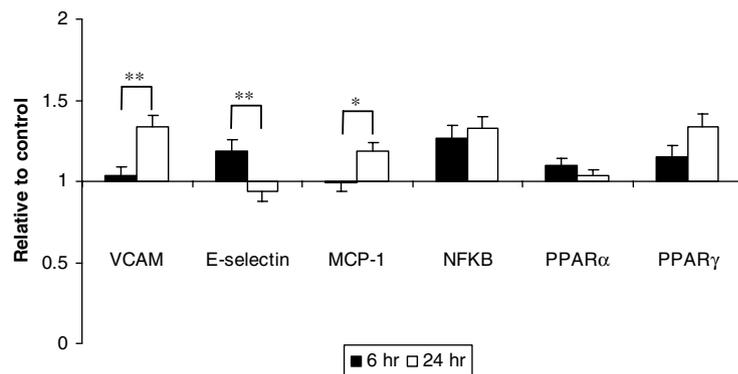


Fig. 1. Effect of fatty acid incubation time on inflammatory gene expression. Change displayed relative to BSA control (arbitrarily set at 1). Values are means with their standard errors represented by vertical bars. Four-factor ANOVA was used to analyse the effects of four variables, which included incubation time. Mean values at 6 h were significantly different from those at 24 h: * $P < 0.02$, ** $P < 0.01$.

Importantly, these results show that the experimental approach used can have a significant and complex impact on reported findings. The comparative effects of SFA, MUFA and *n*-6 and *n*-3 PUFA on endothelial gene expression depend on the specific FA investigated, its length of incubation, the cell stimulation state and the gene investigated. These findings may explain existing disparity in the literature. Future work is required to confirm whether these differences in FA effects that occur under varied environments are of biological relevance.

This work was funded by the EC Framework Programme 6 via the LIPGENE project (FOOD-CT-2003-505944).

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Reasons, motivations and attitudes of individuals aged 18–25 years, trying to lose weight with various methods and with the use of over-the-counter oral solid weight-loss formulations. By A.M.L. ANDRONICOU, A.F. HACKETT, S. MAXWELL, J. RICHARD and J. KRŠKA, ¹*Liverpool John Moores University, Faculty of Education, Community and Leisure and School of Pharmacy and Chemistry, Barkhill Road, Liverpool L17 6BD, UK*

The ideal body type has become increasingly slender¹. The most-widely-accepted weight-loss method is to reduce the energy consumption and increase energy expenditure; the goal of health professionals being to achieve a steady small decrease in weight over a period of time². Nevertheless, members of the public are more likely to want a quick method of weight loss³. In the UK two-fifths of women are actively trying to lose weight, and one in four males get involved⁴. There are a large number of oral solid formulations (pills, capsules, tablets) that can be bought over the counter (OTCWLF) that claim to aid the consumer in achieving weight loss, many of which also claim to allow the individual to 'lose weight fast'. Consumers appear to purchase these products in the search for a solution that requires little change to their normal routine³.

The aim of the present study was to identify the reasons, motivations and attitudes of individuals trying to lose weight and especially those using OTCWLF. The study comprised two parts. During the first part a short questionnaire was completed and BMI measurements were taken for 110 individuals aged 18–25 years of both genders who were trying to lose weight. Less than half (43.7%) were obese or overweight. Furthermore, 13% were underweight (BMI < 20 kg/m²) and eleven (10%) admitted to having experienced an eating disorder. In addition, twenty-six (23.4%) were using OTCWLF to lose weight, of whom none was obese, three (11.5%) were underweight and five (19.2%) admitted to having experienced an eating disorder ($P < 0.05$). Nearly all the subjects ($n = 108$; 98.2%) stated that they were not satisfied with their shape, with no exceptions among the underweight individuals ($P < 0.05$). A significant number of individuals ($n = 21$; 19.1%) thought that they had an excess of weight because of the speed of their metabolism. More than one-quarter of the individuals ($n = 28$; 25.5%) stated that they will use OTCWLF if they do not lose weight with the method that they were currently using. During the second part of the study the subjects who had used OTCWLF were interviewed with a pro-forma questionnaire by telephone. More than half stated that health professionals such as nutritionists, dietitians, general practitioners and pharmacists (including NHS Direct) had little to no knowledge regarding these formulations. Almost all of them ($n = 24$; 92.3%) admitted that they thought that OTCWLF were not a safe method to lose weight and thirteen (50%) were embarrassed that they used them. A large number ($n = 20$; 76.9%) stated that they experienced a side effect that they were not informed about.

OTCWLF are widely available and a substantial minority of individuals use them, including individuals who are underweight or have an eating disorder. Health professionals should lead information delivery on weight loss and especially on OTCWLF so that consumers can make a truly 'informed choice'.

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Associations of body composition with bone mineral density and proximal femoral geometry in sedentary premenopausal women. By C.A. BAILEY¹, A. PARSONS² and K. BROOKE-WAVELL¹,

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Understanding factors that contribute to peak bone mass and bone strength in women is important for osteoporosis prevention. Fat-free mass (FFM) is related to bone mineral density (BMD) in premenopausal women; fat mass (FM) less consistently so¹. Fewer studies have examined relationships between body composition and proximal femur geometric variables that have been related to fracture risk, such as cross-sectional moment of inertia and hip strength index (estimated compressive yield strength:estimated compressive stress of a fall); lower values of which are reported in hip-fracture cases². Furthermore, physical activity may influence both FFM and bone, and hence confound relationships in previous studies that have included physically-active participants. The aim was thus to examine the associations between body composition, BMD and proximal femoral geometry in sedentary premenopausal women.

Participants were eighty-eight healthy premenopausal women who did not regularly engage in physical activity that might influence bone or muscle mass (<1h/week). BMD was assessed and proximal femoral geometry estimated using a Lunar Prodigy Advance dual X-ray absorptiometer (GE Medical Corp., Madison, WI, USA). Body composition was assessed using tetrapolar bioelectric impedance analysis.

The mean age for the participants was 32.8 (SD 8.6) years, mean body mass was 61.8 (SD 10.0) kg and mean stature was 1.63 (SD 0.06) m. Both FM and FFM were positively associated with lumbar spine and hip BMD and femoral cross-sectional moment of inertia and cross-sectional area. Associations for FFM were independent of FM, except the negative association between FFM and hip strength index was not significant after adjustment for FM. However, most associations for FM disappeared after adjustment for FFM, with only the positive association with lumbar spine BMD and the negative association with hip strength index persisting.

Correlations (r) between body composition and bone variables	FM	FM adjusted for FFM	FFM	FFM adjusted for FM
BMD: Lumbar spine	0.39***	0.32**	0.26*	0.11
Femoral neck	0.29**	0.12	0.44***	0.36**
Wards triangle	0.12	-0.05	0.36**	0.35**
Trochanter	0.23*	0.08	0.38***	0.32**
Total hip	0.28**	0.12	0.41***	0.33**
Strength index	-0.37***	-0.30**	-0.26*	-0.11
Cross-sectional moment of inertia	0.28**	-0.02	0.66***	0.63***
Cross-sectional area	0.34**	0.12	0.57***	0.50***

*P<0.05, **P<0.01, ***P<0.001.

FFM was positively related to hip geometry and BMD, whilst FM was not independently so, which is consistent with findings in men³. FM, whilst positively associated with spine BMD, was associated with lower femur strength index, presumably resulting from increased potential stress on bone unaccompanied by compensatory improvements in BMD or geometry.

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Dietary fibre (DF) intakes from food sources in Irish children aged 5–12 years. By S. BANNON, J. WALTON, E.M. HANNON, and A. FLYNN, Department of Food and Nutritional Science, University College Cork, Cork, Republic of Ireland

The objective of the present study was to identify the dietary determinants of DF intake in 5–12-year-old children. Data from the National Children’s Food Survey (NCFS) was used for this purpose.

The NCFS was carried out between April 2003 and April 2004 to establish a database of habitual food and drink consumption in a representative sample of Irish children aged 5–12 years. A 7 d weighed-food record was used to collect food intake data from 594 children (293 boys, 301 girls). Analysis of dietary intake data was carried out using WISP© (Tinuviel Software, Llanfechell, Anglesey, UK) which is based on the 6th edition of *McCance and Widdowson’s The Composition of Foods*¹.

A high prevalence of inadequate intakes of DF has previously been reported in this population group². In this analysis the contribution of food groups (g and %) to mean daily DF intakes by tertile of DF intake (stratified for age and gender) are reported in 5–12-year-old children. The food groups that account for the greatest proportion of the difference in intakes between high (the top third) and low (the bottom third) consumers of DF are reported.

	Low (n 197)		Medium (n 201)		High (n 196)		Difference (high–low)	
	g	%	g	%	g	%	g	%
Veg and veg dishes	0.8	9	1.2	10	2.4	14	1.6	20
Bread and rolls	1.8	22	2.5	21	3.4	21	1.6	20
Breakfast cereals	1.0	12	1.7	14	2.6	15	1.6	20
Fruit and fruit juices	0.7	8	1.2	10	1.9	12	1.2	15
Grains, rice, pasta and savouries	0.7	8	1.1	9	1.2	8	0.5	7
Potatoes and potato products	1.7	20	1.8	15	2.1	13	0.5	6
Other food groups	1.9	21	2.6	21	2.9	17	1.0	12
Total	8.6	100	12.1	100	16.6	100	8.0	100

Mean daily energy intakes were 6.2 MJ (1472 kcal; low consumers), 7 MJ (1665 kcal; medium consumers) and 7.9 MJ (1867 kcal; high consumers).

Bread and rolls, vegetables and vegetable dishes and breakfast cereals each account for 20% of the total difference in DF intake (8 g) between high and low consumers, with fruit and fruit juices accounting for 15% of the difference. For vegetables and vegetable dishes, fruit and fruit juices and bread and rolls this is attributable mainly to a difference in the frequency of consumption, whereas for breakfast cereals high-DF consumers have a greater intake per eating occasion but frequency of consumption is the same (six to seven per week). Although potatoes and potato products contribute 13–20%² to the mean daily intake of DF, they only explain 6% of the difference in intakes between high and low consumers.

These findings identify dietary strategies for increasing DF intake in children that are useful for the development of food-based dietary guidelines.

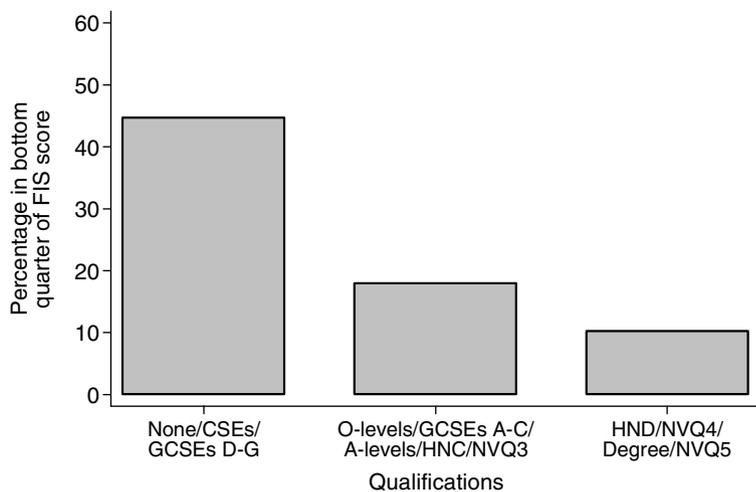
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Women of low educational attainment have lower food involvement and eat fewer fruits and vegetables. By M.E. BARKER¹, W.T. LAWRENCE¹, J.M. WOADDEN² S.C. CROZIER¹ and T.C. SKINNER², ¹MRC Epidemiology Resource Centre, University of Southampton, Southampton General Hospital, Southampton SO16 6YD, UK and ²School of Psychology, University of Southampton, Highfield, Southampton SO17 1BJ, UK

The Southampton Women's Survey has shown that women who leave school with few or no educational qualifications are less likely than women who attain more qualifications at school to have diets that meet current recommendations¹. It is likely that educational attainment is a marker for differences in lifestyles and in the priority given to diet. It is hypothesised that women of low educational attainment may have poorer-quality diets because they have lower 'food involvement', i.e. for a variety of reasons they pay less attention to food during all phases of selection, preparation and consumption. To test this hypothesis Bell and Marshall's food involvement scale² was administered to a sample of 240 women of differing levels of educational attainment, all living and working in Southampton. A subgroup of 127 women was also asked how often they ate fruit and vegetables in an average week. Women's food involvement was found to decrease significantly with decreasing educational attainment (r 0.35, P <0.001). Of the women who had no educational qualifications 42% were in the lowest quarter of the food involvement score, compared with 12% of women with degrees. This relationship is shown in the graph below.

Percentage of women falling in the bottom quartile of the food involvement score at three levels of educational attainment (n 240)



Younger women (r 0.17, P =0.05), and those with more children living in the house (r -0.15, P =0.05) also had lower food involvement. However, only educational attainment remained significantly associated with food involvement score when entered into a multiple regression model (P <0.001). Women with lower scores on the food involvement scale also reported eating fewer fruits and vegetables (r_s 0.43, P <0.001). In a logistic regression analysis the odds of eating fewer fruits and vegetables rose with both lower educational attainment (P <0.001) and lower food involvement scores (P =0.01), suggesting that each has an independent effect.

The data suggest that this measure of food involvement identifies differences between women of high and low educational attainment in their approach to food preparation and consumption. It is also associated with differences in fruit and vegetable consumption, an important indicator of dietary quality. This study is part of an ongoing programme of work in Southampton to investigate the reasons why women of low educational attainment eat a poorer-quality diet.

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A prospective analysis of dietary fat, fibre and energy intake in relation to fatness in childhood. By L. JOHNSON¹, A. MANDER¹, L.R. JONES², P.M. EMMETT² and S.A. JEBB¹, ¹MRC Human Nutrition Research, Cambridge CB1 9NL, UK and ²ALSPAC, University of Bristol, Bristol BS8 1TQ, UK

Worldwide thirty million children are classified as obese and at increased risk of chronic diseases, such as type 2 diabetes, heart disease and cancer¹. Obesity results from a long-term positive energy imbalance, when energy intake (EI) exceeds requirements. The World Health Organization has implicated the consumption of energy-dense foods, which typically have a high fat content, as detrimental for obesity risk and a high fibre intake as a protective of obesity risk². The aim of the present prospective analysis was to assess whether high fat, low fibre or an increased EI are related to greater fat mass in children.

Diet data was collected in a random subsample of children from a prospective cohort study in Avon, England at age 5 (n 523) and 7 (n 682) years using 3 d unweighed-diet diaries. Average daily EI (MJ), fibre density (g/MJ) and fat intake (% EI) were calculated. Misreporting of EI was assessed using EI:estimated energy requirements (EER)³. Body fat mass was estimated at age 9 years using dual-energy X-ray absorptiometry. The association between dietary EI, fat or fibre and body fatness was examined using linear regression of each dietary variable *v.* fat mass adjusted for potentially-confounding variables. In order to adjust for body size, height at age 9 years was included as a covariate in all regression models⁴.

Age (years)...	Model 1*				Model 2†				Model 3‡			
	5		7		5		7		5		7	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
EI (MJ)	0.08	-0.20, 0.36	-0.03	-0.29, 0.23	5.59	4.78, 6.39	5.52	5.08, 6.00	2.78	1.81, 3.76	3.82	3.30, 4.33
Fat (%)	0.02	-0.06, 0.11	0.05	-0.03, 0.12	0.03	-0.05, 0.12	0.08	0.01, 0.15	-0.04	-0.12, 0.03	0.04	-0.01, 0.09
Fibre (g/MJ)	-1.30	-2.21, -0.39	-0.87	-1.63, -0.10	-1.32	-2.21, -0.42	-1.25	-1.99, -0.51	-1.22	-2.01, -0.44	-0.29	-0.82, 0.23

Values are the change in fat mass (kg) for an increase of one unit in EI (MJ), fat (%) or fibre (g/MJ). * Includes gender and height (m) as covariates. † Includes gender, height and EI:EER (mis-reporting of EI) as covariates. ‡ Includes gender, height, EI:EER, maternal education, maternal BMI (kg/m²), child overweight status at baseline, TV watching at age 4.5 years and two of EI, fat and fibre as covariates.

In the fully-adjusted models: an increase of 1 MJ EI at age 5 and 7 years was associated with a 2.8 kg and 3.8 kg rise respectively in fat mass at age 9 years; a 1 g/MJ fall in fibre intake at age 5 years was associated with a 1.22 kg rise in fat mass at age 9 years, however there was no evidence of a similar trend for fibre intakes at age 7 years; there was no evidence of an association between dietary fat intake at either 5 or 7 years and body fat mass at 9 years.

The association between high EI and fat mass is only apparent after controlling for misreporting of EI. The association is attenuated by the inclusion of other confounding factors, but still remains a highly significant determinant of fat mass in childhood. The relationship between fat mass at age 9 years and low fibre intake is strong, although for fibre intakes at age 7 years this association was attenuated after adjustment for other confounders. There was no evidence of a relationship between dietary fat intake and subsequent body fat mass in childhood.

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Intake of total fat, SFA, MUFA and PUFA in Irish children aged 5–12 years. By T. JOYCE and M.J. GIBNEY, *UCD Institute of Food and Health, UCD, Belfield, Dublin 4, Republic of Ireland*

It is well established that there is a relationship between diet and CVD and that the development of coronary atherosclerosis, a risk factor for CVD, begins in childhood¹. Numerous studies have shown a link between dietary fat and CHD^{2,3,4}. Thus, it is essential to assess the intake of fat in childhood. The UK Department of Health⁵ recommends that total fat intake should be $\leq 33\%$ total energy, SFA intake $\leq 10\%$ total energy, MUFA intake $\geq 12\%$ total energy and PUFA intake $\geq 6\%$ total energy. The aim of the present study was to update the National Children's Food Survey database with accurate estimates of SFA, MUFA and PUFA values to obtain intakes of total fat and fatty acids. In addition, compliance with current dietary fat recommendations and the main contributors to fat intake were also analysed.

	Total population		Boys						Girls								
	5–12 years		5–12 years		5–8 years		9–12 years		5–12 years		5–8 years		9–12 years				
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
	(n 594)		(n 293)	(n 145)	(n 145)	(n 148)			(n 301)	(n 151)	(n 150)						
Total fat (g/d)	63.2	16.5	**	65.5	17.9	60.7 ^a	16.6	70.2 ^b	17.8	†††	60.9	14.7	58.2 ^a	13.5	63.5 ^b	15.4	††
SFA (g/d)	27.3	7.8	**	28.4	8.5	26.5 ^a	7.9	30.3 ^b	8.6	†††	26.2	6.9	25.6	6.6	26.9	7.1	ns
MUFA (g/d)	21.6	6.0	**	22.5	6.6	20.6 ^a	6.0	24.3 ^b	6.7	†††	20.8	5.2	19.6 ^a	4.7	21.9 ^b	5.4	†††
PUFA (g/d)	9.2	3.4	ns	9.4	3.4	8.6 ^a	3.1	10.2 ^b	3.4	†††	9.0	3.4	8.3 ^a	3.1	9.6 ^b	3.5	††
% total energy from total fat	33.9	4.2	*	33.4	4.4	33.5	4.3	33.3	4.5	ns	34.5	4.0	34.4	3.9	34.5	4.2	ns
SFA	14.7	2.5	ns	14.5	2.5	14.6	2.6	14.3	2.4	ns	14.9	2.5	15.1	2.5	14.6	2.3	ns
MUFA	11.6	1.8	*	11.4	1.9	11.4	1.8	11.6	2.1	ns	11.8	1.6	11.6	1.5	11.9	1.7	ns
PUFA	4.9	1.3	**	4.8	1.2	4.7	1.3	4.8	1.2	ns	5.1	1.3	4.9 ^a	1.3	5.2 ^b	1.4	†

Comparison of means between all boys and all girls: * $P < 0.05$, ** $P < 0.01$.

Comparison of mean nutrient intakes between age-groups within each gender: † $P < 0.001$, †† $P < 0.01$, ††† $P < 0.05$.

Mean intakes (% energy) of total fat, SFA, MUFA and PUFA were 33.9, 14.7, 11.6 and 4.9 respectively. Boys had significantly higher intakes (% energy) of total fat, MUFA and PUFA ($P < 0.01$) compared with girls. Overall, there was high compliance with total (88%) and monounsaturated (88.2%) fat recommendations; however, compliance with the SFA recommendation was low (5.7%). The main contributors (%) to total fat intakes were whole milk (14.8), meat products (10.5) and sugars, syrups and confectionery (8.3); to SFA were whole milk (21.1), sugars, syrups and confectionery (10.3) and biscuits, cakes, buns and pastries (8.7); to MUFA were meat products (13.1), whole milk (11.3) and sugars, syrups and confectionery (8.6); to PUFA were meat products (11.4), potatoes chipped, fried and roasted (9.9) and spreading fats (other than butter and low-fat spreads; 9.5). These results show that efforts need to be made to modify the fatty acid composition in the diets of Irish children to obtain better compliance with recommendations, especially for SFA.

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Frequency of consumption of added-sugar meals and relationship to percentage total energy from added sugars in a representative sample of Irish adults aged 18–64 years and Irish children aged 5–12 years. By T. JOYCE, S.N. MCCARTHY and M.J. GIBNEY, *UCD Institute of Food and Health, UCD, Belfield, Dublin 4, Republic of Ireland*

Recommendations and guidelines on added sugar intakes have been established in numerous countries based on the documented relationship with dental caries formation and on the hypothesis of micronutrient dilution¹. In their report on diet, nutrition and the prevention of chronic diseases the WHO² highlighted that in addition to population targets given in terms of the amount of free sugars, targets for the frequency of consumption of foods containing free sugars are important. It was recommended that for countries with high intakes of free sugars, the amount of free sugars should be $\leq 10\%$ energy intake and the frequency of consumption of foods and/or drinks containing free sugars should be limited to four times per d². The North/South Ireland Food Consumption Survey (18–64 years, n 1379) and the National Children's Food Survey (5–12 years, n 594) databases were used in this analysis. Adults who consumed one to nine meals per d were included in the analysis, and after the exclusion of under-reporters (energy intake: BMR < 1.05), the final sample contained 950 subjects. In children all meals were included (range one to twelve meals consumed per d) and under-reporters were not analysed. The present study aims to investigate whether there is a quantitative association between the recommended level of $\leq 10\%$ total energy from added sugars intake and frequency of mean daily added-sugar occasions in Irish adults and children.

	Quartiles of mean daily added sugar eating occasions										
	Total		Quartile 1		Quartile 2		Quartile 3		Quartile 4		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Added sugar eating occasions											
Adults	4.0	0.9	2.9 ^a	0.4	3.7 ^b	0.2	4.3 ^c	0.2	5.3 ^d	0.5	***
Children	4.2	0.9	3.1 ^a	0.3	3.9 ^b	0.2	4.4 ^c	0.2	5.3 ^d	0.6	***
Total energy (MJ/d)											
Adults	10.0	2.8	9.0 ^a	2.4	9.5 ^a	2.5	10.2 ^b	2.7	11.2 ^c	3.1	**
Children	7.0	1.5	6.2 ^a	1.4	7.3 ^b	1.5	7.3 ^b	1.5	7.4 ^b	1.3	***
Added sugar (g/d)											
Adults	60.6	35.9	40.6 ^a	22.6	53.2 ^b	29.7	64.3 ^c	34.0	84.0 ^d	44.4	**
Children	65.2	29.7	52.7 ^a	26.7	64.0 ^a	29.2	68.4 ^b	27.7	76.4 ^b	30.5	**
% Total energy from added sugar											
Adults	9.3	4.1	7.1 ^a	3.6	8.6 ^b	3.6	9.8 ^c	4.0	11.7 ^d	3.9	**
Children	14.6	5.4	13.5 ^a	6.0	14.0 ^a	5.0	14.8 ^{ab}	4.7	16.2 ^b	5.5	**

^{a,b,c,d} different superscripts denote significant differences between the means – * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Total adults (n 950), quartile 1 (n 220), quartile 2 (n 238), quartile 3 (n 270), quartile 4 (n 222).

Total children (n 594), quartile 1 (n 154), quartile 2 (n 146), quartile 3 (n 149), quartile 4 (n 145).

In Irish children and adults there was an increase in % total energy from total and added sugars as the frequency of added-sugar eating occasions increased. Children obtained a higher energy intake from added sugars (% energy) than adults (14.6% *v.* 9.3%). A quantitative association between the recommended level of $\leq 10\%$ total energy from added sugar intake and frequency of added eating occasions per d (four times per d) was found in adults only. The main contributors to added sugar intake in adults and children were biscuits, cakes, buns and pastries, carbonated beverages, squashes and cordials, confectionery, sugars, syrups, preserves and sweeteners (adults) and ready-to-eat breakfast cereals (children). This is the first study to assess whether there is quantitative association between the recommended level of $\leq 10\%$ total energy from added sugar intake and frequency of added-sugar eating occasions per d in children and adults.

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Antioxidant vitamin intakes in the progression of gastro-oesophageal reflux disease. By M.L. HAWKINS¹, G.J. DAVIES¹, M.F. CHAPLIN¹, J.F. DILLON², J.P. COTTON² and P.W. DETTMAR³,
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The prevalence of oesophageal adenocarcinoma (OA) is rapidly increasing in the Western world. Scotland has the highest incidence in Europe¹. A high antioxidant status is known for its protective effects in preventing the development of cancer at numerous sites².

The aim of the present study was to investigate possible differences in dietary intakes of vitamins and minerals known for their antioxidant status in three groups of patients, those with: non-erosive reflux disease (NERD); erosive reflux disease (ERD); Barrett's oesophagus (BO), the pre-malignant condition for OA. A total of 102 patients were asked to complete a 7 d estimated dietary record. Patients were recruited from the Gastroenterology Clinic at Ninewells Hospital, Dundee, and ethical approval was given by the Tayside Research Ethics Committee. No dietary advice was given to patients. The main findings are summarised in the Table.

Mean nutrient intake (/d)	NERD		ERD		BO	
	Males (n 21)	Females (n 26)	Males (n 11)	Females (n 12)	Males (n 19)	Females (n 13)
Vitamin A (retinol equivalents, µg)	1109*	754	605	766	657	1029
Carotene (µg)	3568†	1905	1320	1930	1430	3033
Vitamin C (mg)	94	84	88	96	82	172‡
Vitamin E (mg)	8.8	6.6	8.4	8.6	8.3	8.8

Mean value was significantly different from male intakes in the ERD and BO groups: * $P < 0.05$.

Mean value was significantly different from male intakes in the ERD and BO groups: † $P < 0.05$.

Mean value was significantly different from female intakes in the NERD and ERD groups (ANOVA): ‡ $P < 0.05$.

The data suggest that for male patients as gastro-oesophageal reflux disease (GORD) progressed to BO intakes of vitamin A and carotene were significantly lower in those with ERD and BO than in those with NERD. Conversely vitamin C intakes for females were significantly higher in patients with BO compared with patients with NERD and ERD. Despite not reaching statistical significance vitamin E intakes increased as the disease progressed in females, but decreased in males. Mineral intakes were not significantly different between genders in the three patient groups. It has previously been well documented in the literature that Caucasian males have a higher prevalence of BO and OA compared with females^{3,4}. These findings indicate that males having lower intakes of key antioxidant vitamins may be predisposed in the progression of GORD to BO and OA. The observation that females have higher intakes could be explained by recent dietary changes as a result of ill health. Larger population studies are needed to confirm this hypothesis.

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Exercise-induced changes in postprandial leptin concentrations are related to changes in fat and carbohydrate oxidation rates. By F.L. BURTON¹, D. MALKOVA¹, M.J. CASLAKE² and J.M.R. GILL¹, ¹Institute of Diet, Exercise and Lifestyle (IDEAL) and ²Department of Vascular Biochemistry, University of Glasgow, Glasgow G12 8QQ, UK

Plasma leptin concentrations increase in proportion to fat mass. However, it has also been shown that leptin concentrations change acutely in response to energy intake restriction and exercise, suggesting that leptin may be further regulated by energy-balance status. However, plasma leptin concentrations have also been shown to decrease following exercise without an associated energy deficit¹, although this is not unequivocal². The aim of the present study therefore was to investigate the effects of acute exercise, with and without energy deficit, on postprandial plasma leptin concentrations. It is hypothesised that exercise-induced changes in leptin would be mediated by changes in fat and carbohydrate balances, rather than changes in overall energy balance, and therefore correlate with exercise-induced changes in fat and carbohydrate oxidation rates.

Thirteen men (age 40 (SD 8) years, BMI 31.1 (SD 3.0) kg/m²) completed three 2 d trials in random order. On day 1 subjects rested (Con), walked briskly to expend 27 kJ/kg body mass (Exe-D) or completed the same walk with all energy expended replaced (Exe-NED). On day 2 subjects arrived after a 12 h fast and rested for 8.5 h. During this time breakfast and lunch were provided (each with 97 g carbohydrate, 33 g fat, 28 g protein and 3.3 MJ energy). Regular blood samples were collected for measurement of leptin. Indirect calorimetry with a ventilated hood was used to determine whole-body fat and carbohydrate oxidation. For 2 d before and on day 1 of each trial subjects avoided alcohol, planned exercise (other than the exercise trial walks) and all their food and drink was provided.

Time-averaged postprandial leptin concentrations were significantly lower following Exe-D and Exe-NED compared with Con. No significant differences were seen between Exe-D and Exe-NED (ng/ml; Con 10.98 (SE 2.39); Exe-D 9.12 (SE 1.90); Exe-NED 10.23 (SE 2.25); $P < 0.05$ for Exe-D v. Con and for Exe-NED v. Con). Postprandial fat oxidation over the 8.5 h was higher for Exe-D and Exe-NED compared with Con and also for Exe-D compared with Exe-NED (g; Con 37.0 (SE 2.0); Exe-D 44.6 (SE 2.3); Exe-NED 40.6 (SE 2.1); $P < 0.05$ for Con v. Exe-D, for Con v. Exe-NED and for Exe-D v. Exe-NED). Postprandial carbohydrate oxidation over the 8.5 h was lower following Exe-D and Exe-NED compared with Con. No significant differences were seen between Exe-D and Exe-NED (g; Con 102 (SE 4.9); Exe-D 86.1 (SE 4.8); Exe-NED 93.7 (SE 3.9); $P < 0.05$ for Exe-D v. Con and for Exe-NED v. Con). A significant negative correlation was observed between the exercise-induced change in postprandial leptin concentrations and postprandial fat oxidation ($r -0.44$, $P < 0.05$) with a reciprocal positive correlation between the exercise-induced change in postprandial leptin concentrations and postprandial carbohydrate oxidation ($r 0.51$, $P < 0.01$).

In conclusion, previous moderate exercise lowered postprandial leptin concentrations independently of energy deficit. Furthermore, exercise-induced changes in fat and carbohydrate oxidation rates, and therefore fat and carbohydrate balances, were associated with changes in postprandial leptin concentrations. Thus, these data provide further insight into the interrelationships between substrate metabolism and circulating leptin concentrations following exercise.

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Macronutrient, fibre and salt intakes in supplement users and non-users in Irish children aged 5–12 years. By E. WALSH, E.M. HANNON and A. FLYNN, *Department of Food and Nutritional Science, University College Cork, Cork, Republic of Ireland*

The objective of the present study was to compare intakes and compliance with macronutrient, fibre and salt recommendations in nutritional-supplement users and non-users.

The National Children's Food Survey (NCFS) was carried out between April 2003 and April 2004 to establish a database of habitual food and drink consumption in a representative sample of Irish children aged 5–12 years. A 7 d weighed-food record was used to collect food intake data from 594 children (293 boys, 301 girls). Analysis of dietary intake data was carried out using WISP© (Tinuiel Software, Llanfechell, Anglesey, UK), which contains *McCance and Widdowson's The Composition of Foods, 6th Edition*¹. Nutritional-supplement use was recorded during the 7 d by the respondent and/or their parents or guardians in the food diary. The percentage of subjects meeting guidelines for intakes of macronutrients, fibre and salt were compared in supplement users and non-users.

Recommendation	Percentage of individuals meeting nutrient recommendations			
	Boys		Girls	
	Supplement users (n 80)	Non-users (n 213)	Supplement users (n 66)	Non-users (n 235)
Fat (% TE ≤35)	71	68	62	59
SFA (% TE ≤11)	16	12	6	10
MUFA (% TE ≥13)	21	19	21	20
PUFA (% TE ≥6.5)	9	9	20*	9
Carbohydrate (% TE ≥50)	68	72	73	66
Dietary fibre (>age of child+5)	50	33	32	26
Salt (<target level)†	34	33	46	46

TE, total energy.

Value was significantly different higher than that for non-users: * $P < 0.05$.

† Population target levels² (g): 5–6 year olds, 3; 7–10 year olds, 5; 11–12 year olds, 6.

In our previous studies we have shown that in this population users of nutritional supplements have a lower prevalence of inadequate intakes for a number of micronutrients, especially vitamin A, folate and Fe³. In the present study supplement users had better compliance than non-users with recommendations for PUFA (girls only) and fibre (boys only). With these exceptions there were no differences in compliance with guidelines for macronutrients, fibre or salt.

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Polyphenol content and antioxidant activity of beer. By V.J. CLARKE¹, D. VAUZOUR¹, M.H. GORDON¹, J.M. AMES², R. MULLER³ and J.P.E. SPENCER¹, ¹*Department of Food Biosciences, The University of Reading, Whiteknights, Reading RG6 6AP, UK*, ²*School of Biological and Food Sciences, Queen's University Belfast, Belfast BT9 5AG, UK* and ³*Brewing Research International, Lyttel Hall, Nutfield, Surrey RH1 4HY, UK*

Research in the last decade strongly supports a role for polyphenols in the prevention of degenerative diseases, particularly CVD¹. Proposed mechanisms include inhibition of the oxidation of LDL, an important step in the aetiology of atherosclerosis and CHD² and effects on cell signalling or gene expression³. Polyphenols are the most abundant antioxidants in the diet, with intakes as high as 1 g/d⁴, principally from fruits, vegetables, cereals, chocolate, tea, coffee and red wine. One source that is often overlooked is beer, which is polyphenol-rich, and is regularly consumed. Studies have shown that beer increases plasma antioxidant capacity in human subjects⁵ and in patients suffering from hypercholesterolemia and CHD, short-term moderate beer consumption was cardioprotective⁶. There are however, vast differences in composition between beers, and some evidence that darker beers have higher antioxidant activity *in vitro* resulting from interactions between sugars, amino acids and phenolic acids⁷.

The overall aim of the present study is to investigate the bioavailability and metabolism of beer phenolics and their ability to protect LDL against oxidation. Initially, an investigation of the compounds present in beer, their relative antioxidant activity and how this differs in lager, ale and stout was performed. Ethyl acetate extracts and aqueous extracts were prepared from three commercial beer samples. Separation and identification of polyphenols and other potential compounds of interest was performed using reverse-phase (RP) HPLC. Antioxidant activity was determined using the ferric-reducing antioxidant power (FRAP) assay. Preliminary results indicated that the ale extracts possessed the most compounds of interest with the highest antioxidant activity; therefore, these extracts were subjected to further analyses. Centrifugal partition chromatography (CPC) was used to separate ale extracts into seventeen fractions of differing composition.

RP-HPLC was used for the separation of CPC fractions and subsequent identification and quantification of compounds belonging to different classes of polyphenols (hydroxycinnamates, hydroxybenzoates, flavonoids; including one not previously reported in beer, but known to inhibit LDL oxidation) and also other compounds of interest (furans) (Fig. 1A). Concentrations of compounds contained within CPC fractions have been correlated with antioxidant activity (Fig. 1B), as measured using the FRAP assay (Fig. 1C). CPC fractions have been analysed by liquid chromatography–(electrospray ionization)–MS, confirming detection of several phenolic compounds by mass.

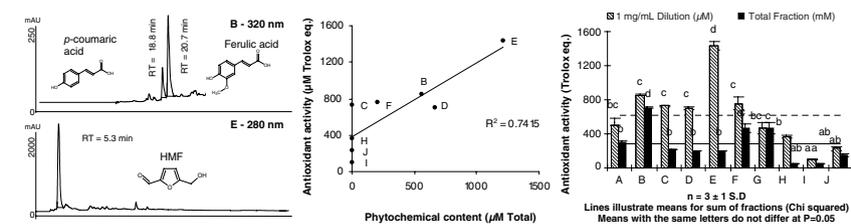


Fig. 1. Ale Organic Extract: (A) HPLC chromatograms of CPC fractions B & E showing several identified compounds together with their chemical structures (B) Correlation of CPC fractions phytochemical content with antioxidant activity (C) Antioxidant activity values of CPC fractions as determined using the FRAP assay.

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Effect of acute intervention with flavonoid-rich juice on the plasma antioxidant capacity. By C. NIWAT, T.W. GEORGE, M.H. GORDON and J.A. LOVEGROVE, *Hugh Sinclair Unit of Human Nutrition, School of Chemistry, Food Biosciences and Pharmacy, The University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, UK*

Epidemiological data provide evidence that consumption of a diet rich in fruit and vegetables is associated with a reduction in the incidence of CVD¹. One possible mechanism for the protective effect of fruits and vegetables is the antioxidant activity of several compounds, including vitamins, minerals, fibre and other phytochemicals². Flavonoids are a group of phenolic compounds with strong antioxidant activity that are present in fruits, vegetables and other plant foods and play a role in the reduction of the incidence of chronic diseases such as CVD³. However, very little is known about the amount of flavonoids absorbed from the diet and the reliability of plasma biomarkers⁴. The present study set out to investigate the pharmacokinetics of flavonoid uptake and the beneficial effect of an acute intervention with flavonoid-rich juices on markers of oxidative stress and cardiovascular risk factors.

Twenty-four healthy free-living volunteers with low habitual fruit and vegetable intake participated in the study, including twenty males and four females, with an age range of 28–64 years. The study was a single-blind randomized postprandial study. Volunteers were asked to consume a low-flavonoid diet for 5 d before the study day and fast overnight the day before the study. The volunteers were randomly allocated to consume either 400 ml flavonoid-rich juice or 400 ml flavonoid-poor juice, which had the same sugar content. Blood and urine samples were taken at time intervals after the ingestion of the juice and throughout the study day for 8 h. The subjects were asked to return to give blood and urine samples the following morning (24 h) and again in the evening (32 h). After an interval of 1 month they were asked to repeat the procedure with the juice from the other arm of the study.

The antioxidant capacity of plasma was measured before and up to 8 h after consumption of the fruit juice using the ferric-reducing antioxidant potential (FRAP) assay and the oxygen radical absorbance capacity (ORAC) assay. It was observed that the area-under-curve (AUC) for the FRAP response increased significantly by 9.1% during the 4 h period after ingestion of the flavonoid-rich juice ($P=0.001$), although this was not observed after consumption of the flavonoid-poor juice. In addition, the AUC from the ORAC assay was significantly higher after ingestion of the flavonoid-rich juice compared with the flavonoid-poor juice ($P=0.05$).

The present study provided evidence that acute ingestion of flavonoid-rich juice can significantly increase the plasma antioxidant capacity in human subjects.

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An *in vitro* investigation into the cancer-protective role of lycopene. By S. ELGASS¹, G. SCARLETT², A. COOPER¹ and M. CHOPRA¹, ¹*School of Pharmacy and Biomedical Science and* ²*School of Biological Sciences, University of Portsmouth, Portsmouth PO1 2DT, UK*

Since its discovery in 1986 the transcription factor NF- κ B has been found to play an important role in inflammation, cellular growth and apoptosis, and is therefore involved in a variety of disorders such as cancer and CVD. Numerous external stimuli, including TNF α , have been shown to lead to NF- κ B activation by phosphorylation of its inhibitor (inhibitory subunit of NF- κ B (I κ B) α) via I κ B kinases¹.

TNF α -induced NF- κ B activation is the major factor in resistance to apoptosis in PC3 prostate carcinoma cells, and a high serum concentration of TNF α is an indicator of poor prognosis in patients with prostate cancer². Lycopene, a natural plant carotenoid, is suggested to have an anti-cancer role³. In the present study the effect of lycopene on prostate cells after stimulation with TNF α has been investigated by following changes in I κ B α levels. Prostate cell lines PC3, DU145 (aggressive androgen-resistant carcinoma cells), LNCaP (androgen-sensitive carcinoma cells) and PNT2 (normal immortalised) were grown in RPMI medium (Sigma-Aldrich, Poole, Dorset, UK) supplemented with 10% (v/v) foetal bovine serum. At 60% confluence the cells were switched to 1% (v/v) FBS for 12 h. Cells were then treated with TNF α and/or lycopene delivered in 0.25% tetrahydrofuran. Proteins were extracted on ice in 1% (v/v) NP40 lysis buffer (50 mmol/l Tris, 2 mmol/l EDTA, 100 mmol/l NaCl and 1% NP40 in dH₂O). Using Western blotting proteins were separated via SDS-PAGE and probed with I κ B α , p50 and p65 antibodies and a horseradish (*Armoracia rusticana*, syn. *Cochlearia armoracia*) peroxidase-conjugated secondary antibody.

Basal NF- κ B expression in the four different prostate cells lines varied considerably, with the aggressive cells lines PC3 and DU145 having the highest expression, followed by LNCaP. The normal prostate cells PNT2 showed the lowest basal expression. The present study confirmed the effect of TNF α on the degradation of I κ B α in all cell lines. Western blotting for I κ B α revealed that prolonged exposure of cells to TNF α (0–30 min) as well as increasing concentrations of the stimulant (5–40 μ g/l) leads to a gradual reduction in I κ B α levels. Simultaneous treatment of cells with equimolar concentrations of TNF α and lycopene resulted in a reduction in I κ B α degradation (Figure 1).

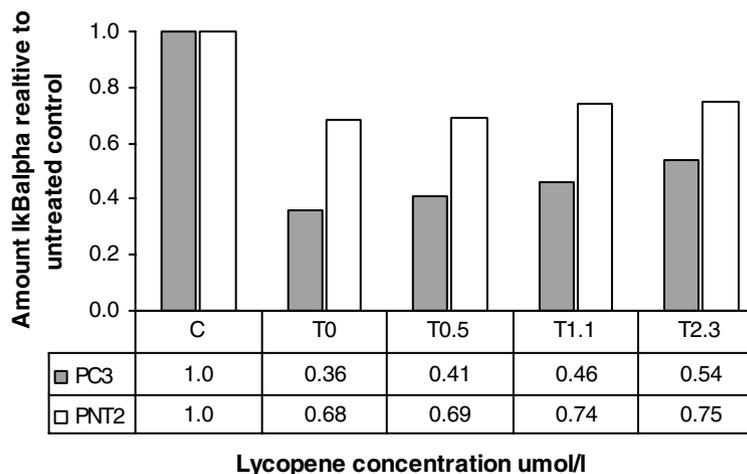


Fig. 1. Effect of simultaneous treatment of prostate cells lines PC3 and PNT2 with 10 μ g/l TNF- α and increasing concentrations of lycopene on the level of I κ B α *in vitro*. T denotes addition of 10 μ g/l (0.5 μ mol/l) TNF- α . C denotes untreated control.

Treatment with TNF α had no effect on overall levels of NF- κ B subunits p50 and p65 in Western blotting and did not affect mRNA levels of NF- κ B as determined via real-time PCR.

In conclusion, the results demonstrate that the simultaneous *in vitro* treatment of PC3 cells with TNF α and a physiologically-attainable concentration of lycopene (0.5–2.3 μ mol/l) causes inhibition of TNF α -induced I κ B α degradation in prostate carcinoma cells in a dose-dependent manner.

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'He says 'I ain't fat. You are. I don't need to diet': who is controlling the food choices of women with low educational attainment? By W. LAWRENCE, M. BARKER and THE FOOD CHOICE GROUP, *University of Southampton, MRC Epidemiology Resource Centre, University of Southampton, Southampton General Hospital, Southampton SO16 6YD, UK*

The Southampton Women's Survey has shown that women with low educational attainment are more likely to eat an unvaried and unhealthy diet than women with high educational attainment¹. From a review of the literature it is hypothesised that psychological, social, environmental and historical factors would be key influences on food choices. The aim of the study was to identify differences in influences on the food choices of women of low and high educational attainment. Eleven focus groups were conducted; eight with women of low educational attainment held at local community centres (Sure Start) and voluntary support groups for women with young children; two with women of high educational attainment recruited from women interviewed for the Southampton Women's Survey²; and one using a convenience sample of women of high educational attainment with young children for comparison with women of low educational attainment who all had children.

Thematic analysis revealed that women of low educational attainment felt they had less control over their own and their family's food choices. This appeared to be related to receiving less support from their partners and/or children for providing a healthy diet. Women of low educational attainment also demonstrated lower self-esteem, and more uncertainty regarding the links between healthy eating and future health outcomes. Women's perceptions of the cost of healthy food, the need to avoid waste, being trapped at home surrounded by opportunities to snack and having limited skill and experience with food, all contributed to their loss of control over their own and their family's food choices.

Illustrations of where study findings fit Bandura's social cognitive model.

Concepts in Model	Key predictors of food choice	Illustrative quotations from focus group discussions
Perceived control	Lack of control over food choices	I tend to do freezer foods. I have a problem with my partner and my son. They don't eat a lot of fruit and veg. Like I cook meals and I just get fed up of doing it 'cos they won't eat it, so I don't bother half of the time. Which is naughty, but ...
Mastery experiences	Learning to cook	Do you know what, I was never allowed in the kitchen as a child.
Vicarious experiences	Social models of healthy eating	I still buy crisps and that lot, 'cos my partner eats 24 in one day ... a multipack - that's his in one night.
Emotional state	Self-esteem	I think it comes back to how you feel about yourself in the end because if you feel important, then you'll cook yourself a meal. Whereas your children are important to you, friends, family, whatever are important to you. That's why you cook ... I don't feel that way about myself so I don't bother.
Impediments	Cost	Fruit and veg is expensive. It's a shame they can't make that cheaper, rather than make all the crap food special offers.
Impediments	Work vs home environments	I eat a lot on a Monday, pick a lot on a Monday night 'cos my husband goes out. You know, I'm at home on my own and it's just sooo boring.
Facilitators	Social support (lack of)	He says, 'I ain't fat, you are. I don't need to diet'.
Outcome expectations	Nutrition - long-term health link	I think there's a certain limit to it yeah, it's good to be healthy but I don't push it ... you can be too healthy.

Bandura's social cognitive model³ of behaviour provides an appropriate framework for considering the links between these influences. It has now been used to guide the development of a structured questionnaire for Phase 2 of this research, to quantify the relationship between diet and these social and psychological factors. Findings from this will inform the design of an intervention to improve the diets of women of low educational attainment.

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Socio-economic differences in diet- and health-related attitudinal variables among young Dublin women. By D.M.A. McCARTNEY, M.T. O'NEILL, J. WALSH, K.M. YOUNGER and J.M. KEARNEY, *School of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland*

A sample population of 138 young female respondents aged 18–35 years were recruited from twenty-seven areas across Dublin from September to November 2006. These respondents were categorised into socially 'advantaged' (*n* 20) and 'disadvantaged' (*n* 118) cohorts for comparative purposes, based on their geographical area of recruitment. Socio-economic data, including occupational social class, education, household structure, accommodation, medical card entitlement and income, were collected for each respondent to confirm their 'advantaged' or 'disadvantaged' designation.

Attitudinal data concerning general issues, health and diet were also collected for each respondent. Subjects were asked to indicate how often they thought about their life in the future to assess future salience. Subjects' health locus of control was also assessed by indicating the extent to which they felt their health was influenced by fate (chance locus), outside factors (external locus) and their own behaviour (internal locus). In relation to dietary stages of change¹, respondents were also asked to indicate which stage of change (pre-contemplation, contemplation, decision, action, maintenance or relapse) best described them at that point in time. Finally, some of the potential barriers to following a healthy diet were investigated.

Univariate Pearson's χ^2 tests were conducted to examine differences in each of these attitudinal variables between the socially 'advantaged' and 'disadvantaged' groups. The Table describes the attitudinal differences observed.

	'Advantaged' (%)	'Disadvantaged' (%)	<i>P</i>
Future salience			
Consider life in 1 month rarely or not very often	15.0	30.5	
Consider life in 1 month fairly or very often	85.0	69.5	0.248
Consider life in 10 years rarely or not very often	75.0	56.4	
Consider life in 10 years fairly or very often	25.0	43.6	0.188
Health locus of control			
Chance Locus	0.0	22.0	0.034
External Locus	0.0	14.4	0.039
Internal Locus	100.0	96.6	0.705
Stages of dietary change			
Action or maintenance	55.0	29.1	0.043
Pre-contemplation	5.0	17.1	0.293

These data indicate no statistically significant difference in future salience between the 'advantaged' and 'disadvantaged' young women. However, those women in the 'disadvantaged' group are significantly more likely to believe that their health is determined by chance or by external factors, than their more-'advantaged' peers. Additionally, the 'disadvantaged' women are significantly less likely to be in the 'action' or 'maintenance' stages of dietary change.

In relation to potential barriers to healthy eating, Fisher's exact χ^2 analysis revealed that a greater percentage of those with low and intermediate education cite poor dietary knowledge (19.8% *v.* 0%) (*P*=0.008) as a barrier, compared with their more-educated peers. However, a much lower percentage of those with low or intermediate education cited 'busy lifestyle' as a barrier to healthy eating than their more-educated counterparts (39.7% *v.* 69.0%) (*P*=0.006).

These findings indicate that interventions that improve dietary knowledge, and that raise awareness of, and emphasise, the role of diet in health, remain important when seeking to improve the diets of young 'disadvantaged' women. Further interventions that facilitate healthy eating, such as price reduction of healthy food, may also likely to yield improvements in dietary behaviour among this group.

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The relationship between diet and CVD risk factors in Ghanaian populations from London and Accra. By M. OWUSU, M. PUFULETE and J. THOMAS, *Nutritional Sciences Research Division, King's College London, 150 Stamford St, London SE1 9NH, UK*

The burden of CVD, in particular stroke, is increasing in Ghana and the West African sub-region. This increase has been associated with the emergence of risk factors caused by changing lifestyle as a result of urbanisation. Migration to affluent societies is thought to promote these risk factors. The aim of the present study was to compare dietary patterns of a first-generation Ghanaian migrant population in the UK with an urban population in Accra, Ghana, to determine whether differences in diet were associated with differences in biomarkers of CVD risk. The specific focus was on markers of folate status including plasma homocysteine, which is related to stroke but has not been studied much in this population.

Eighty subjects in London were age and gender-matched to 160 subjects in Accra. Dietary intake was assessed by 24h recalls and a food-frequency checklist. Information on use of supplements was obtained from a questionnaire on health and lifestyle. The 24h recalls were analysed using Microdiet version 1.2 (Downlee Systems Ltd, Salford, UK), which included updated data on the nutritional composition of commonly-consumed Ghanaian dishes. Fasting blood samples were collected for the determination of plasma homocysteine, serum and erythrocyte folate, serum vitamin B₁₂, TAG, HDL- and LDL-cholesterol, plasma glucose and insulin.

Dietary intake of macronutrients and selected micronutrients are shown in the Table. Mean intake of total fat, protein, SFA, MUFA, dietary cholesterol and vitamin B₁₂ were significantly higher in subjects from London than in subjects from Accra ($P<0.05$). Carbohydrate and dietary folate intakes were significantly lower in London than in Accra ($P=0.001$). There were more supplement users in London than in Accra (36% v. 13%; $P=0.001$).

	London (n 80)		Accra (n 160)		P
	Mean	SD	Mean	SD	
Energy intake(kJ/d)	9122	3114	9752	3223	0.145
Protein (% energy)	17.2	7.8	15.2	5.8	0.04
Total fat (% energy)	32.3	7.2	28.6	6.9	0.001
SFA (% energy)	06.1	2.9	4.8	2.9	0.001
MUFA (% energy)	07.7	5.5	5.6	1.9	0.001
Carbohydrates (% energy)	50.6	8.7	54.9	8.8	0.001
Cholesterol (mg/d)	82.1	82.8	35.1	33.9	0.001
Dietary folate (µg/d)	500	208	573	186	0.001
VitaminB ₁₂ (µg/d)	7.0	6.2	5.2	4.3	0.008

% energy, % contribution of nutrient to total energy intake.

Compared with subjects from Accra, subjects from London had significantly higher serum and erythrocyte folate concentrations (by 26% and 33% respectively; $P=0.001$) and significantly lower plasma homocysteine concentrations (by 26%; $P=0.001$). Serum HDL-cholesterol was significantly higher (by 17%; $P=0.001$) and LDL-cholesterol was significantly lower (by 11%; $P=0.02$) for subjects from London than for those from Accra.

Migration to the UK results in an improvement in biomarkers of folate status, despite the fact that reported dietary intake of folate was lower in subjects from London. However, mean contributions of folic acid from supplements and fortified food to daily folate intakes were 20% in London and 9% in Accra. This difference highlights the superior bioavailability of folic acid relative to natural folates in foods and the important contribution of fortified foods in the diet. Migration to the UK is also associated with an improved lipid profile despite the fact that reported fat intake including SFA was significantly higher in London ($P=0.001$). However, differences in fatty acid profiles between the groups are likely to account for these results, since it is now well established that the type rather than the total amount of fat is an important determinant of plasma cholesterol concentrations.

Determinants of early introduction to solid foods in a sample of healthy term infants. By R. TARRANT¹, M. SHERIDAN-PEREIRA², K.M. YOUNGER¹ and J.M. KEARNEY¹, ¹*School of Biological Sciences, Dublin Institute of Technology, Kevin's Street, Dublin 8, Republic of Ireland and* ²*The Coombe Women's Hospital, Dept of Paediatrics, The Coombe, Dublin 8, Republic of Ireland*

Early introduction to solids is globally considered an unsafe and inappropriate weaning practice for all infants. The WHO recommends exclusive breast-feeding and delaying the introduction of solid food in an infant's diet until 6 months post partum to promote optimal infant health¹. This public health recommendation is endorsed in Ireland; however, scientific evidence is lacking on the proportion of infants weaned onto solid foods prematurely (≤ 12 weeks). The objective of the present study was to identify the determinants of early introduction to solids (≤ 12 weeks)² in a sample of healthy term infants.

A cross-sectional prospective study was conducted that involved the recruitment of 561 pregnant mothers from a large Dublin maternity hospital, of which 401 healthy Irish-born mothers met the criteria for the current data analyses. Quantitative data were recorded at 6 weeks and 6 months post partum, detailing the mothers' weaning practices and the reasons for such feeding choices.

Of the infants 23% were consuming solid foods by 12 weeks post partum. Several factors were significantly associated with early introduction to solid foods in the univariate analysis (Table). Following a logistic regression analysis the main determinants were identified, including the age-group (15–24-year-old mothers; OR 4.51 (95% CI 1.29, 15.78)), mothers who completed education to primary or secondary level (OR 6.78 (95% CI 2.32, 19.77)), infants who were formula fed at 12 weeks (OR 3.91 (95% CI 1.06, 4.40)) and mothers who reported antenatally that they intended to introduce solids <13 weeks post partum (OR 7.33 (95% CI 3.17, 19.95)).

Factors associated with early weaning onto solids ($P>0.001$)	
Maternal	Age, social class, educational attainment, marital status of mothers, health insurance status during antenatal care, planned or unplanned pregnancy
Health	Smoking status during pregnancy
Behaviours	Folic acid supplementation during pregnancy
Antenatal	Attendance at antenatal classes Mothers' antenatal intention in relation to approximate timing of first solids post partum
Feeding mode	Breast-feeding initiation Mode of feeding at 12 weeks (any breast-feeding v. formula feeding) Whey- v. casein-based formula feed consumed at 6 weeks

Results demonstrate that the introduction to solid foods by 12 weeks of age is a common weaning practice among Irish mothers. To improve compliance with current infant feeding recommendations younger mothers with lower educational attainment should be targeted. Findings strongly suggest that educating all mothers in the antenatal period about the correct weaning time is a key modifiable determinant of early weaning.

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Uptake and secretion of micellarised lutein from spinach (*Spinacia oleracea*) using a Caco-2 intestinal cell model. By L.P. O’SULLIVAN, L. RYAN and N.M. O’BRIEN, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Lutein is a xanthophyll carotenoid that is abundant in green leafy vegetables such as spinach (*Spinacia oleracea*). The present study employs both an *in vitro* digestion procedure and a Caco-2 cell model to investigate the bioaccessibility, uptake and secretion of lutein from three samples of spinach (fresh, frozen and canned). Each sample was untreated, boiled or microwaved. Samples were then homogenised, subjected to an *in vitro* digestion procedure and digesta were ultracentrifuged to isolate the aqueous micellar fraction. Lutein content of the digestate and micelles was quantified by HPLC. The transfer of lutein from the digestate to micelles was defined as bioaccessibility. Micelles generated from the *in vitro* digestion were adjusted to contain 0.1 µM-lutein with sterile media and were added to Caco-2 monolayers cultured on a 0.4 µm pore size membrane on a transwell plate. Micelles were incubated for 4 h, after which time media were removed and monolayers were washed twice with Hank’s balanced salt solution. Monolayers were then incubated with media containing chylomicron-stimulating compounds for 16 h. Carotenoids from monolayers and basolateral chamber media were extracted and analysed by HPLC.

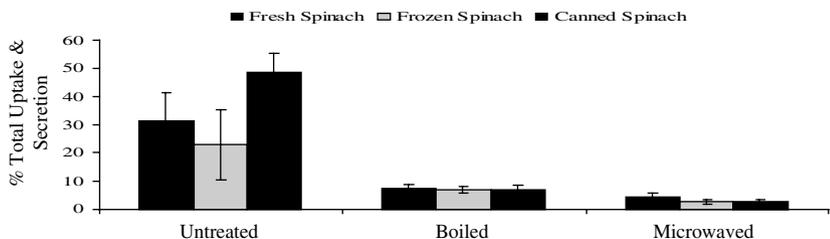


Fig. 1. The percentage total uptake and secretion of 0.1 µM-micellarised lutein from spinach in Caco-2 intestinal cells (*n* 3). Values are means with their standard errors represented by vertical bars.

Fig. 1 shows the percentage total uptake and secretion of micellarised lutein from spinach in the Caco-2 cell model. This system mimics the intestinal absorption of lutein *in vivo*, thus giving an indication of the potential availability of lutein from spinach. Lutein in micelles prepared from untreated spinach samples had the highest percentage cellular uptake and secretion. The percentage uptake and secretion of micellar lutein was highest from the canned spinach (48). These data are in line with a human study in which the bioavailability of lutein from whole-leaf spinach was reported to be 45%¹. For all spinach samples boiling resulted in an increase in the lutein content of the micelles (increase in bioaccessibility; data not shown); however, when micellar content was adjusted to 0.1 µM the cellular uptake and secretion was decreased compared with the untreated samples (Fig. 1). Microwaving all spinach samples decreased the amount of lutein in the micelles (data not shown) and after adjustment of micellar content cellular uptake and secretion was also decreased (Fig. 1). Further work is required to determine what other carotenoids may be released following boiling or microwaving and to what extent the carotenoids may have become isomerised during the cooking processes.

The research was funded by Science Foundation Ireland.

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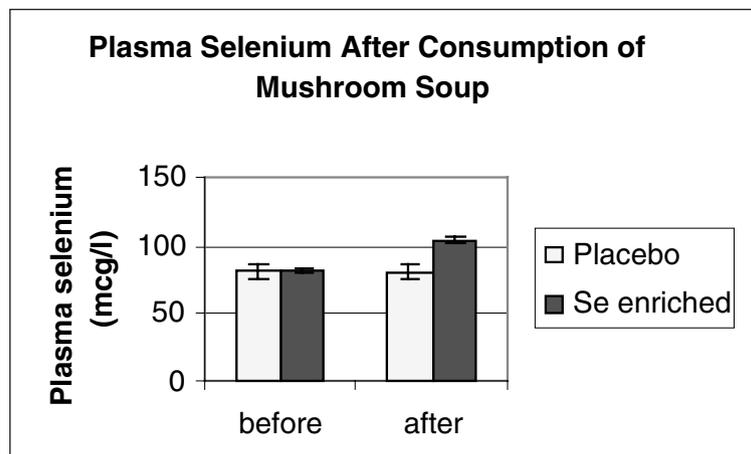
Bioavailability and speciation of selenium from selenium-enriched mushrooms. By M. RAYMAN¹, F. ANGUS¹ and H. GOENAGA-INFANTE², ¹*School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK* and ²*LGC Limited, Queens Road, Teddington, Middlesex, UK*

The relatively low intake of Se in the UK has encouraged the creation of Se-enriched functional foods. One such food is the common mushroom, which can be cultivated readily in the presence of additional Se. However, previous published information has suggested that bioavailability of Se from mushrooms is fairly low¹, calling into question their usefulness as a possible new source of dietary Se. The aim, therefore, was to see whether consumption of Se-enriched mushrooms could increase Se status in adults.

Se-enriched *Agaricus bisporus* mushrooms were grown by Hughes Mushrooms (Dungannon, Co. Tyrone, UK) in the presence of additional Se to give 2.5 µg Se/g fresh wt. Control mushrooms were grown for comparison. Each type of mushroom was made into a soup, which was frozen in preparation for the bioavailability trial. The Se content of the mushrooms and the soups was determined.

Power calculations showed that 21 subjects would be required per group. Fifty-two healthy volunteers aged 18–70 years who had not taken (in the last 6 months), or were not taking, Se supplements and were not allergic to milk or mushrooms were randomly allocated to two groups, ensuring an even spread by gender and age. They consumed their normal diet for 1 week avoiding high-Se foods. On day 7 they provided a blood sample, which was separated, coded and frozen for later analysis. Each lunchtime for 14 d, according to their group allocation, they consumed a measured portion of either placebo soup or Se-enriched mushroom soup containing 200 µg Se. They provided a second blood sample on completion of the trial. Subjects, researchers and analysts were blinded to the type of soup.

Mean plasma Se (µg/l), measured by inductively-coupled plasma MS, did not change in the placebo group, (initial 81.4 (SE 3.0) v. final 80.7 (SE 5.0); NS), but rose significantly in those consuming the fortified soup (81.4 (SE 1.5) v. 104.2 (SE 2.7); *P*<0.0001) as shown in the figure below.



Speciation of an aqueous extract of the Se-enriched mushrooms showed that Se was present both as selenomethionine (24.3% of total Se), and as the potent anti-cancer agent, *Se*-methyl-selenocysteine (0.17% of total Se).

It is concluded that eating Se-enriched mushrooms is a satisfactory way of increasing Se status.

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Vitamin D status and bone mineral density in adolescents: The Young Heart's Project. By T. HILL¹, A. COTTER¹, J. WALLACE³, P.J. ROBSON³, C. BOREHAM³, W. DUBITZKY³, L. MURRAY⁴, A. FLYNN¹, M. KIELY¹ and K.D. CASHMAN^{1,2}, ¹Department of Food and Nutritional Sciences, ²Department of Medicine, University College, Cork, Republic of Ireland, ³Northern Ireland Centre for Food and Health, University of Ulster, Coleraine, UK and ⁴Department of Epidemiology and Public Health, Queens University, Belfast, UK

Vitamin D status is considered to be an important determinant of peak bone mass in adolescents. Studies examining the relationship between vitamin D status and bone mineral density (BMD) in large representative samples of adolescents are scarce. Thus, the objective was to examine whether vitamin D status, as measured by serum 25-hydroxyvitamin D (25(OH)D), in adolescents has any influence on BMD.

The present study of vitamin D status and BMD in adolescents used data and blood samples from subjects in the Young Hearts II cohort of adolescents¹, which had bone mass, vitamin D status, dietary and lifestyle data available (*n* 926). BMD was measured by dual-energy X-ray absorptiometry at the non-dominant forearm and dominant heel. Serum 25(OH)D was measured by enzyme immunoassay (IDS Ltd, Boldon, Tyne and Wear, UK). Dietary data was collected using the diet-history method. Height and weight were measured in all subjects and questionnaires were used to assess smoking habits, usual alcohol consumption and physical activity patterns. Multivariate linear models were used to examine the relationship between BMD of the non-dominant forearm and dominant heel and low, moderate and high vitamin D status. Covariates in the models included: height; weight; pubertal status; physical activity score; alcohol intake; smoking status; Ca intake. Fruit intake was also included as a covariate in the multivariate analysis for the 12-year-old girls, as this was previously shown to have a significant association with BMD². A significant interaction between age and gender on BMD and vitamin D status was found ($P < 0.05$), which justified analysing each age-gender group separately.

	Forearm BMD [‡]							
	Boys (<i>n</i> 244)		Girls (<i>n</i> 236)		Boys (<i>n</i> 214)		Girls (<i>n</i> 232)	
	β [‡]	SE	β	SE	β	SE	β	SE
Unadjusted								
D1 ^{‡‡}	0.006	0.007	0.020*	0.008	0.008	0.011	0.021*	0.009
D2 ^{‡‡‡}	0.000	0.008	0.009	0.008	-0.006	0.011	0.010	0.009
Adjusted for physical, lifestyle and dietary variables [†]								
D1	0.002	0.007	0.018*	0.008	0.005	0.009	0.018*	0.008
D2	-0.002	0.007	0.014	0.008	-0.001	0.009	0.010	0.009

* $P < 0.05$.

[†] Covariates: height, weight, pubertal status, physical activity score, alcohol intake, smoking status, Ca intake (fruit intake was included as a covariate in the 12-year-old girl group).

[‡] β is the estimated unstandardized regression coefficient. For boys, low vitamin D status was defined as a serum 25(OH)D level < 50.1 nmol/l and a high vitamin D status as serum 25(OH)D level > 75.3 nmol/l. For girls, low vitamin D status was defined as a serum 25(OH)D level < 46.3 nmol/l and a high vitamin D status as a serum 25(OH)D level > 74.1 nmol/l.

^{‡‡} D1 compares the high-vitamin D-status group with the low-vitamin D-status group.

^{‡‡‡} D2 compares the high-vitamin D-status group with the moderate-vitamin D-status group.

In both unadjusted and adjusted analysis 12- and 15-year-old girls with a high vitamin D status had significantly higher forearm BMD than did the girls with a low vitamin D status (unadjusted analysis; β 0.020 (95% CI 0.004, 0.037) and β 0.021 (95% CI 0.003, 0.039) respectively) and (adjusted analysis; β 0.018 (95% CI 0.003, 0.033) and β 0.018 (95% CI 0.001, 0.034) respectively). No other significant associations were observed. In conclusion, maintaining a moderate to high serum 25(OH)D level (i.e. > 50 nmol/l) may be important for bone health in adolescent girls.

The work was supported by funding made available through the Higher Education Authority under their Strand 1: North South Programme for Collaborative Research.

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Differential expression of cancer-related genes in omental adipose tissue in human obesity. By V. CATALÁN¹, J. GÓMEZ-AMBROSI¹, C. SILVA², M.J. GIL³, J.L. HERNÁNDEZ-LIZOÁIN⁴, J. BAIXAULI⁴, F. ROTELLAR⁴, J.A. CIENFUEGOS⁴, J. SALVADOR² and G. FRÜHBECK^{1,2}, ¹Metabolic Research Laboratory and Departments of ²Endocrinology, ³Biochemistry and ⁴Surgery, Clínica Universitaria de Navarra, University of Navarra, Pamplona, Spain

Obesity is associated with different types of cancer that significantly increase the morbidity and mortality of patients with cancer^{1,2}. Although obesity is a multi-factorial heterogeneous condition, fat accumulation in the visceral depot has been shown to be highly associated with cancer development risk³. Different explanations have been proposed for the detrimental effects of obesity on mutagenesis but the underlying adipose-dependent molecular mechanisms involved in this association have not been completely disentangled.

The aim of the present study was to gain an insight into the potential molecular effects of visceral adipose tissue (VAT) in cancer development by comparing the pattern of gene expression of VAT from lean and obese volunteers using DNA microarrays. VAT biopsies were obtained by laparoscopic surgery from six male patients (44.2 (SE 6.3) years). RNA was extracted and pooled for the lean (BMI 23.4 (SE 0.8) kg/m²) and obese (BMI 37.3 (SE 2.5) kg/m²) groups. Some metabolic characteristics of the subjects are presented in the Table.

	Body fat (%)		Glucose (nmol/l)		Insulin (pmol/l)		HOMA		TAG (mg/l)		Leptin (ng/ml)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Lean	20.2	2.4	4.8	0.1	55	16	1.96	0.58	680	60	5.9	1.1
Obese	40.3	4.4	5.4	0.2	79	33	3.17	1.40	1100	190	17.5	0.4
<i>P</i>	*		*		NS		NS		*		*	

Differences between the lean and obese groups were significant (Mann-WhitneyU test): * $P < 0.05$.

From the 153 genes with a > 2 -fold variation, 89 (8% of the total number) were up regulated and 64 (6% of the total number) were down regulated in the VAT from patients who were normoglycaemic, normotensive and obese. The majority of the genes with altered expression encode proteins involved in cell proliferation, angiogenesis, metabolism and signal transduction. Three major functions related to cancer development were preferentially identified in genes with up-regulated expression in obese patients: cell proliferation; cell motility; metabolism. In addition, the down-regulation of genes involved in cell cycle or cell death may be exerting an impact on the association of obesity and cancer.

Up-regulated genes			Down-regulated genes		
Biological process	Fold change	UniGene symbol	Biological process	Fold change	UniGene symbol
Cell proliferation	3.5	VEGFB	Cell cycle	2.7	CDKN2B
	3.2	IGF2		2.5	CDKN1C
	2.9	FGF1	2.2	CDKN2D	
	2.7	FGFR1	Cell death	5.0	IGFBP5
	2.6	NME1		2.5	IGF2R
2.5	IL3	2.0		PTGER3	
Cell motility	2.4	MAPK9	Signal transduction	3.4	IFNAR1
	2.1	GRPR		2.8	NGFR
	2.1	TGFB1		2.0	TGFB3R
	2.7	HGFA			
	2.3	BDKRB1			
Metabolism	2.2	DRD2			
	2.8	ITPR3			
	2.5	LRP5			
	2.1	PGK1			

VAT from obese patients showed an altered expression profile of genes involved in cancer development, thereby providing evidence of some of the genetic clues involved in gene expression level changes in this tissue. Further molecular studies are necessary to better understand the contribution of adiposity-related derangements to carcinogenesis.

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Differences in fatty acid-binding protein (FABP) 3 and 4 mRNA expression in skeletal muscle and subcutaneous adipose tissue between normal-birth-weight and low- and high-birth-weight porcine offspring at days 7 and 14 of postnatal life. By A. MOSTYN¹, P.J. WILLIAMS¹, J.C. LITTEN², K.S. PERKINS³, A.M. CORSON³, L. CLARKE³ and M.E. SYMONDS¹, ¹Centre for Reproduction and Early Life, Institute of Clinical Research, University Hospital, Nottingham NG7 2UH, UK, ²School of Agricultural, Policy & Development, University of Reading, Reading RG6 6AR, UK and ³Faculty of Natural Sciences, Imperial College London, Wye, Kent TN25 5AH, UK

Size at birth has been shown to have important consequences on skeletal muscle and adipose tissue development¹. The transport of fatty acids from the plasma membrane to the intracellular organelles involved in fatty acid utilisation is performed by members of the FABP family. FABP may regulate lipid metabolism and other cellular processes such as gene transcription, cellular signalling, growth and differentiation. The aim of the present study was to examine whether birth weight influences the expression of mRNA for FABP3 and FABP4 in subcutaneous adipose tissue (SAT) and skeletal muscle (SM) at days 7 and 14 of postnatal life.

Eleven sows of similar body weight and parity were housed individually in a temperature-controlled barn and allowed to give birth naturally. On the first day of life piglets were ranked according to birth weight and three from each litter were assigned to small (S; n 11), normal (N; n 11) or large (L; n 11) groups. Piglets were weighed on days 0, 7 and 14. Equal numbers of male and female piglets were distributed amongst the groups. Animals were humanely killed with an overdose of barbiturate anaesthetic at either day 7 (n 15) or day 14 (n 18) of age. SAT and SM (biceps femoris) were rapidly dissected, placed in liquid N₂ and stored at -80 °C until analysed. The mRNA abundance of FABP3, FABP4 and 18S within SAT and SM was quantified by real-time PCR. Results are expressed as mean values and standard errors normalised to 18S and were analysed by one-way ANOVA using SPSS version 12 (SPSS, Chicago, IL, USA).

On all days of the study S piglets were lighter than the L piglets (P<0.05), but S piglets were only lighter than N piglets on days 0 and 7¹. The Table shows the molecular results; there were no significantly-different results from SM on day 7 or SAT on day 14 (data not shown).

		SM						SAT					
		S		N		L		S		N		L	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Day 7	FABP3							21.6	8.7 ^a	115.2	26.3 ^{ab}	32.2	9.0 ^b
	FABP4							1.3	0.5 ^a	55.0	11.4 ^{ab}	11.6	5.0 ^b
Day 14	FABP3	1.2	0.3 ^a	8.2	1.8 ^{ab}	3.2	0.4 ^b						
	FABP4	2.0	0.4 ^a	9.2	1.3 ^{ab}	2.5	0.7 ^b						

^{a,b} Means within rows with common superscript letters were significantly different (P<0.05).

Altered expression of FABP3 and FABP4 in SAT and SM between N and both S and L offspring indicates that alterations in fatty acid utilisation may occur in those offspring. Further work is needed to examine whether these changes may be associated with the onset of metabolic disease in later life as a result of differences in fat deposition and processing in offspring that are not within normal-birth-weight boundaries.

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Identification of putative protein biomarkers of dietary supplementation with soya foods. By M.C.Y. WONG^{1,2}, J. WHEELER³, K.S. LILLEY⁴, P.W. EMERY¹, V.R. PREEDY¹ and H. WISEMAN¹, ¹Nutritional Sciences Research Division, King's College London, London SE1 9NH, UK, ²Nutrition, Dietetics and Food Sciences Division, School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH, UK, ³National Institute for Biological Standards and Control, Potters Bar EN6 3QG, UK and ⁴Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, UK

It has previously been shown that consumption of soyabean isoflavones in foods or supplements significantly decrease oxidative stress and improve cognitive function in healthy human subjects^{1,2}. It is hypothesized that pathways affected by soyabean isoflavones can be elucidated using 2-D gel electrophoresis to identify changes in the serum protein profiles of subjects following soya food consumption.

Twenty-two healthy female subjects (aged 18–35 years) were fed for 2 weeks on a diet in which soya products were incorporated to provide an average of 60 mg total isoflavones/d. Venous blood was collected at baseline and at the end of treatment periods. Serum samples were analysed by 2-D fluorescence difference gel electrophoresis using Cy3 and Cy5 minimal-labelling dyes. A total of 50 µg protein was focused on each 240 mm immobilized pH gradient strip, non-linear gradient pH 3–10. The second dimension was run on 240 mm × 240 mm × 1 mm 12% (v/v) SDS-PAGE gels. The images were captured by a Typhoon 9410 scanner (Amersham Biosciences, Little Chalfont, Bucks., UK) and analysed using DeCyder 2D version 6.5 software (GE Healthcare, Piscataway, NJ, USA). A preparative gel with 500 µg pooled protein loading was also prepared and the spots of interest were excised and digested with trypsin. The identities of these proteins of interest were obtained by peptide mass fingerprinting using liquid chromatography–MS/MS based on Mascot database.

In total, 1178 spots were located and matched. Using the biological variation analysis module within the software, eleven proteins were found to be significantly altered in concentration (six down regulated and five up regulated; P<0.05 in all cases, based on paired *t* tests). In particular, apoE and caeruloplasmin were shown to be significantly increased, while α-1-acid glycoprotein was shown to be significantly decreased after 2 weeks of soya food consumption. The increase in caeruloplasmin concentration may improve the antioxidant capacity, while the increase in apoE may alter the lipid profile and thus reduce the initiation of lipid peroxidation. The reduction in α-1-acid glycoprotein concentration may also suggest a role for soyabean isoflavones in immunomodulation. These novel findings show that soya food consumption alters protein expression, particularly in relation to oxidative stress and lipid metabolism.

We thank Dr Eleanor Lynn for carrying out the diet intervention part of the study.

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Hepatic proteomics in IL-1R1^{-/-} mice that are resistant to obesity-induced insulin resistance following a high-fat diet. By B.D.E. ROOS¹, K. ROSS¹, G. RUCKLIDGE¹, S. TOOMEY², J. BROWNE² and H.M. ROCHE², ¹Rowett Research Institute, Division of Vascular Health, Aberdeen, UK and ²Nutrigenomics Research Group, Department of Clinical Medicine, St James's Hospital, Dublin, Republic of Ireland

Obesity is associated with a state of chronic low-grade inflammation that has been implicated in the development of atherosclerosis and insulin resistance. In the present study links between obesity, insulin resistance and the pro-inflammatory response were investigated in IL-1R1^{-/-} mice that have a compromised macrophage response. For this, eight IL-1R1^{-/-} mice and eight C57Bl/6 control mice were fed a high-fat diet (60% energy from fat) for 18 weeks, after which serum glucose, insulin and TAG concentrations were measured and proteomics performed on hepatic tissue.

Both groups gained similar weight after the high-fat diet; however, the IL-1R1^{-/-} mice were protected against insulin resistance. IL-1R1^{-/-} mice had significantly lower serum glucose ($P < 0.001$), serum triglyceride ($P < 0.01$) and serum NEFA ($P < 0.001$) concentrations compared with control mice. HOMA levels, an index of insulin resistance, and revised QUICKI, an index of insulin sensitivity, were improved in the IL-1R1^{-/-} group ($P < 0.01$) compared with control mice.

Table 1. Fasting levels of serum triglycerides, glucose, insulin and NEFA, HOMA and revised QUICKI, in IL-1R1^{-/-} mice ($n = 7$) and C57Bl/6 control mice ($n = 8$) fed a high fat diet for 18 weeks

	IL-1R1 ^{-/-} mice	C57Bl/6 control mice
Triglycerides (mmol/L)	0.62 ± 0.15**	0.89 ± 0.16
Glucose (mmol/L)	5.82 ± 0.46***	8.05 ± 0.73
Insulin (µU/mL)	34.68 ± 13.09	50.38 ± 16.59
NEFA (µmol/L)	574.58 ± 34.42***	775.63 ± 88.69
HOMA-IR	9.09 ± 3.77**	18.13 ± 6.54
Revised QUICKI	0.20 ± 0.01***	0.18 ± 0.01

Values are means ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Proteomics revealed that thirty-five hepatic proteins were up or down regulated in IL-1R1^{-/-} mice, the majority of which were involved in pathways related to inflammation, glucose metabolism, oxidative stress and methionine metabolism. For example, anti-inflammatory proteins like hepatic HSP70, superoxide dismutase and peroxiredoxin were increased in IL-1R1^{-/-} mice, whereas fructose-1,6-bisphosphatase protein levels were decreased, suggesting a decrease in gluconeogenesis and underwriting the increased insulin sensitivity. Senescence protein 30 levels were increased in IL-1R1^{-/-} mice indicating decreased aging and a lower responsiveness to apoptosis compared with control mice.

In conclusion, the systems biology approach indicates that disrupting components of the IL-1-mediated inflammatory response results in a significant protection from obesity-induced insulin resistance.

The effect of *trans*-10, *cis*-12 conjugated linoleic acid on gene-expression profiles related to lipid metabolism in human Caco-2 cells. By E.F. MURPHY¹, G.J.E.J. HOOIVELD³, M. MULLER³, R.A. CALOGERO⁴ and K.D. CASHMAN^{1,2}, ¹Department of Food and Nutritional Sciences and ²Department of Medicine, University College, Cork, Republic of Ireland, ³Nutrition, Metabolism and Genomics Group, Division of Human Nutrition, Wageningen University, The Netherlands and ⁴Bioinformatics and Genomics Unit, Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy

Conjugated linoleic acid (CLA) is predominantly found in milk and meat of ruminant animals as a mixture of CLA isomers. We have previously shown that the *trans*-10, *cis*-12 CLA isomer has profound genome-wide effects on gene expression in Caco-2 cells, whereas the *cis*-9, *trans*-11 isomer (the most-abundant natural isomer) has no effect¹. In that study we explored gene expression patterns of relevance to carcinogenesis and Ca transport¹, and for which functional data exists; however, gene ontology analysis also showed that the process of lipid metabolism was significantly altered by *trans*-10, *cis*-12 CLA. Thus, in the present study we conducted a more in-depth investigation of the effect of *trans*-10, *cis*-12 CLA on gene-expression profiles related to lipid metabolism.

Caco-2 cells were grown for 14 d in media containing 80 µmol linoleic acid, *cis*-9, *trans*-11 CLA isomer or *trans*-10, *cis*-12 CLA isomer¹. RNA was isolated from the cells, labelled and hybridised to the Affymetrix U133 2.0 Plus arrays (Affymetrix, Santa Clara, CA, USA; 54645 sequences). The list of differentially-expressed genes was generated using a false discovery rate <0.05 together with an absolute fold-change threshold of 1.6. Microarray quality control and statistical validation were performed using Bioconductor² and selected genes were verified with quantitative RT-PCR. Differentially-expressed genes were categorized according to gene ontology using EASE³.

The *trans*-10, *cis*-12 CLA isomer induced alterations in the expression of genes involved in a number of lipid metabolism-related processes including: TAG metabolism; cholesterol transfer to HDL; gluconeogenesis; β-oxidation. In particular, a number of genes encoding proteins that are involved in mediating lipid and cholesterol transport in the intestine were found to be either significantly up regulated (fatty acid-binding protein (FABP)-1, 2.04-fold; FABP-6, 2.94-fold; apoA-IV, 6.98-fold) or down regulated (ATP-binding cassette, sub-family A, member 1, 4.63-fold; apoB mRNA editing enzyme, 1.76-fold) in Caco-2 cells treated with *trans*-10, *cis*-12 CLA compared with linoleic acid-treated control cells.

In the previous study the microarray analysis highlighted a number of gene-expression patterns in relation to enhanced Ca transport in Caco-2 cells¹. In the present study there is evidence of potential adverse effects related to intestinal HDL biogenesis and glucose metabolism in Caco-2 cells. These observations supported by evidence in animal and human studies warrant further investigation, including follow-up with functional studies.

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Interaction between dietary fatty acids and genetic variation in the metabolic syndrome. By C. PHILLIPS¹, L. GOUMIDI², S. BERTRAIS³, J. FERGUSON¹, R. McMANUS¹, S. HERCBERG³, D. LAIRON², H.M. ROCHE¹ and R. PLANELLAS², ¹Nutrigenomics Research Group, School of Medicine, Institute of Molecular Medicine, Trinity College Dublin at St James Hospital, Dublin 8, Republic of Ireland, ²Department of Human Nutrition, UMR INSERM 476/INRA 1260-Faculté de Médecine de la Timone, Marseille, France and ³UMR INSERM U557/INRA/CNAM, ISTNA-CNAM, Paris, France

The metabolic syndrome (MS) is a common multi-component disorder resulting from genetic and environmental components. The diverse clinical characteristics of the disease suggest that multiple genes are involved. Dietary fat is an important environmental factor that can modify the risk of the MS. Inter-individual variability in response to dietary modification is determined by genetic factors. However, there are limited data that indicate that an individual's genetic background interacts with their dietary fat exposure, affecting their risk of the MS.

The need for a large gene–nutrient analysis has been addressed by LIPGENE (Diet, genomics and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis), an EU Sixth Framework Programme Integrated Project (www.lipgene.tcd.ie). The aim of the LIPGENE prospective case–control candidate-gene study was to determine the interaction between polymorphisms implicated in the pathogenesis of the MS and biomarkers of habitual dietary fat composition.

The LIPGENE cohort consists of 1754 matched case and control subjects. Plasma fatty acid composition (14:0 to 22:6n-3) was determined as a biomarker of habitual dietary fat intake. Candidate genes were selected because of an *a priori* hypothesis about their aetiological role in the MS. Haplotype-tagged polymorphisms and those identified in association studies were included, resulting in the selection of 768 polymorphisms for 178 genes. Genotyping was conducted by Illumina Inc. (San Diego, CA, USA).

Preliminary single single-nucleotide polymorphism (SNP) analysis (Table) revealed significant differences in allele and/or genotype frequencies of thirteen polymorphisms of genes involved in inflammation, vascular function and fatty acid, glucose and lipid metabolism between cases and controls.

Table. LIPGENE single SNP analysis and OR for the significant associations ($P < 0.01$) with the MS

Gene	Symbol	rs no.	Pathway	OR
Acetyl-CoA carboxylase β	ACACB	rs4766587	Fatty acid metabolism	1.31
Long-chain acyl-CoA synthetase 1	ACSL1	rs9997745	Fatty acid metabolism	0.77
Complement component 3	C3	rs1047286	Inflammation	1.33
		rs2230199		1.31
Lymphotoxin α	LTA	rs915654	Inflammation	1.29
TNF α	TNF α	rs1800629	Inflammation	0.76
Transforming growth factor β 1	TGFB1	rs2241715	Inflammation and vascular function	0.79
ApoB	APOB	rs512535	Lipid metabolism	0.82
HMG-CoA reductase	HMGCR	rs10038095	Lipid metabolism	1.28
Lipoprotein lipase	LPL	rs1059611	Lipid metabolism	1.35
Paraoxanase 1	PON1	rs662	Lipid metabolism	1.25
Upstream stimulatory factor	USF1	rs2073653	Lipid and glucose	1.13
		rs1556259		1.14

HMG-CoA, hydroxymethylglutaryl-CoA.

In conclusion this study has identified a number of genetic variants associated with the MS. Understanding the interaction between these polymorphisms and dietary fatty acids may provide novel insights into the pathogenesis and progression of the disease. Only with a full understanding of the multiple gene-gene, gene-nutrient and gene-nutrient-environment interactions can the molecular basis of the MS be solved in order to reduce the risk and minimise the adverse health effects.

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Oestrogen receptor genotype and response of bone-health indices to dietary phyto-oestrogen intervention in European post-menopausal women. By K.M. SEAMANS¹, S. CUSACK¹ and K.D. CASHMAN^{1,2} on behalf of The PHYTOS Consortium, ¹Department of Food and Nutritional Sciences, and ²Department of Medicine, University College Cork, Cork, Republic of Ireland

Dietary phyto-oestrogens have been proposed to exert a protective effect against post-menopausal bone loss, although the results from intervention studies have been conflicting¹. Dietary phyto-oestrogens have been shown to interact with oestrogen receptor (OR) genotype in relation to cardiovascular health variables². The objective of the present study was to examine baseline associations between OR α and β genotype and bone mineral density (BMD) and markers of bone turnover in post-menopausal European women and, furthermore, to examine the effect of OR genotype on the response of these bone health variables to 12-month phyto-oestrogen supplementation.

Baseline data on nutrient intake, serum 25-hydroxyvitamin D, urinary pyridinoline and deoxypyridinoline (markers of bone resorption), plasma bone-specific alkaline phosphatase and amino terminal propeptide of type I collagen (markers of bone formation), as well as BMD of the whole body (WB) and lumbar spine (LS), for 242 post-menopausal women from three centres in Europe (France, Netherlands and Italy) participating in a 12-month phyto-oestrogen intervention trial (the PHYTOS study) were used for the present study. Genomic DNA was isolated from whole blood and subjected to PCR followed by *Xba* I and *Pvu* II (OR α) and *Alu* I (OR β) restriction digests. Multiple regression analysis and two-way ANOVA were used to assess baseline associations between genotypes and bone indices and possible genotype–phyto-oestrogen treatment interactions on the 12-month change in bone variables.

Genotype frequencies of PHYTOS participants

	Genotype	n	%
OR α (<i>Pvu</i> II)	PP	56	23.1
	Pp	111	45.9
	pp	75	31.0
OR α (<i>Xba</i> I)	XX	46	19.0
	Xx	98	40.5
	xx	98	40.5
OR β (<i>Alu</i> I)	GG	98	45.1
	GA	90	41.5
	AA	29	13.4

OR α and OR β genotype frequencies were similar to that reported for Caucasian women. At baseline there was no association between OR α (*Xba* I and *Pvu* II separately, or combined) or OR β genotype and BMD (WB, LS) or markers of bone turnover, after controlling for potential confounding factors. Moreover, there was no interaction between OR α (*Xba* I and *Pvu* II separately, or combined) or OR β genotype and phyto-oestrogen supplementation in relation to 12-month changes in BMD (WB, LS) or markers of bone turnover.

The findings suggest that OR (α or β) genotype did not appear to be a major determinant of bone health indices, or influence the response of bone to phyto-oestrogen intervention in post-menopausal European women.

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Feeding a diet with conjugated linoleic acid (CLA)-enriched beef improves the diabetic phenotype in ob/ob mice. By S. TOOMEY¹, J. McMONAGLE¹, A. MOLONEY² and H.M. ROCHE¹, ¹Nutrigenomics Research Group, School of Medicine, Institute of Molecular Medicine, Trinity College Dublin, Republic of Ireland and ²Teagasc, Grange Research Centre, Dunsany, Co. Meath, Republic of Ireland

The metabolic syndrome defines a clustering of metabolic irregularities, including obesity, insulin resistance and dyslipidaemia, which is associated with a high risk of type 2 diabetes mellitus (T2DM) and CVD. CLA refers to a family of positional and geometric isomers of linoleic acid (18: 2n-6). Animal-feeding studies have shown that synthetic forms of *cis*-9, *trans*-11 CLA (c9,t11-CLA) have protective effects on T2DM and CVD risk factors, reducing cholesterol and TAG concentrations, inhibiting the development of atherosclerosis and improving insulin sensitivity.

To date, all work in this area has focused on synthetic CLA sources. The natural dietary sources of CLA are dairy products and ruminant meats, in which most of the CLA present is the c9,t11-CLA isomer. Thus, the aim of the present study is to investigate the effect of high-c9,t11-CLA beef (produced as a result of greater pasture feeding) on risk factors associated with the metabolic syndrome and to determine its relative efficacy compared with the synthetic form of the fatty acid.

Thirty male ob/ob mice were randomly assigned to one of three dietary treatments as shown in the Table for a 28 d period. Both the beef- and synthetic-CLA diets significantly reduced serum glucose ($P<0.05$), cholesterol ($P<0.05$), TAG ($P<0.05$), and NEFA ($P<0.05$) concentrations compared with the control low-CLA-beef diet. The CLA diets also significantly reduced plasma IL-6 ($P<0.05$) levels and increased plasma adiponectin concentrations ($P<0.05$) compared with the controls.

Table. Whole-body metabolic data

Diet ...	Low-CLA beef (n 7)		High-CLA beef (n 8)		Low-CLA beef and synthetic CLA (n 7)	
	Mean	SE	Mean	SE	Mean	SE
Glucose (mmol/l)	16.63	0.76	14.07*	1.04	15.28*	1.48
Cholesterol (mmol/l)	5.14	0.42	5.83*	0.17	5.86*	0.46
TAG (mmol/l)	1.49	0.06	1.38*	0.05	1.36*	0.04
NEFA (mmol/l)	0.70	0.07	0.36*	0.07	0.45*	0.06
IL-6 (pg/ml)	1626	138	1168*	168	1151*	109
Adiponectin (mg/ml)	7198	635	9961*	226	9212*	706

Mean values were significantly different from those for the control group (low-CLA-beef diet): * $P<0.05$.

The preliminary results of the present study suggest that beef enriched with CLA may have beneficial effects on glucose metabolism, insulin sensitivity and mediators of inflammation, which are all key metabolic markers of T2DM and the metabolic syndrome. Additional analysis is required to determine the molecular basis of these effects.

The effect of dietary source of very-long-chain n-3 PUFA on the EPA and DHA concentrations of edible chicken tissues. By R.A. GIBBS, C. RYMER and D.I. GIVENS, *Nutritional Sciences Research Unit, School of Agriculture Policy and Development, University of Reading, PO Box 237, Earley Gate, Reading RG6 6AR, UK*

The consumption of oil-rich fish in the UK is poor (~27% adults are consumers)¹ and current intakes of very-long-chain (VLC) n-3 PUFA have been shown to be sub-optimal at approximately 244 mg/d for UK adults². With the increasing emphasis on the human health benefits of VLC n-3 PUFA there is a potentially important role for enriched animal-derived foods in increasing population intakes. Poultry meat is a key target for enrichment because of its widespread consumption. Enrichment of chicken meat with VLC n-3 PUFA can be achieved primarily by the addition of fish oil or fish meal to broiler diets; however, the sustainability of using oil-rich fish for this purpose can be questioned.

The main objective of the present study was to compare the effect of different sources of VLC n-3 PUFA in growing broiler diets on the enrichment of white and dark meat. It was hypothesised that marine algae could be a potentially valuable source of DHA, and could yield similar levels of enrichment when compared with a diet supplemented with fish oil. To test this hypothesis 144 Ross 308 mixed-gender chicks were purchased (P.D. Hook Hatcheries Ltd, Bampton, Oxon, UK) and reared on a proprietary chick crumb for 21 d, when the birds were randomly allocated to one of twenty-four cages, six per cage and each cage allocated one of six experimental diets, giving four cages per diet. The diets were either control (CON) containing soyabean oil, fresh fish oil (FFO), encapsulated fish oil (EFO), low algae (ALG1), medium algae (ALG2) and high algae (ALG3). Dietary Unsaturated fatty acid (UFA), EPA and DHA concentrations are shown in the Table below.

Diet	UFA concentration (g/kg feed)	EPA concentration (g/kg feed)	DHA concentration (g/kg feed)
CON	24	0	0
FFO	23	2.5	5
EFO	22	1.25	5
ALG1	30	0	2.5
ALG2	32	0	5
ALG3	39	0	7.5

At 42 d the birds were humanely slaughtered and skinless samples of breast and leg tissue harvested from two birds per cage. The samples were analysed for fatty acid concentrations using GC-MS. Statistical analysis (ANOVA) was performed and a summary of the results is given in the Table below.

Fatty acid	Tissue	Fatty acid concentration (mg/100 g per tissue)						SE	P		
		CON	FFO	EFO	ALG1	ALG2	ALG3		Diet (D)	Tissue (T)	D × T
EPA	White	4.0	30.6	18.4	8.9	6.1	8.8	5.3	<0.001	<0.001	0.001
	Dark	4.0	52.5	57.7	12.2	10.0	33.6				
DHA	White	24.2	129.0	121.5	111.0	146.8	187.1	13.1	<0.001	>0.05	>0.05
	Dark	7.9	119.7	139.2	91.7	93.2	184.1				

Dietary treatment had a significant ($P<0.001$) effect on mean EPA and DHA in white and dark meat, with both EPA and DHA concentrations being higher for the FFO, EFO and ALG diets than those for the CON diet. It should be noted that dietary EPA levels were greater for the FFO and EFO diets than for the ALG diets, and therefore higher concentrations of EPA are reflected in the white and dark meat of the birds from the fish oil diets. In summary, marine algae appear to yield similar levels of enrichment to fish oil when compared with the CON diet, and therefore indicates that marine algae could potentially be used as an alternative to fish to enrich chicken meat with VLC n-3 PUFA.

This work is part of LIPGENE, an EU Sixth Framework Programme.

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Survey of packed lunches in British primary schoolchildren age 8–9 years. By C.E.L. EVANS¹, J.D. THOMAS¹, D.C. GREENWOOD² and J.E. CADE¹, ¹*Nutritional Epidemiology Group and* ²*Biostatistics Unit, Centre for Epidemiology and Biostatistics, University of Leeds, 30–32 Hyde Terrace, Leeds LS2 9LN, UK*

On average 45% of school pupils take a packed lunch from home equating to 5.5×10^9 lunches packed for children each year in the UK. With concerns over the nutritional content of school meals currently high on the political agenda, packed lunches may be viewed by parents as a healthier alternative. However, the 2004 School Lunch Box Survey for the Food Standards Agency¹ found that fruit was contained in just half of lunchboxes belonging to primary schoolchildren in English schools. In addition the majority of lunch boxes contained savoury snacks or confectionary, or both. The School Meals Review Panel² (SMRP)-recommended food-based standards for packed lunches provided by school caterers include the five food groups: starch, protein, dairy, fruit and vegetables, and a number of restrictions on drinks, savoury snacks and desserts. A national survey of packed lunches taken to school was undertaken and the results were compared with the new standards introduced in September 2006.

The survey included year 4 pupils in eighty-nine randomly-selected primary schools in England, Scotland, Wales and Northern Ireland, conducted in June 2006. A total of 1294 children aged 8–9 years had food items in their packed lunch weighed before and after lunch, to assess what they had eaten for lunch. Foods were coded in a specifically-designed system to assess nutrient intakes. The results were then compared with the current food and nutrient standards. The food based results showed that the majority of packed lunches surveyed did not meet the SMRP requirements for packed lunches provided by schools. Only 1% (fourteen) of the children in this survey met all the SMRP 2006 standards for packed lunches.

The nutrient based results are compared with the SMRP standards in the table below.

Nutrient	Mean food provided 2006		SMRP standards		% meeting standards
Energy (kJ)	2604	34% EAR	2328	30% EAR	41
Total protein (g)	18.1	64% RNI	8.5	≥30% RNI	90
Non-milk extrinsic sugars (g)	n/a		16.3	≤11% energy	n/a
Total sugar (g)	53.4	34%	n/a		n/a
Total fat (g)	21.0	30% energy	21.6	≤35% energy	59
Saturated fat (g)	8.21	12% energy	6.8	<11% energy	46
Fibre (g)	2.06	14% RNI	4.5	≥30% RNI	26
Na (mg)	868	43% SACN	600	≤30% of SACN	30
Vitamin A (µg)	179.4*	23% RNI	200	≥40% RNI	19
Vitamin C (mg)	33.9*	100% RNI	12	≥40% RNI	79
Folate (µg)	49.7*	32% RNI	60	≥40% RNI	68
Ca (mg)	228.2*	41% RNI	220	≥40% RNI	53
Fe (mg)	2.43	28% RNI	3.5	≥40% RNI	13
Zn (mg)	2.07	30% RNI	2.8	≥40% RNI	21

n/a, Not applicable; EAR, estimated average requirement; RNI, reference nutrient intake; SACN, Scientific Advisory Committee on Nutrition guideline. * Geometric mean.

More than 50% of children did not meet the recommended levels of saturated fat, fibre, sodium, vitamin A, iron or zinc. Similar results were obtained when amounts consumed in the packed lunch were compared with 30% of the RNI for 7–10 year olds.

In conclusion, children's packed lunches appear to contain foods too high in sodium and too low in vitamins and minerals. Although NMES were not measured there was also an indication that sugar levels were higher than the recommended standards.

Thank you to the Food Standards Agency for commissioning this research project and the National Foundation for Educational Research for the survey work in schools.

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Effects of acute consumption of fruit- and vegetable-juice shots on vasodilation and risk factors for CVD. By T.W. GEORGE, C. NIWAT, M.H. GORDON and J.A. LOVEGROVE, *Department Of Food Biosciences, University Of Reading, Whiteknights, Reading RG6 6AP, Berks., UK*

UK adults are advised to consume five 80 g portions of fruit and vegetables daily for the prevention of chronic diseases. However, despite these recommendations average consumption is less than three portions daily¹. Additionally, it is suggested that fruit juice should only count as one portion regardless of how much is consumed. This recommendation is based on the juicing process removing much of the fibre content of the fruit and vegetables and releasing more of the sugars present inside the cells. However, some of the beneficial effects of fruit and vegetables are accredited to compounds that are also present in many fruit juices², for example, vitamin C³ and polyphenolic compounds⁴. Since 1974 the general consumption of vegetables has remained reasonably constant and there has been a modest increase in fruit consumption⁵. However, the most marked observation has been the constant annual increase in fruit-juice consumption, from 30 g *per capita* per week in 1974 to 284 g *per capita* per week in 1999. Thus, these juice products may be a potential source of beneficial phytochemicals.

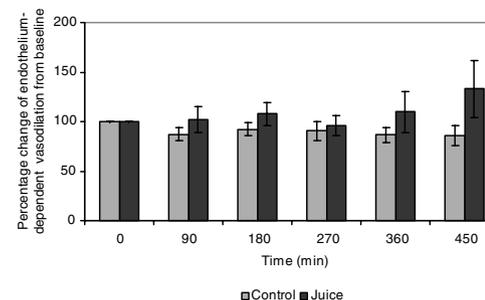
An investigation was undertaken into the effects of acute consumption of 400 ml concentrated-fruit-juice shots on bioavailability, antioxidant status and risk factors for CVD. The effects on vasodilation, assessed by laser Doppler imaging (LDI), are described here. The study was a single-blind randomised controlled cross-over dietary-intervention study with a 4-week washout period. Four 100 ml concentrated-fruit-juice shots or control drink (fruit-flavoured squash matched for sugar composition) were consumed on the morning of the study day by twenty-four volunteers. The volunteers had consumed a low-flavonoid diet for the preceding 5 d. Blood samples were taken at baseline and at twelve intervals during the day. Additional blood samples were taken in the morning and evening of the following day. Urine samples were collected at baseline and at two-hourly intervals for the 8 h of the study day followed by overnight collection and a further collection during the day following juice consumption. Measurement of biochemical variables in the blood and urine were assessed along with a real-time measure of vascular tone using LDI with iontophoresis.

Five LDI measurements were recorded at 90 min intervals following juice consumption. The results for endothelium-dependent vasodilation are shown in the figure opposite. The overall trend was for a protective effect of fruit juice compared with the control drink, but this did not reach statistical significance. Measurement of plasma total nitrate–nitrite showed a significant effect of treatment ($P=0.001$). There was no effect of treatment on total cholesterol, HDL-cholesterol, TAG or NEFA. There was no effect of treatment on plasma glucose, but the subjects showed a lower glycaemic response to the juice.

Overall, the current study provided evidence that acute consumption of 400 ml concentrated-fruit-juice shots increased plasma nitrate–nitrite, with a trend towards increased vasodilation as assessed by LDI.

The authors acknowledge funding from Unilever Bestfoods.

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A novel approach to dietary analysis: exploration of food intake at the meal level. By A.P. HEARTY and M.J. GIBNEY, *Nutrition Unit, School of Agriculture, Food Science and Veterinary Medicine, UCD, Belfield, Dublin 4, Republic of Ireland*

In general, the examination of the human diet is based at the food level, i.e. the examination of the individual foods consumed by the population, despite the obvious fact that foods are eaten together in certain combinations forming meals. Certain statistical approaches such as cluster and factor analysis have been employed in an attempt to explain the prevailing dietary patterns of a population¹, but these again are based at the food level. One reason why dietary analysis is based at the food level is because of the structure of dietary databases, which are in general not amenable to analysis at the meal level. In order to deal with this bottleneck, detailed reconstruction of the North/South Ireland Food Consumption Survey (NSIFCS) database² was required. This survey of 1379 adults aged 18–64 years used a 7 d food diary to estimate food intakes, and the meal type was also recorded. Foods consumed were categorised into sixty-two food groups. Originally, each row in the database represented a single food group consumed, resulting in a total of 222 404 rows. This was then aggregated per meal, resulting in each row representing a single meal consumed. Using the software package Clementine v9.0 (SPSS, Chicago, IL, USA) the data were manipulated in order to group the foods per meal as a comma-separated string variable. This allowed the database to be examined at the ‘meal’ level.

a) Current food consumption database structure for one person on one day

Time (hours)	Meal definition	Food group	Food group description
08.30	Breakfast	08	Breakfast cereal
08.30	Breakfast	12	Low fat milk
08.30	Breakfast	06	Wholemeal bread
08.30	Breakfast	20	Butter
08.30	Breakfast	55	Tea
08.30	Breakfast	12	Low fat milk
11.15	Morning snack	60	Carbonated beverage
11.15	Morning snack	10	Cakes/pastries
13.20	Lunch light meal	05	White bread
13.20	Lunch light meal	15	Cheese
13.20	Lunch light meal	20	Butter
13.20	Lunch light meal	28	Salad vegetables
13.20	Lunch light meal	57	Coffee
19.00	Dinner main meal	23	Potatoes
19.00	Dinner main meal	27	Green vegetables/carrots
19.00	Dinner main meal	38	Beef/lamb/pork
19.00	Dinner main meal	52	Sauces/dressings
19.00	Dinner main meal	59	Water

b) Food consumption database structure at the ‘meal level’ for the same person

Time (hours)	Meal definition	Meal code	Meal description
08.30	Breakfast	(08,12,06, 20,55,12)	Cereal and wholemeal bread
11.15	Morning snack	(60,10)	Confectionary/snack
13.20	Lunch light meal	(05,15,20, 28,57)	Cheese salad sandwich
19.00	Dinner main meal	(23,27,38, 52,59)	Potatoes, vegetables and red meat

A total of 49 671 meals were consumed in the NSIFCS, with an average of 4.5 foods per meal. Based on the sixty-two food groups these meals were further aggregated into 17 928 unique meals. The examination of food combinations at the meal level provides an approach to deal with the complexity and unpredictability of the diet. In order to further explore meal patterns and the applications of this type of analysis to public health nutrition (e.g. its potential role in food-based dietary guidelines) data-mining techniques will be employed (e.g. association rules analysis, neural networks and decision trees).

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A longitudinal study of tracking of adiposity from early adolescence to young adulthood: Northumberland, UK (ASH 17). By A.E. HOSSACK, E. STAMP, E.S. NORTHOVER, J.C. MATHERS and A.J. ADAMSON, *Human Nutrition Research Centre, Newcastle University, Newcastle upon Tyne NE2 4HH, UK*

There is a worldwide increase in the prevalence of overweight and obesity, both of which have devastating consequences on health. Recent studies have indicated that childhood characteristics, and in particular BMI, are predictors of adult health¹.

The aim of this study was to determine the extent to which overweight and obesity track from age 12 years to 17 years.

A group of 424 11 year olds were recruited from seven schools in Northumberland, UK in 2000 (ASH 11)². Of the 2000 sample 192 (16–18 years) consented to take part in 2005 in this follow-up study. Height and weight were measured at both time-points and BMI (kg/m²) was calculated; percent body fat and waist and hip circumference were measured in 2006 only. BMI was classified using Cole cut-offs³ at age 12 years and 17 years. Pearson correlation was used to measure the strength of association between BMI at age 12 years and 17 years.

Of the 192 subjects followed up at age 17 years 29% (25% males (M); 28% females (F)) were overweight or obese at age 12 years. This compares with 30% (27% M; 31% F) of the 424 subjects in the original cohort. Further analysis showed (Table 1; Figure 1) that of the participants who were followed up in this study, 58% (n=32) of those overweight or obese at age 12 yrs remained overweight or obese at age 17 yrs. At 17 years 24% (n=46) (31% M; 20% F) of 192 subjects were overweight or obese (Table 1). The relationship between overweight and obesity at 12 years and 17 years can be modelled using the regression equation:

$$\begin{aligned} \text{Log}_e \text{ BMI (kg/m}^2\text{) at 17 years} \\ &= 1.19 + 0.65 \times \text{log}_e \text{ BMI at 12 years (M),} \\ &= 0.79 + 0.78 \times \text{log}_e \text{ BMI at 12 years (F).} \end{aligned}$$

There was a highly significant association between BMI at 12 years and at age 17 years for both M ($P < 0.001$, $R = 0.619$) and F ($P < 0.001$, $R = 0.738$).

Table 1 Number of overweight and obese at age 12 and 17 years

		BMI class 12 years			
		Normal (n)	Overweight (n)	Obese (n)	Total (n)
BMI class 17 years	Normal	123	22	1	146
	Overweight	12	23	4	39
	Obese	2	3	2	7
Total		137	48	7	192

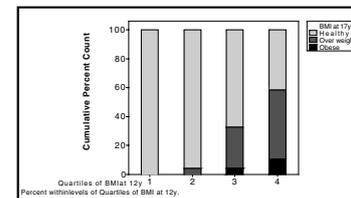


Figure 1 Tracking of BMI at age 17 within quartiles at age 12

These findings show that BMI tracks significantly between ages 12 years and 17 years, and underlines the importance of the prevention of overweight and obesity in early adolescence or childhood.

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Low-income groups' perceived barriers to healthy eating: a qualitative study. By S. KLEEMANN, O. HAUGHEY and K. YOUNGER, *Dublin Institute of Technology, Kevin St, Dublin, Republic of Ireland*

Dietary patterns of low socio-economic status (SES) individuals are important because there is an inverse relationship between dietary quality and prevalence of chronic disease¹. Gaining an understanding of the obstacles encountered by low SES individuals is vital when designing nutrition interventions. Reinforcing positive nutritional behaviours and identifying and attempting to alleviate barriers to healthy eating are crucial to sustaining adequate health².

The present study aimed to use qualitative methodology to identify perceived barriers that exist for women of low SES to achieve a 'healthy eating diet'. The participating women (*n* 23) were living in inner-city Dublin communities. Seventeen females of low SES participated in three focus-group discussions (FGD). A focus group was also conducted with a stakeholder group comprising six women for comparison.

The qualitative research involved using triangulation of data in the form of focus groups and an interviewer-assisted questionnaire. Thematic analysis identified six key factors as barriers to healthy eating. The most prevalent barrier discussed was low motivational levels, followed by lack of self control, limited time and finally taste.

Low motivation levels were the most common barrier cited in the FGD. Some of the women had made a conscious decision to eat unhealthily and suggested that only a major health risk would prompt a change. Self control was a commonly-mentioned barrier in both this study and the Pan EU survey³. The women reported the difficulties that they encountered when trying to give up the food they liked. Their cravings for unhealthy food presented a major obstacle in achieving a healthy diet. Giving into these cravings was reported as being a source of guilt and failure.

Subjects also reported environmental barriers such as family influences and confusion in relation to nutritional messages. All the women showed an interest in improving their own and their families' diets. However, many of the changes that they had tried to institute had been met with resistance from their partners and, most especially, from their children. The difficulties in motivating the rest of the family to adopt a healthy eating lifestyle proved to be a major barrier for most of the women. This highlights the need for support and motivation from others in order to achieve a healthy diet.

Twelve of the seventeen participants in the focus groups felt that they had sufficient nutritional knowledge and understood what a healthy diet entailed. It appears that although the participants were aware of the basic principles of healthy eating, such as eating more fruit and vegetables and decreasing fat intake, the more complex terminology and language that is frequently used in the media leads to some bewilderment. The stakeholder focus group suggested that a means of overcoming this confusion was to use 'simple language' in nutrition education. This suggests that when forming nutrition education programmes the community should be consulted, allowing the information to be tailored appropriately.

For dietary change to be successful motivation levels must be high. Individuals must also feel equipped to deal with feelings of lack of willpower and self control. Thus, emotional support and encouragement are vital. The information derived from the present study should inform the design of nutrition education programmes and campaigns. The results also support the concept that qualitative analysis should be carried out to assess the barriers that exist in a community setting before a nutrition education programme is initiated.

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The prevalence of underweight and probable undernutrition in first-trimester pregnant women. By J.C. ABAYOMI^{1,2}, H. WATKINSON², J. TOPPING¹ and A.F. HACKETT², ¹*Liverpool Women's Hospital, Crown Street, Liverpool L8 7SS, UK* and ²*Liverpool John Moores University, Faculty of Education, Community and Leisure, Barkhill Road, Liverpool L17 6BD, UK*

Underweight and inadequate weight gain in pregnancy is related to health risks for both mother and foetus. Weight gain during the first trimester is thought to have a greater influence on birth weight, than weight changes during the remaining 6 months¹. Adverse perinatal outcomes associated with low maternal BMI and low weight gain are associated with poor growth and development during infancy and childhood, and also with an increased risk of developing disorders in later life such as hypertension and CVD^{2,3}. The aim of the present study was to determine the prevalence of underweight among women booking-in for antenatal care at a Liverpool maternity hospital. A report was compiled consisting of height, weight, age and parity for 7981 women who had booked in between July 2004 and June 2005. BMI was calculated and women were categorised according to BMI. Data for >1000 women (13.4%) had to be disregarded because an appropriate height or weight was not recorded. Average BMI was 25.4 (range 14.3–65.7) kg/m², with 267 women (3.8%) underweight and probably malnourished (BMI <18.5 kg/m²) and a further 743 (10.7%) possibly malnourished (BMI <20.0 kg/m²)⁴. There was also a high prevalence of overweight and obesity (44%).

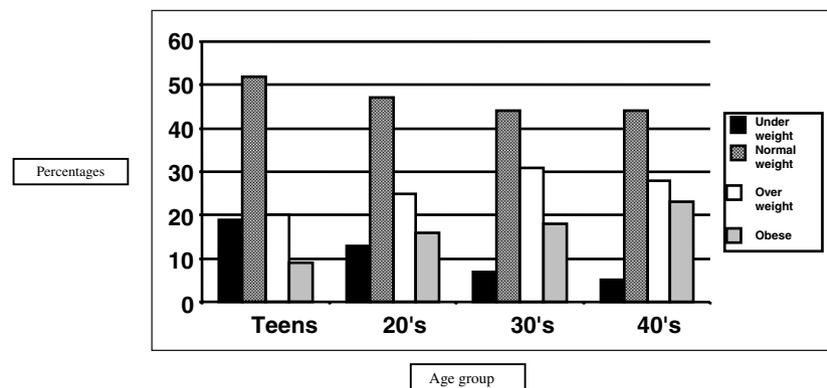


Fig. 1. Prevalence of underweight in different age-groups.

Underweight was significantly associated with younger age and primiparity (*P*<0.001), although some older and multiparous women were also underweight (Fig. 1). None of these women were referred to the dietitian for intervention. Dietary advice during pregnancy may help to improve eating habits and promote optimal weight gain. This approach would not only enhance maternal and foetal health by improving nutritional intake, but might also prevent complications of pregnancy, such as low birth weight, interuterine growth retardation and reduce risks of chronic disease later in life.

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Do women reduce alcohol, tea and coffee consumption, and stop smoking when pregnant? Longitudinal data from the Southampton Women's Survey. By S.E. BORLAND, S.M. ROBINSON, S.R. CROZIER, K.M. GODFREY, H.M. INSKIP and the SWS STUDY GROUP, *MRC Epidemiology Resource Centre, University of Southampton, Southampton SO16 6YD, UK*

Pregnant women are advised to drink $\leq 1-2$ units alcohol no more than one to two times per week, to limit their intake of caffeine and not to smoke¹. Retrospective data on alcohol and smoking before and during pregnancy provide evidence of some reduction in pregnancy², but prospective data on change in alcohol consumption, smoking and caffeine intake are limited.

Using data from two cohort studies, the aims of the present study were twofold: to examine the change in intake of alcohol, tea and coffee, and in smoking when women become pregnant; to compare these factors during pregnancy with data collected 10 years earlier.

The Southampton Women's Survey (SWS) is a longitudinal study of 12 500 non-pregnant women aged 20–34 years living in Southampton, with follow-up during pregnancy and beyond. The data presented were collected at three time-points: pre-pregnancy; early pregnancy; late pregnancy. On each occasion alcohol, tea and coffee intake over the previous 3 months and current smoking status were recorded. There were 1987 pregnancies between 1998–2003 data are presented for 1484 women who had data collected at all time-points. Pregnancy data from SWS were compared with similar data collected on 620 women at the same hospital in 1991–2 (PAH Study)³.

Characteristic	SWS			PAH	
	Pre-pregnant	Early pregnancy	Late pregnancy	Early pregnancy	Late pregnancy
Alcohol (% total)					
≤4 units/week	47	90	95	96	97
21 units/week	45	9	5	4	3
>21 units/week	8	1	0.1	0.2	0
Tea and coffee (no. of times per week; median)	28.0	11.5	14.0	21.0	28.0
Smoking (% total)	27	15	14	26	22
Gestational age (weeks; median)		11.7	34.6	15.3	32.7

Compared with the pre-pregnant period SWS women had halved tea and coffee intake, markedly reduced alcohol intake and half the smokers had given up smoking by early pregnancy. Between early and late pregnancy alcohol consumption and smoking were further reduced whilst tea and coffee intake was slightly increased. SWS participants were consuming less tea and coffee during pregnancy than their counterparts 10 years earlier and rates of smoking in pregnancy were lower. Alcohol intake during early pregnancy was higher in SWS than 10 years earlier, but this may be related to the later gestational age at the time of assessment in the PAH study.

Evidence suggests that most women comply with advice to reduce their intake of caffeine and alcohol, and many women stop smoking when they become pregnant. Encouragingly, the present study suggests that compliance with guidelines is good.

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Fish eating in pregnancy in relation to depression during and after pregnancy. By P. EMMETT¹, C. STEER², J. HIBBELN³, J. DAVIS⁴ and J. GOLDING², ¹*Department of Social Medicine* ²*Department of Community-based Medicine, University of Bristol, Bristol BS8 1TQ, UK,* ³*National Institutes of Health, USA and* ⁴*University of Illinois, USA*

Studies have shown that low levels of *n-3* fatty acids in the blood are associated with depression. The main source of *n-3* fatty acids (DHA) in the Westernised diet is from fish, particularly oily fish, and although the body can manufacture DHA from dietary precursors, sources of preformed DHA appear still to be important.

Subjects were pregnant women taking part in Avon Longitudinal Study of Parents and Children recruited in 1991–2 in a prescribed geographical area of south-west England. These women completed an FFQ that asked about consumption of fifty commonly-consumed foods, food groups and drinks, including three questions about fish eating, covering white fish, oily fish and shellfish¹.

Self-completion questionnaires administered at 18 and 32 weeks of pregnancy and 8 weeks and 8 months post partum contained questions from the Edinburgh postnatal depression score (EDPS)^{2,3}. These could be used to assess the level of depressive symptoms in the woman at these time points and assign them a depression score. The questionnaire also gained information about the woman's educational attainment, age, housing tenure, smoking, parity, life events etc., which may confound any association.

Data were available for up to 9966 women with singleton births surviving to 1 year. *n-3* Fatty acid intake was estimated from frequency of intake of the three fish types. An EPDS>12 indicated the presence of a high level of depressive symptoms. The prevalence rate for high levels of depressive symptoms was 12.6, 14.2, 9.4 and 8.1% at 18 and 32 weeks of pregnancy and 8 weeks and 8 months post partum respectively. Odds ratios (OR) for having high levels of depressive symptoms were assessed by amount of *n-3* fatty acid from fish.

		<i>n-3</i> Fatty acids from fish (>1.5 g/week; OR 1)						<i>P</i> for trend
		None		0.1–0.4 g/week		0.4–1.5 g/week		
		OR	95%CI	OR	95%CI	OR	95%CI	
18 weeks an:	Unadj	1.64	1.34, 2.00	1.61	1.34, 1.94	1.14	0.98, 1.33	<0.0001
(<i>n</i> 9966)	Adj	1.24	1.00, 1.55	1.35	1.10, 1.64	1.03	0.87, 1.22	0.004
32 weeks an:	Unadj	1.97	1.63, 2.38	1.64	1.37, 1.96	1.31	1.13, 1.52	<0.0001
(<i>n</i> 9960)	Adj	1.54	1.25, 1.89	1.37	1.13, 1.66	1.20	1.03, 1.41	<0.0001
8 weeks pp:	Unadj	1.24	0.98, 1.57	1.18	0.95, 1.46	1.07	0.90, 1.27	0.04
(<i>n</i> 9538)	Adj	0.99	0.77, 1.29	1.02	0.81, 1.30	1.00	0.83, 1.20	0.97
8 months pp:	Unadj	1.64	1.28, 2.10	1.25	0.98, 1.58	1.10	0.91, 1.33	<0.0001
(<i>n</i> 9120)	Adj	1.43	1.09, 1.87	1.12	0.87, 1.44	1.07	0.87, 1.31	0.013

An, antenatal; pp, post partum.

Greater levels of depressive symptoms at 18 & 32 weeks of pregnancy and at 8 months post partum were associated with consuming no *n-3* fatty acids from fish even after adjustment for twelve social and economic factors ($P=0.003$ for interaction).

These results are in line with the recommendation to eat fish particularly oily fish during pregnancy.

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Folate status in pregnant women in relation to compliance with current folic acid recommendations for the prevention of neural-tube defects. By B. McNULTY¹, K. PENTIEVA¹, B. MARSHALL², M. WARD¹, A.M. MOLLOY³, J.M. SCOTT³ and H McNULTY¹, ¹Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine BT52 1SA, UK, ²Maternity Unit, Causeway Hospital, Coleraine BT52 1HS, UK and ³Department of Immunology & Biochemistry, Trinity College, Dublin, Republic of Ireland

Folate has a well-established protective role against first occurrence and recurrence of neural-tube defects (NTD), resulting in clear recommendations to take folic acid supplements from before conception up to the 12th gestational week¹. These recommendations have now been in place for 15 years in the UK and elsewhere; however, over this period there has been no detectable decline in NTD across thirteen different European countries including the UK². In contrast, NTD rates have declined considerably in the USA and Canada where mandatory folic acid fortification has been in place since 1998. In the present study the aim was to investigate folic acid supplement usage in pregnant women and to explore the relationship between the timing of folic acid supplementation and folate status at the end of the first trimester. The participants were 154 pregnant women aged 18–35 years with singleton pregnancies who reported they had taken folic acid supplements in the first trimester of pregnancy and had no known current pregnancy complications. The women were recruited at approximately the 14th gestational week, and a non-fasting blood sample was collected and a screening questionnaire was administered to determine folic acid usage during the first trimester of pregnancy.

	Preconception (n 38)		0–6 Gestational weeks (n 62)		>6 Gestational weeks (n 54)		P*
	Mean	SD	Mean	SD	Mean	SD	
Duration of folic acid usage (weeks)	23.4 ^a	7.9	9.1 ^b	2.1	6.3 ^c	1.9	0.000
Red Cell folate (nmol/l)	1412 ^a	394	1033 ^b	375	989 ^b	419	0.000
Homocysteine (μmol/l)	5.6	1.7	5.9	2.2	6.4	2.7	0.197

^{a,b,c} Means in rows with different superscript letters were significantly different. * Values compared using ANOVA with Bonferroni *post hoc* following log transformation of data for normalisation purposes.

The Table shows that red cell folate was significantly lower at the end of the first trimester in those women who commenced folic acid after conception compared with women who commenced folic acid before conception. Red cell folate status was found to be significantly correlated with the total number of weeks of reported folic acid usage (r 0.328; $P=0.001$; data not shown). Of the 154 participants recruited on the basis that they were taking folic acid in the first trimester, only 25% had actually followed the current recommendations correctly, i.e. commenced it preconceptionally. In conclusion, the present study shows that folic acid recommendations to prevent NTD are not being followed correctly in 75% of pregnant women who report taking folic acid supplements. The folate status of such women is unlikely to have offered optimal protection against NTD at the critical period when the neural tube is closing, given that red cell folate levels reflect status over the previous 3–4 month period. These findings support the need for mandatory fortification, as the current practice is not meeting the recommendations for the prevention of NTD even in women who are aware of folic acid.

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Homocysteine and related B-vitamin concentration in pregnant women recruited as part of the Seychelles Child Development Study. By J.M.W. WALLACE¹, M.P. BONHAM¹, E.M. DUFFY¹, P.J. ROBSON¹, M. WARD¹, H. McNULTY¹, P.W. DAVIDSON², G.J. MYERS², T.W. CLARKSON², C.F. SHAMLAYE³, A.M. MOLLOY⁴, J.M. SCOTT⁴ and J.J. STRAIN¹, ¹Northern Ireland Centre for Food and Health, University of Ulster, Coleraine BT52 1SA, UK, ²University of Rochester School of Medicine and Dentistry, USA, ³Ministry of Health, Republic of Seychelles and ⁴Department of Biochemistry, Trinity College Dublin, Republic of Ireland

Pregnancy is associated with a lower homocysteine concentration (tHcy) and with changes in related B-vitamins¹; most likely as a result of hormonal factors and fetal requirements. The majority of studies to date have been undertaken in populations with Western dietary patterns, where fortification of foods is common. The aim of the present study was to assess tHcy, and associated folate and vitamin B₁₂ status, in pregnant women recruited as part of the Seychelles Child Development Study.

Pregnant women (n 226), recruited through antenatal clinics, provided blood samples at enrolment (week 13 (SD 4) of gestation), week 28 of gestation and at delivery. Cord blood was obtained from a subset of participants (n 135). Serum tHcy and related B-vitamin concentrations were assessed.

	Enrolment		Week 28		Delivery		Cord blood	
	Median	5th, 95th percentile	Median	5th, 95th percentile	Median	5th, 95th percentile	Median	5th, 95th percentile
		tHcy (μmol/l)		5.83 ^a		4.0, 10.4		6.84 ^b
Folate (nmol/l)†	14.1 ^a	5.51, 38.3	9.01 ^b	2.8, 30.7	40.2*	12, 88.3	435*	174, 1139
Vitamin B ₁₂ (pmol/l)†	250 ^a	128, 493	221 ^b	107, 507	435*	174, 1139		

Data were analysed by repeated measures analysis of variance and *post-hoc* analyses with Bonferroni's correction. ^{a,b,c} Values within rows with different superscript letters were significantly different over time ($P<0.05$). Values were significantly different from those at delivery: * $P\leq 0.01$. † Folate and vitamin B₁₂ analysis was not undertaken at enrolment.

Maternal tHcy was lower in pregnancy than at delivery, while folate and vitamin B₁₂ status declined towards delivery. Maternal folate status was lower than has been observed in Western countries^{1,2} such that at delivery 30% of the women had a folate concentration <6.1 nmol/l, the accepted cut-off for deficiency. Despite the low maternal status, cord blood folate was similar to that reported in Western populations². Clearly, fetal requirements for these nutrients are paramount, such that fetal status is maintained, even in the face of low maternal status. However, given the high prevalence of low folate status in mothers, advice to pregnant women in the Seychelles on consumption of folic acid is needed to improve maternal status.

This study was supported by the US National Institute of Environmental Health Sciences, National Institutes of Health.

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Effects of the chronic consumption of fruit- and vegetable-juice shots on oxidative stability of plasma. By S. WARONPHAN, E. PATERSON, T.W. GEORGE, M.H. GORDON and J.A. LOVEGROVE, *Hugh Sinclair Unit of Human Nutrition, School of Chemistry, Food Biosciences and Pharmacy, The University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, Berks., UK*

CVD is a major contributor to mortality and morbidity from degenerative disease, with increases in death recorded each year. Epidemiological studies have shown that a high consumption of fresh fruits and vegetables is associated with a reduction in the level of oxidative stress and CVD risk. Chu and Liu¹ have reported that a daily intake of one serving of fruit and vegetables decreases the risk of CVD by 4%. Since 1989 the US National Academy of Sciences has recommended consumption of at least five portions (400 g) of fruit and vegetables daily, similar recommendations have also been made by the WHO^{2,3}. However, only 13% of males and 15% of females in the UK and 17% of 15 000 Americans surveyed reach this level of intake⁴. Oxidation of LDL has been recognised as an early stage in the development of atherosclerosis, which leads to CVD. An increasing number of studies of the antioxidant effect of phytochemicals in fruits and vegetables, including the retardation of the susceptibility of LDL to oxidation both *in vitro* and *ex vivo*, have been reported⁵.

A dietary intervention study was conducted to investigate the effects of 5 portions of fruit and vegetables in the form of liquid juice shots on antioxidant status. The effects of consumption of concentrated juice shots for a 6-week period on bioavailability, antioxidant status and risk factors for CVD were investigated. The study was a single-blind randomised controlled cross-over dietary intervention study involving two 6-week intervention periods with juice shots or control (fruit-flavoured squash), with an 8-week washout period. Thirty-nine volunteers (fifteen males, twenty-four females) with an age range of 30–70 years participated in the study. Fasted blood samples and morning spot urine samples were collected before and after each intervention period and biochemical variables were assessed. The juice shots were found to contain the antioxidant components summarised in the table below.

Antioxidant components (mL juice shots)	Vie shot strawberry	Vie shot orange
Ascorbic acid (mg/100mL)	35.96 (sd 1.35)	48.49 (sd 1.97)
Total phenolics (mg gallic acid equivalents (GAE)/mL)	1.92 (sd 0.12)	1.33 (sd 0.05)
Anthocyanins (µg cyanidin-3-O-glucoside/mL)	142.17 (sd 9.84)	43.99 (sd 5.82)
ORAC (oxygen radical absorbance capacity) (µm Trolox equivalent/mL)	67.02 (sd 3.90)	51.02 (sd 7.17)
LDL oxidation (% increase in lag phase time/100 ng juice shots)	49.80 (sd 7.19)	38.77 (sd 7.89)

Plasma oxidative stability was assessed by the susceptibility of LDL to oxidation *ex vivo* and by the ORAC, Trolox equivalent antioxidant capacity (TEAC) and ferric-reducing antioxidant potential (FRAP) assays, which showed no significant effect of treatment on oxidative status. Additionally, there were no correlations between oxidative stability assays; ORAC, TEAC, FRAP and LDL oxidation. However, incubation of the juice shots extract directly with isolated LDL caused a significant increase in the lag-phase time relative to the control ($P=0.001$). Concentrations of extract at 0.5 µm-GAE caused an increase in the percentage change of lag-phase time relative to the control as shown in the table.

In conclusion, there were no effects from consumption of fruit- and vegetable-juice shots on the susceptibility of LDL to oxidation or on oxidative stability as assessed by the ORAC, TEAC and FRAP assays. Bioactive compounds may not have accumulated at sufficient levels in plasma during the intervention period or the metabolites may have reduced antioxidant activity.

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Effects of breakfast on physiological and psychological responses to foods eaten later in the day.

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In a previous study in non-obese women the reported energy intake (EI) was significantly lower during the 2-week period when they ate breakfast, while during the 2 weeks when they omitted breakfast there was a rise in fasting total cholesterol and LDL-cholesterol and postprandial insulin resistance¹.

Several factors have been suggested to explain the observed association between eating breakfast and EI, including the enhanced satiety eating breakfast may produce, resulting in reduced subsequent EI, or the effects that eating or omitting breakfast may have on the physiological and psychological responses to foods eaten later in the day.

The purpose of the present study was to investigate whether eating or omitting breakfast affected subsequent EI and metabolic responses to foods eaten later in the day.

Ten normal-weight (BMI 23.4 (sd 1.9) kg/m²) non-smoking males (mean age 25.8 (sd 8.0) years) who were self-reported regular breakfast eaters were recruited to take part in a randomised cross-over trial. Subjects were assigned to receive breakfast (Rice Krispies (Kelloggs, Warrington, UK) and semi-skimmed milk equivalent to 10% individual daily energy requirement) at approximately 08.00 hours or to miss breakfast (OB) in a random order after an overnight fast. A standard 1045 kJ (250 kcal) liquid preload containing 21, 39 and 41% total energy from protein, carbohydrate and fat respectively was served to subjects at approximately 11.00 hours. Blood samples were taken at baseline (fasting), before consuming the preload and at 15 min intervals for 90 min. At approximately 12.30 hours the subjects were provided with a pasta-based test meal in small portions in excess of the anticipated requirement and instructed to eat as much as they wished until they felt comfortably full. Visual analogue scales of mood and appetite were completed at baseline, before and after the preload and lunch.

EI at the *ad libitum* test meal was significantly lower in the breakfast-eating condition compared with the OB condition (mean intake 5250 kJ (1256 kcal) v. 6149 kJ (1471 kcal); $P<0.01$).

Plasma glucose response (incremental area under curve) to the standard liquid preload was significantly greater in the OB condition ($P<0.05$). Subjects reported feeling less full, greater hunger and desire to eat before consuming the preload and, although consuming the preload increased fullness and reduced hunger and desire to eat, differences were still evident between conditions. However, observed differences had become non-significant by the time the test meal was served.

These data suggest that subjects who regularly eat breakfast compensate when they miss breakfast by consuming more at an *ad libitum* lunchtime test meal than when they have consumed breakfast, despite being provided with a mid-morning energy-containing liquid preload in both conditions.

Missing breakfast appears to produce a greater glucose response to the same standard preload than consuming breakfast.

Greater hunger and reduced fullness and desire-to-eat ratings were observed in the OB condition but differences were not significant between conditions by the time the test meal was served.

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Colon-available raspberry extract exhibit anti-cancer effects on *in vitro* models of colon cancer. By E.M. COATES¹, G. POPA¹, C.I.R. GILL¹, M. McCANN¹, G.J. McDUGALL², D. STEWART² and I. ROWLAND¹, ¹*Northern Ireland Centre for Food and Health, University of Ulster, Coleraine BT52 1SA, UK and* ²*Scottish Crop Research Institute Quality, Health and Nutrition Programme, Mylnfield, Invergowrie, Dundee DD2 5DA, UK*

Epidemiological studies consistently show a strong association between consumption of fruit and vegetables and reduced risk of human pathologies such as CVD and cancer¹. Cancers of the digestive tract are amongst those most susceptible to diet-related modifications, particularly colo-rectal cancer (CRC). The majority of sporadic cases of CRC (about 75%) are directly influenced by diet², and dietary modification may be a feasible strategy to reduce CRC risk³. This potential protective effect of dietary fruit and vegetables may be a result of the biological activities of dietary fibre, vitamins, minerals and/or phytochemicals⁴. The chemopreventive nature of the latter group has been attributed in part to their antioxidant components⁴. Berries, especially raspberries, are a rich source of these antioxidant components, including polyphenols, anthocyanins and ellagitannins⁴. Several studies using berries and berry extracts have shown reduced levels of DNA damage and proliferation in immortalised human colon-cancer cells^{5–8}. However, these extracts may not have a realistic phytochemical profile as some of the antioxidant components may be sensitive to digestion, and it is not known whether there is a synergistic interaction between the various components. The aims of the present study were to examine the effects of a ‘colon-available’ raspberry extract (CARE) on a series of events that are biologically relevant to CRC.

A CARE was prepared by subjecting raspberry extract to a digestion procedure that mimicked the physiochemical conditions of the upper gastrointestinal tract. The resulting CARE, consisting of phytochemicals stable enough to undergo the digestive process, was assessed for anti-cancer properties in a series of *in vitro* systems that model the important stages of colon carcinogenesis, initiation, promotion and invasion.

The phytochemical composition of CARE was monitored using LC–MS. The CARE was reduced in anthocyanins and ellagitannins compared with the original raspberry juice but enriched in other polyphenols and polyphenol breakdown products that were more stable to gastrointestinal digestion. H₂O₂-induced DNA damage was measured using the comet assay, and the protective effect of a range of CARE concentrations (0–50 µg/ml GAE) recorded. Cell proliferation was recorded by monitoring the numbers of cells in different phases of the cell cycle, and evaluating the effect of different CARE concentrations on the different phases. Barrier function was assessed by recording the trans-epithelial resistance of CaCo-2 cell monolayers at various concentrations of CARE. Finally, invasion was recorded using the matrigel invasion assay and the potential to prevent migration of invasive cancer cells was assessed at different concentrations of CARE.

Initiation: CARE caused significant protective effects against DNA damage induced by H₂O₂ in HT29 colon-cancer cells. Promotion: CARE significantly decreased the population of HT29 cells in the G₁ phase of the cell cycle, effectively reducing the number of cells completing the cell cycle. However, CARE had no effect on epithelial integrity (barrier function). Invasion: CARE caused significant inhibition of HT115 colon-cancer cell invasion.

A considerable weight of evidence has been gathered that suggests that consumption of fruit and vegetables is advantageous to health and may help to prevent chronic diseases such as cancer¹. The data obtained in the *in vitro* study supports this view and provides insight into the possible stages at which raspberry phytochemicals may act to halt the progression of cancer. It has been shown that raspberry phytochemicals likely to reach the colon can inhibit several important stages in CRC development, i.e. initiation, promotion and invasiveness, when applied *in vitro*. Further insights into the anti-cancer effects of CARE may require the use of a suitable animal model.

Comparison of dietary assessment methods within a population suffering from gastro-oesophageal reflux disease. By M.L. HAWKINS¹, G.J. DAVIES¹, M.F. CHAPLIN¹, J.F. DILLON², J.P. COTTON² and P.W. DETTMAR³, ¹*Nutrition Research Centre, London South Bank University, London SE1 0AA, UK,* ²*Ninewells Hospital & Medical School, Dundee DD1 9SY, UK and* ³*Technostics, The Deep Business Centre, Kingston-upon-Hull, East Yorkshire HU1 4BG, UK*

Dietary records have long been identified as being a superior method of dietary assessment compared with FFQ^{1,2}.

The aim of the present study was to test the validity of data collected using FFQ and food record diaries within a patient population with gastro-oesophageal reflux disease. Eighty-three Caucasians (thirty-nine males and forty-four females) were recruited from Tayside in Scotland from the Gastroenterology Clinic at Ninewells Hospital, Dundee. Patients completed a FFQ recalling their habitual eating habits over the previous 12 months and a 7 d estimated-diet record. The main findings are summarised in the Table.

Nutrient	FFQ (mean; /d)	7 d dietary record (mean; /d)	P value
Protein (g)	94.4	76.2	<0.01
Carbohydrate (g)	293	238	<0.01
NSP (g)	17.8	13.1	<0.01
Energy (kJ)	9167	7491	<0.01
Cholesterol (mg)	292	238	<0.01
Potassium (mg)	3776	3152	<0.01
Calcium (mg)	1164	928	<0.01
Magnesium (mg)	334	279	<0.01
Phosphorus (mg)	1565	1296	<0.01
Iron (mg)	14.8	11.9	<0.01
Selenium (ug)	58	45	<0.01
Iodine (ug)	232	164	<0.01
Retinol (ug)	646	457	<0.01
Vitamin A (ug)	1117	840	<0.01
Thiamin (mg)	1.9	1.6	<0.01
Riboflavin (mg)	2.6	1.9	<0.01
Niacin (mg)	24.9	20.4	<0.01
Vitamin B ₆ (mg)	3	2	<0.01
Vitamin B ₁₂ (mg)	8.6	4.8	<0.01
Folate (µg)	349	284	<0.01

The findings show that the use of FFQ significantly overestimated intakes of specific nutrients compared with an estimated 7 d diet record, which is in accord with previous studies³. This raises questions about the use of FFQ as a research tool in estimating nutrient intakes in epidemiological studies.

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The relationship between plasma antioxidants and respiratory health in young adulthood: The Northern Ireland Young Hearts Project. By C.E. NEVILLE¹, J.V. WOODSIDE¹, L.J. MURRAY², G.W. CRAN², C.A.G. BOREHAM³, P. McCARRON² and I.S. YOUNG¹, ¹Nutrition and Metabolism Research Group, Centre for Clinical and Population Sciences, Queen's University Belfast, Belfast BT12 6BJ, UK, ²Epidemiology and Public Health Research Group, Queen's University Belfast, Belfast BT12 6BJ, UK and ³UCD Institute for Sport and Health, Dublin, Republic of Ireland

There is growing evidence to suggest that antioxidants contribute to respiratory health¹. Furthermore, high intakes of fruit and vegetable have been found to have positive effects on lung function in older age-groups². Few population-based studies have examined the relationship between plasma antioxidant levels and respiratory health at a younger age. The aim of the present study was to assess whether plasma antioxidant levels are related to lung function at young adulthood and to examine the association between plasma antioxidants and fruit and vegetable intake.

The current study included 216 males and 165 females aged 20–25 years from Northern Ireland. Habitual dietary intake was assessed using the diet-history method. Anthropometric measurements were taken and lifestyle factors were assessed by questionnaire. Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) were measured by spirometry (Micro Medical Ltd, Rochester, Kent, UK). A fasting blood sample was obtained and stored at –80°C. Plasma samples were analysed for retinol, α - and γ -tocopherol and the carotenoids including lutein, zeaxanthin, α - and β -carotene, β -cryptoxanthin and lycopene, using HPLC³. Multivariable linear regression analyses were carried out with adjustment for age, BMI, physical activity, smoking status, pack years of smoking, alcohol intake, education and total daily energy intake. Retinol, lutein, zeaxanthin and β -cryptoxanthin were significant independent predictors of both FVC and FEV1 in males. No other antioxidants were significantly associated with FVC or FEV1 in males. In females no independent relationships were observed between plasma antioxidant levels and lung function. A further multivariable regression analysis found that in females, plasma β -cryptoxanthin and α -carotene were positive predictors of fruit intake while zeaxanthin was a negative predictor of fruit intake. In females, plasma retinol and lutein were positively associated with vegetable intake while α -tocopherol, β -cryptoxanthin and lycopene were negatively associated with vegetable intake. In contrast to α -tocopherol, plasma β -cryptoxanthin showed a positive independent association with fruit intake in males. No significant associations were evident between plasma antioxidants and vegetable intake in males.

Variables ($\mu\text{mol/l}$)	Males (n 216)				Females (n 165)			
	FVC		FEV1		FVC		FEV1	
	B	SE	B	SE	B	SE	B	SE
Retinol	303.09*	133.00	233.33*	107.31	53.16	92.51	-1.478	70.51
γ -Tocopherol	-14.13	83.24	-47.23	67.015	6.50	70.58	-65.17	53.37
α -Tocopherol	3.423	10.58	3.522	8.525	5.94	7.69	0.831	5.858
Lutein	1865.92*	883.14	1481.47*	712.01	437.19	575.67	274.57	437.67
Zeaxanthin	6062.13*	2329.82	4830.14*	1878.47	1713.63	1834.24	984.45	1395.37
β -Cryptoxanthin	1789.60*	725.22	993.65	589.03	102.25	364.13	106.90	276.61
α -Carotene	1308.45	861.21	542.41	696.96	909.53	714.40	392.29	544.74
β -Carotene	253.76	214.64	112.19	173.40	277.48	164.89	65.32	126.32
Lycopene	104.73	114.76	57.40	92.59	74.06	96.70	9.17	73.77

B, unstandardised regression coefficients. * $P < 0.05$.

The present study clearly suggests that specific plasma antioxidants are positive contributors to respiratory health and that this effect may be exerted through both fruit and vegetable intake. However, further research is warranted in order to fully elucidate the relationship between plasma antioxidants and respiratory health.

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Investigation of dietary factors and faecal water biomarkers in relation to colorectal cancer. By J. PEARSON¹, C. GILL¹, A. McGLYNN¹ and I. ROWLAND², ¹Northern Ireland Centre for Food and Health, Centre for Molecular Biosciences, University of Ulster, Cromore Road, Coleraine, Co. Londonderry, BT52 1SA. ²Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, University of Reading, Reading, UK, RG6 6AH

Colorectal cancer (CRC) is a common form of cancer that can occur sporadically or be hereditary. CRC development is a multi-step process of mutation accumulation. CRC can be affected by various risk factors introduced through lifestyle choices. A major risk factor for CRC is diet as it can affect cell proliferation rates, apoptosis rates and the effect of carcinogens on the initiation and promotion phases of cancer development. A diet that is high in animal fats and red meats and low in fruit, fibre and vegetables may increase the risk of CRC development^{1,2}. The converse of this is also true. The diet is important as it can introduce many different compounds into the colon that may alter the risk of CRC development, such effects may be modulated through the activity of the faecal water.

The risk of CRC development can be assessed through various biomarkers, including faecal water genotoxicity³. Faecal water is the aqueous phase of faeces and is a proposed biomarker of risk for CRC development. Many mutagens can be found in faecal water such as bile acids, fatty acids, *N*-nitroso compounds, fecapentanes and heterocyclic aromatic amines. The characteristics and properties of faecal samples can also be used as biomarkers of colorectal cancer. It is thought that a higher faecal weight will decrease toxicity due to the dilution of potential carcinogens⁴. Faecal pH is also a risk factor for CRC with a higher pH thought to increase the risk of cancer development⁵.

An observational study is being carried out to determine if these faecal water biomarkers are affected by any changes that may occur in the diet over a 12-month period. Faecal samples and 4-day food diaries were obtained at 12 time-points from healthy, non-smoking volunteers. These will be analysed using different bioassays. The activity of faecal water will be assessed using several cell-based models that may reflect stages of CRC development and progression. These assays include cytotoxicity, genotoxicity, invasion and epithelial resistance assays³. The various assays show if a particular compound or changes in the diet has an effect on the toxicity of faecal water.

Preliminary data from the crude samples shows that faecal weight and pH. Faecal weight varies over the sampling period both inter- and intra-individually. The mean faecal weight, per volunteer, over the year ranges between 17.01 g \pm 6.6 and 163.07 g \pm 80.38. However faecal pH remains relatively constant throughout the study, ranging from 6.14 \pm 0.32 to 8 \pm 0.25. Further data from the bioassays is to follow.

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Evaluation of quinoa (*Chenopodium quinoa* Willd.) in coeliac disease. By V. ZEVALLOS¹, P.J. CICLITIRA¹, T. SULIGOJ¹, L.I. HERENCIA² and H.J. ELLIS¹, ¹Nutritional Science, King's College London, The Rayne Institute, St Thomas Hospital, Lambeth Road, London SE1 7EH UK and ²Departamento de Producción Vegetal, Universidad Politécnica de Madrid, Ciudad Universitaria 28440, Madrid, Spain

Coeliac disease (CD) is a life-long intolerance to gluten, triggered by the prolamin fractions of wheat, rye, barley and oats in some individuals. This enteropathy affects the small bowel mucosa of genetically-predisposed individuals, causing loss of villous architecture and malabsorption. Its prevalence in Europe is approximately 1 in 100. The only effective treatment is to permanently follow a gluten-free diet (GFD). CD is more frequent in patients with type I diabetes mellitus than in the general population. Patients need to maintain good glycaemic control whilst adhering to a GFD. It is important to increase the availability of appropriate products for this type of diet. Quinoa is an annual Andean pseudocereal of the *Chenopodiaceae* family, which has been used in the human diet for millennia. Quinoa has low glycaemic index¹ and offers great nutritional value and appropriate baking properties. Considering quinoa's distant botanical relationship to wheat, it is unlikely that it can harm patients with CD. However, there are little experimental data. In addition, quinoa contains prolamin fractions that could be potentially toxic to individuals with CD, thus there is reluctance among health professionals to accept this potentially-useful food source as safe for gluten-intolerant individuals.

Four murine monoclonal antibodies to known coeliac toxic gluten proteins and peptides were previously produced, i.e. CDC3 and CDC7 (anti-high molecular weight glutenins)², PN3 (anti A gliadin 31–49) and CDC5/6 (anti α -gliadin 57–75)³. A dot immunobinding assay, using these antibodies was used to detect possible gluten-like contamination of nominally gluten-free quinoa flour. Fifteen quinoa cultivars were ground to flour and prolamin fractions were extracted using 40% (v/v) ethanol. Three commercially-obtained wheat starches of known gluten content (acceptable for a GFD, borderline and unacceptable) were similarly extracted and all samples were spotted onto nitrocellulose paper and exposed separately to the four antibodies, enzyme-linked anti-mouse secondary antibody and chromogen. The intensity of the resulting dots was assessed by eye.

The acceptable wheat starch had no reaction with any of the four antibodies; however, the borderline and unacceptable wheat starches had moderate and strong reactions respectively with all four antibodies. Ten quinoa samples were completely negative, equivalent to a wheat starch acceptable for patients with CD, when tested with antibodies raised against both coeliac toxic gliadin and glutenins. Cultivars Ayacuchana, LP-4B, INIA Pasankalla, Witulla and Rojo Achachino gave borderline or positive results with one or both of the anti-gliadin antibodies. These five cultivars will be tested by quantitative monoclonal antibody ELISA to determine whether the levels of prolamin are above the 20 mg/kg recommended for naturally gluten-free foods⁴.

The present early study suggests that several quinoa cultivars may be safe for patients with CD. Further *in vitro* and *in vivo* studies are required to confirm which cultivars may be safe for individuals who are gluten intolerant.

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Cultural differences in the sources of dietary Ca in post-menopausal Caucasian and South Asian women living in Blackburn, Lancashire, UK. By S.R. MITRA¹, P.C. FOSTER¹, P.A. JUDD², B. ELLAHI³ and N.M. LOWE¹, ¹Department of Biological Sciences, University of Central Lancashire, Preston PR1 2HE, UK, ²Lancashire School of Health and Postgraduate Medicine, University of Central Lancashire, Preston PR1 2HE, UK and ³Department of Biological Sciences, University of Chester, Chester CH1 4BJ, UK

In the UK dairy foods provide the greatest contribution to dietary Ca intake; however, the contribution from other food groups may vary according to ethnic dietary choices. Mojtahedi *et al.*¹, studying Ca intake and source in age- and socio-economic status-matched older black and white women, found that fortified cereals were a more important source of Ca for black women than for white women. There is a paucity of information about ethnic differences in dietary habits and preferences in relation to Ca intake between Caucasians and South Asians. The differences in the food sources of Ca were explored as part of a study examining risk factors for osteoporosis in post-menopausal Caucasian and South Asian women in the UK.

Dietary intake was assessed in seventy-six South Asian women and forty-seven Caucasian women by an interviewer-administered 225-item FFQ and analysed using a food composition database (WinDiets; Univation Ltd, Aberdeen, UK). Twenty-seven of the South Asian women and two Caucasian women were found to be under-reporting energy intake (EI; EI: BMR<1.1) and therefore their data were omitted from dietary analysis. The mean age (years) of the forty-nine South Asian and forty-five Caucasian participants included was 56.3 (SE 0.64) and 60.0 (SE 0.53) respectively. Ca intake (mg/d) of the South Asian women was 772 (SE 35.2), significantly lower than that of the Caucasian women (1090 (SE 60.9); $P<0.0005$).

Food group	Mean daily intake of food groups (g)			Mean daily Ca intake from food groups (mg)			Ca intake as percentage of total Ca intake		
	Caucasian	South Asian	P	Caucasian	South Asian	P	Caucasian	South Asian	P
Milk	280 (21.8)	283 (24.8)	0.94	337 (26.2)	337 (29.5)	0.99	29.9 (2.10)	40.6 (2.59)	0.002
Milk products	72.9 (8.93)	40.9 (8.62)	0.01	104 (13.6)	64.5 (15.4)	0.06	9.10 (1.19)	8.35 (1.69)	0.72
Cheese	17.4 (2.36)	2.06 (0.80)	0.0005	101 (13.1)	7.88 (2.31)	0.0005	8.77 (1.05)	1.09 (0.34)	0.0005
Cereals	287 (17.8)	591 (307)	0.35	241 (17.8)	193 (13.0)	0.03	23.0 (1.65)	26.7 (1.88)	0.13
Green leafy vegetables	33.6 (4.34)	22.5 (2.31)	0.02	14.7 (1.88)	27.0 (3.06)	0.001	1.41 (0.19)	3.70 (0.44)	0.0005
Other vegetables	398 (16.3)	230 (12.3)	0.0005	74.7 (3.81)	49.4 (2.76)	0.0005	7.46 (0.62)	7.04 (0.50)	0.59
Meat, egg and fish	151 (12.8)	138 (15.0)	0.51	41.2 (5.54)	41.2 (8.30)	0.99	3.75 (0.35)	5.22 (0.74)	0.08

Values are means with their standard errors in parentheses. Statistical analysis was by independent sample *t* test.

Intake of cheese for Caucasians was significantly higher than that for the South Asians. Although the total quantity of cereal foods consumed by the South Asians was higher, the Ca from cereals was significantly lower because fewer fortified cereals were eaten. The percentage contribution from cereals to Ca intake in both groups was second only to that from milk. Ca from green leafy vegetables was significantly higher in South Asians than in Caucasians.

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Trends in phytate and fibre (NSP) consumption over seventeen adult years in a British birth cohort. By A. McCARRON¹, C.J. PRYNNE¹, A.M. STEPHEN¹ and M.E.J. WADSWORTH², ¹MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge CB1 9NL, UK and ²MRC National Survey of Health and Development, University College and Royal Free Medical School, London WC1 6BT, UK

Phytate is classified as a naturally-occurring compound that can have a considerable impact on the nutritional properties of foods¹. The major nutritional concern regarding phytate in the diet is its ability to chelate and precipitate minerals, leading to negative effects on mineral absorption from the intestine. It has been shown that the bioavailability of minerals such as Fe, Zn, Ca and Mg can be affected by high intakes of phytate. The major dietary sources of phytate are whole grains, cereals and legumes, which are also the main dietary sources of fibre or NSP. Fibre and phytate are closely linked, as phytate is located in the outer layers of the grain. Thus, an increased intake of fibre is often accompanied by a rise in phytate intake.

The MRC National Survey of Health and Development (1946 British Birth Cohort) has provided information on diet at three time points in adulthood over 17 years: 1982, 1989 and 1999². A total of 1253 subjects completed dietary records of ≥ 3 d at each time point. These records were coded and analysed for phytate and NSP content using the in-house programs based on McCance and Widdowson's *The Composition of Foods*, 4th and 6th editions. Phytate content of foods was added to the database, with some values being obtained from the literature³ while others, such as those for composite dishes, were calculated from the ingredients.

	n	1982		1989		1999	
		Mean	95% CI	Mean	95% CI	Mean	95% CI
Phytate (mg/d): Men	562	670 ^a	630, 700	670 ^a	640, 700	730 ^b	700, 770
Women	691	570 ^a	540, 600	570 ^a	540, 590	660 ^b	640, 690
NSP (g/d): Men	562	13.5 ^a	13.1, 13.9	14.3 ^b	13.8, 14.8	15.5 ^c	15.0, 15.9
Women	691	11 ^a	10.7, 11.3	12.5 ^b	12.1, 12.8	14.3 ^c	13.9, 14.6

^{a,b,c} Mean values with unlike superscript letters were significantly different ($P < 0.05$).

Phytate intakes of both men and women did not change between 1982 and 1989 but rose significantly between 1989 and 1999 ($P < 0.005$). NSP intakes show a clear rising trend from 1982 to 1999, all three time points were significantly different ($P < 0.05$). Intakes of both phytate and NSP were greater in men compared with women in all three years but the rise between 1989 and 1999 was more marked in women. Women aged 53 years and post-menopausal may have increased their fibre intake for health reasons or to counteract problems with constipation.

Investigation of the diets in this cohort² has shown increased consumption of cereal foods such as pasta, rice and wholegrain products while that of potatoes declined. Although total bread consumption fell, a greater proportion of wholemeal bread was consumed. There was also a rise in the consumption of ready-to-eat high-fibre breakfast cereals. This increased consumption of whole grains was mainly responsible for the rise in phytate intake between 1989 and 1999.

The fibre intake of the cohort in 1999 was very close to that of the age-matched subjects of the National Diet and Nutrition Survey⁴. This intake is still lower than the recommended average of 18 g/d⁵. However, these results show that an increase in fibre intake would be accompanied by a less-desirable rise in phytate with its concomitant effect on the bioavailability of minerals.

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Influence of low-glycaemic sweetener isomalt on metabolic variables and vascular risk factors in patients with type 2 diabetes. By I. HOLUB¹, A. GOSTNER¹, S. HESSDOERFER¹, S. THEIS³, G. BENDER², B. WILLINGER², B. ALLOLIO², G. KOZIANOWSKI³ and W. SCHEPPACH¹, ¹University of Wuerzburg, Department of Medicine II, Division of Gastroenterology, Germany, ²University of Wuerzburg, Department of Medicine I, Division of Endocrinology, Germany and ³Suedzucker AG, Obrigheim, Germany

The objective of the current study was to examine the long-term effect of foods with low-glycaemic carbohydrates instead of higher-glycaemic ingredients in patients with type 2 diabetes. Target markers comprised metabolic control and risk variables typical for individuals with diabetes. The low-glycaemic carbohydrate was the polyol isomalt, which was used instead of sucrose and/or starch hydrolysates.

Thirty-one patients with type 2 diabetes mellitus received a diet with foods containing 30 g isomalt/d instead of higher-glycaemic carbohydrates for 12 weeks. They were only allowed metformin and/or thiazolidindiones as oral antidiabetics. Otherwise, the participants maintained their usual diet during the test phase, but were instructed to refrain from additional sweetened foods or diabetic products. Before, after 6 weeks and on completion of the study blood samples were taken and analysed for metabolic and risk markers as well as clinical routine variables.

All thirty-one patients completed the study. The test diet was well accepted and tolerated. After 12 weeks a significant reduction was observed in glycated Hb (HbA1c), fasting blood glucose, insulin, proinsulin, C-peptide, HOMA-IR (measure of insulin resistance), as well as TAG and oxidized LDL as arteriosclerosis risk variables. Routine variables, blood lipids and clotting variables did not show marked changes.

Blood variables (12h postprandial)	baseline		6 weeks isomalt		12 weeks isomalt		P-value (Friedman)	norm value
	Mean	SD	Mean	SD	Mean	SD		
Glucose (mg/dl)	175.9	35.8	153.6	30.5	160.7	47.1	0.004	60–110
HbA1c (%)	7.4	0.7	7.2	0.7	7.1	0.9	<0.001	4.3–6.1
Fructosamine (μmol/l)	291	44	280	44	269	50	<0.001	<285
Insulin (μU/ml)	34.3	16.3	32.2	17.6	24.5	10.6	<0.001	<20
C-Peptide (ng/ml)	4.11	1.59	3.78	1.62	3.15	1.16	<0.001	1.06–3.53
Proinsulin (pmol/l)	39.4	19.8	37.8	23.1	30.1	15.6	0.044	<11
HOMA-IR	14.9	7.3	12.3	7.1	10.1	6.3	<0.001	–
CRP (mg/dl)	0.47	0.39	0.39	0.36	0.45	0.42	n.s.	0–0.5
Ox. LDL (U/l)	66.9	21.3	63.0	17.8	54.5	16.4	<0.001	–
TAG (mg/dl)	211.4	122.8	181.1	102.0	195.8	121.9	0.014	<200
NEFA (mmol/l)	0.8	0.2	0.8	0.1	0.6	0.2	0.032	♂: 0.1–0.45
	0.6	0.2	0.5	0.1	0.5	0.2	n.s.	♂: 0.1–0.60
Uric acid (mg/dl)	6.3	1.6	6.5	2.2	5.5	0.9	0.041	♂: 2.4–5.7
	5.9	1.3	5.5	1.4	5.5	1.3	0.002	♂: 3.4–7.0
Body weight (kg)	97.7	17.6	96.7	18.7	96.4	18.5	0.001	–

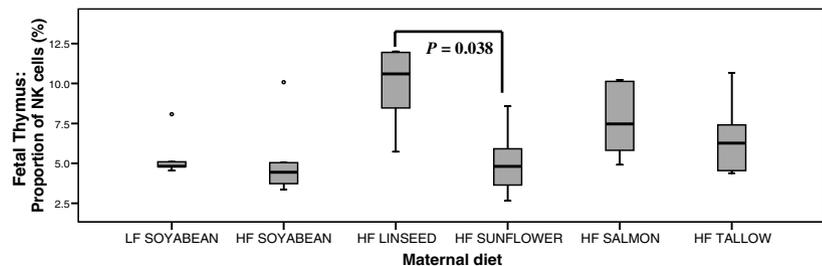
The present study is in agreement with findings of previous controlled studies in human subjects showing beneficial effects of low-glycaemic diets on, for example, HbA1c and fasting glucose in patients with type 2 diabetes. Although effects other than low-glycaemic dietary intervention might have added to the metabolic improvement, these effects could be assumed to be negligible considering that the measured variables usually get worse in general practice, not better as in this study.

n-3 Fatty acids in the maternal diet during rat pregnancy alter expression of cell surface markers on cytotoxic T-cells in both the mother and the fetus and increase the proportion of natural killer cells in the fetal thymus. By C.E. CHILDS, T. ROMIJN, U. ENKE and P.C. CALDER, *Developmental Origins of Health and Disease Research Division and Institute of Human Nutrition, School of Medicine, University of Southampton, Southampton SO16 7PX, UK*

Epidemiological studies have demonstrated associations between the consumption of *n-3* and *n-6* fatty acids during childhood and the risk of asthma. A plausible mechanism for these observations is the role of *n-6* and *n-3* fatty acids in eicosanoid production, which may lead to alterations in immune responses. The present study investigated whether the fatty acid composition of the diet consumed by pregnant rats affects markers of maternal and fetal immune competence.

Female Wistar rats were fed throughout pregnancy on either a low-fat (LF) diet (30 g soyabean oil/kg diet) or one of five higher-fat (HF) diets (130 g fat/kg diet) diets (six rats per diet); fat sources were beef tallow, sunflower oil, soyabean oil, linseed oil or salmon oil. Thus, the HF diets differed according to fatty acid composition, *n-6:n-3* fatty acids and type of *n-3* fatty acid present. At day 20 of gestation maternal and fetal tissues were collected for flow cytometric analysis of cell populations present and relative expression of cell surface markers. Differences between dietary groups were assessed using one-way ANOVA with Bonferroni correction for multiple comparisons.

Maternal and fetal thymus weights were not different according to diet but maternal spleen weight was higher in those animals fed beef tallow or salmon oil ($P=0.002$). There was no effect of diet on the proportions of different cell types present in maternal thymus or spleen or fetal thymus, except natural killer (NK) cells in the latter ($P=0.024$). Maternal diets rich in *n-3* fatty acids were associated with a greater proportion of NK cells (% total lymphocytes) in the fetal thymus (see Figure).



There were significant effects of diet on the level of expression of CD8 on cytotoxic T-cells in the maternal spleen ($P=0.002$) and thymus ($P=0.038$) and in the fetal thymus ($P=0.042$). There were also significant effects of diet on the level of expression of CD3 on cytotoxic T-cells in the maternal spleen ($P=0.005$) and fetal thymus ($P=0.001$) and on the level of expression of CD4 on helper T-cells in the maternal thymus ($P=0.045$). The linseed oil diet tended to result in the highest levels of marker expression.

Altering the pattern of fatty acids consumed during rat pregnancy causes differences in expression of cytotoxic T-cell markers in both the maternal and fetal immune system, and affects the proportion of NK cells within the fetal thymus. Diets rich in *n-3* fatty acids result in higher expression of cytotoxic T-cell markers and a greater proportion of fetal thymic NK cells. It is not clear what the functional effect of these changes is, but both cytotoxic T-cells and NK cells are involved in defence against viral infection.

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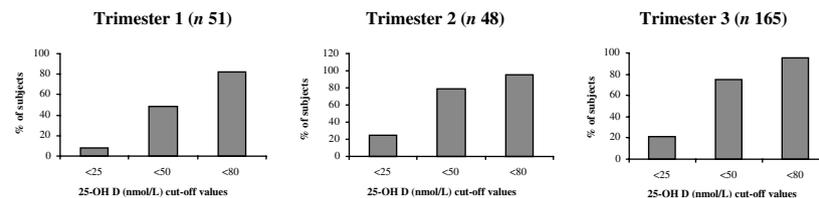
Vitamin D status in women living in Ireland at different stages of pregnancy. By S. MULDOWNNEY¹, K.D. CASHMAN^{1,2}, J.R. HIGGINS³, T. MOORE⁴ and M. KIELY¹, ¹Departments of Food & Nutritional Sciences, ²Medicine, ³Obstetrics & Gynaecology and ⁴Biochemistry, University College Cork, Cork, Republic of Ireland

Low vitamin D status has been reported in the elderly¹, adolescents² and young adults³ in Ireland. Pregnant women are a subgroup of young adults at particular risk of low vitamin D status, because of the additional Ca requirements for foetal development, which leads to an increased physiological requirement for vitamin D to promote Ca absorption. Most studies that have identified widespread vitamin D insufficiency during pregnancy in Western countries have been carried out in immigrant and dark-skinned women who are at particular risk when resident in northern latitudes^{4,5}.

The aim of the present study was to measure serum 25-hydroxyvitamin D (25-OH D) levels in women living in Ireland, at different stages of pregnancy, throughout the year and to compare their 25-OH D status with international cut-off levels. Serum samples were collected from 264 women, aged 18–44 years (of whom >95% were white-skinned), who attended obstetric services in Cork at various stages of pregnancy between March 2004 and July 2006. Laboratory analysis of serum 25-OH D was completed using an ELISA method (OCTEIA[®] 25-OH D; IDS Ltd, Boldon, Tyne and Wear, UK).

Mean serum 25-OH D was 43.1 (sd 22) nmol/l. As expected, 25-OH D levels were significantly lower ($P<0.0001$) in winter (Nov–Mar; 32.1 (sd 14) nmol/l) than in summer (Apr–Oct; 48.2 (sd 23) nmol/l), although the seasonal difference was not as pronounced as expected. Mean serum 25-OH D also differed significantly ($P<0.0001$) according to the pregnancy trimester; mean values were 54.5 (sd 24), 39.4 (sd 18) and 40.6 (sd 21) nmol/l for trimesters 1, 2 and 3 respectively.

Overall, 71% of women were vitamin D insufficient when 50 nmol/l⁶ was used as the cut-off value, which was influenced by pregnancy trimester (see Figure). Over 90% of women in winter and 61% in the summer had 25-OH D levels <50 nmol/l.



Widespread vitamin D insufficiency is apparent among white-skinned pregnant women in Ireland, which has implications for foetal and infant development. Nutritional requirements for vitamin D during pregnancy and lactation require urgent research attention to support public health recommendations.

This study was funded by the Irish Government Department of Agriculture, Food and Rural Development through the Food Institutional Research Measure under the National Development Plan.

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Fatty acid status of a high-fish-consuming population of pregnant women from Seychelles. By M.P. BONHAM¹, E.M. DUFFY¹, J.M.W. WALLACE¹, P.J. ROBSON¹, G.J. MYERS², P.W. DAVIDSON², T.W. CLARKSON², C.F. SHAMLAYE³ and J.J. STRAIN¹, ¹Northern Ireland Centre for Food and Health (NICHE), Department of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, UK, ²University of Rochester School of Medicine and Dentistry, Rochester, USA and ³Ministry of Health, Box 52, Seychelles

In pregnancy there is an increased demand for DHA and arachidonic acid (AA) as a result of increased accretion of maternal, placental and fetal tissue¹, yet concentrations of these fatty acids in pregnancy are reported to decrease as pregnancy progresses². The Seychelles are an archipelago of islands in the Indian Ocean and the population is one of habitual fish consumers, but no data are currently available on alterations in fatty acid status during pregnancy in this population. The aim of the present study was to examine changes in fatty acid status during pregnancy in women reported to consume high amounts of fish, and to describe fatty acid profiles of breast milk in the same women.

Pregnant women (*n* 300) were recruited at their first visit to antenatal clinics. Serum samples (*n* 196) were collected at 28 weeks of gestation and at delivery. Breast-milk samples (*n* 166) were collected at 1 month post partum. All samples were analysed for fatty acids using GC-MS. Diet diaries (4 d) and a food-use questionnaire (*n* 273) were completed at 28 weeks of gestation and estimated mean daily fish and DHA intakes were 75 g and 260 mg respectively.

Fatty acids	28 weeks serum (<i>n</i> =196) median (25, 75 percentile)	Delivery serum (<i>n</i> =196) median (25, 75 percentile)	One month breast milk (<i>n</i> =166) median (25, 75 percentile)
Arachidonic acid (% by weight of total)	2.46 (2.16, 2.90)	2.40 (2.02, 2.83)	0.27 (0.21, 0.37)
Eicosapentaenoic acid (% by weight of total)	0.07 (0.04, 0.11)	0.04 (0.04, 0.08)	0.02 (0.01, 0.02)
Docosahexaenoic acid (% by weight of total)	0.72 ^a (0.56, 0.91)	0.62 ^b (0.48, 0.81)	0.25 (0.17, 0.37)

Results are medians and 25th and 75th percentiles. ^{a,b}Values with unlike superscript letters were significantly different after adjusting for maternal age, parity and BMI ($P < 0.05$).

Serum concentrations of DHA decreased significantly from 28 weeks of gestation to delivery ($P = 0.003$). During pregnancy, therefore, even in the presence of a high fish intake, maternal DHA concentrations decline in the third trimester of pregnancy, even though estimated intakes of DHA in Seychelles, based on the major fish dietary sources alone, are well above current recommendations for pregnancy in the UK, Europe and the USA. The median AA:DHA was 1:1 in breast milk and is consistent with current recommendations for optimal DHA and AA intakes for formula-fed infants.

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Iron status of pregnant women and their neonates participating in the Seychelles Child Development Nutrition Study. By E.M. DUFFY¹, M.P. BONHAM¹, J.M.W. WALLACE¹, P.J. ROBSON¹, G.J. MYERS², P.W. DAVIDSON², T.W. CLARKSON², C.F. SHAMLAYE³ and J.J. STRAIN¹, ¹Northern Ireland Centre for Food and Health (NICHE), Department of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, UK, ²University of Rochester School of Medicine and Dentistry, Rochester, USA and ³Ministry of Health, Box 52, Seychelles

The Seychelles Child Development Nutrition Study (SCDNS) is a large prospective study investigating the relationship between diet and methylmercury (MeHg) toxicity, specifically focusing on cognitive development in children. Nutritional factors in the Seychellois diet, which is rich in ocean fish, may impact on the neurodevelopment of children as well as possibly influencing the effects of prenatal exposure to MeHg. One candidate nutrient that may have such effects is Fe. The Fe status of pregnant women and their newborns participating in the SCDNS were determined in the present study. Ferritin and soluble transferrin receptor (sTfR) concentrations were measured in both maternal and newborn cord blood samples and total body Fe stores (TBI) were calculated¹. A 4-d food diary was administered to establish maternal habitual dietary intake.

n...	Enrolment 270			Delivery 232			Cord blood 140		
	Mean	SD	95% CI	Mean	SD	95% CI	Mean	SD	95% CI
Ferritin (ng/ml)	52.2	49.9	46.1, 58.1	39.2	41.8	33.9, 44.7	119.3***	86.5	105.2, 134.1
sTfR (nmol/l)	22.7 ^a	6.8	21.9, 23.5	36.3 ^b	16.7	34.1, 38.6	54.1***	19.8	50.8, 57.4
TBI (mg/kg)	15.4	3.2	15.0, 15.8	15.0	2.7	14.6, 15.3	6.8***	5.3	5.9, 7.8

^{a,b}Values with unlike superscript letters were significantly different over time ($P < 0.001$). Mean values were significantly different from those at enrolment and delivery: *** $P < 0.001$.

There was no significant change in ferritin or TBI between enrolment and delivery, although there was a significant increase in sTfR ($P < 0.001$), suggesting increased Fe demand. Ferritin and sTfR were significantly higher in cord blood ($P < 0.001$), whereas TBI was significantly lower in cord blood as compared with maternal blood at both enrolment and delivery ($P < 0.001$). Parity had a significant effect on sTfR ($P = 0.003$) and ferritin ($P = 0.035$) at delivery, with increasing parity suggesting increased Fe demands. The average intake of Fe was 9.5 mg/d.

On average, the determinants of Fe status were within normal range in the SCDNS. Fe deficiency as defined ferritin < 12 ng/ml, was present in $\leq 19\%$ of individuals on entering pregnancy; however, at no stage during pregnancy did the mother have negative body Fe stores. At delivery there appeared to be an increased Fe requirement with increasing parity. Approximately 10% of cord TBI values were < 0 mg/kg, suggesting Fe deficiency in these infants.

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Associations between bone turnover and inflammatory status in young overweight adults. By A. LUCEY¹, G. PASCHOS¹, K.D. CASHMAN^{1,2}, J.A. MARTÍNEZ³, I. THORSODDITIR⁴ and M. KIELY¹, ¹Departments of Food and Nutritional Sciences and ²Medicine, University College Cork, Republic of Ireland, ³Department of Physiology and Nutrition, University of Navarra, Spain and ⁴Unit for Nutrition Research, Landspítali University Hospital, Iceland

Biomarkers of systemic inflammation such as serum IL-6 and C-reactive protein (CRP) are relatively low in individuals with high intakes of *n*-3 fatty acids. They are elevated in obesity and can be reduced by weight loss. Weight loss had been shown to induce bone resorption¹. IL-6 is implicated in the pathophysiology of osteoporosis² and associations between high-sensitivity CRP (hsCRP), bone turnover and fracture have been observed in 50–80 year olds³.

The present study has examined associations between bone turnover markers and IL-6 and hsCRP before and after an 8-week dietary intervention that included fish or fish oil as part of an energy-restricted diet (–30%) in 20–40-year-old adults (BMI 27.5–32.5 kg/m²). Participants (*n* 276) were randomised to one of four groups: placebo (sunflower oil capsules; 3 g/d); cod (3 × 150 g/week); salmon (3 × 150 g/week); fish oil capsules (3 g/d). Serum levels of IL-6, hsCRP and the biomarkers of bone formation (osteocalcin (OC) and bone-specific alkaline phosphatase (BAP)) and bone resorption (crosslaps (CTx) and urinary N-telopeptide of type I collagen (NTx)) were measured at baseline and end point.

After adjusting for age, gender, BMI and smoking status, baseline concentrations of hsCRP but not IL-6 were negatively associated with OC, BAP CTx and NTx (Table). Post-intervention, mean weight loss was 5.8 (range 0–15) %. Adjusting for country, gender and weight loss (kg), levels of OC, IL-6 and hsCRP decreased (*P*<0.05) while levels of CTx and NTx increased (*P*<0.05) from baseline to end point (repeated measures analysis). Weight loss influenced the changes in bone turnover and systemic inflammation, while none of the dietary treatments influenced changes in these biomarkers. Changes in hsCRP during the study were not associated with changes observed for the bone turnover markers, although a modest association was observed between the decrease in hsCRP and the increase in CTx (β 0.173 (SE 0.087); *P*=0.051).

Baseline concentrations	Unadjusted		Adjusted*		
	<i>r</i>	<i>P</i>	β	SE	<i>P</i> *
hsCRP v.					
OC (ng/ml)	-0.237	<0.001	-0.044	0.011	<0.001
BAP (U/l)	-0.129	0.032	-0.034	0.015	0.027
CTx (ng/ml)	-0.118	0.05	-0.050	0.027	0.069
NTx (nm BCE/mM cr)	-0.162	0.007	-0.076	0.028	0.008
IL-6 v.					
OC (ng/ml)	-0.102	0.09	-0.037	0.023	0.103
BAP (U/l)	-0.023	0.706	0.012	0.031	0.714
CTx (ng/ml)	-0.003	0.961	0.002	0.051	0.961
NTx (nm BCE/mM cr)	-0.041	0.498	-0.037	0.057	0.520

BCE, bone collagen equivalent; cr, creatinine; *r*, Pearson's correlation coefficient.

*adjusting for age, gender, BMI, and smoking status.

While the change in bone turnover markers on weight loss did not appear to be influenced by reductions in systemic inflammation, interesting associations between markers of systemic inflammation and bone turnover in healthy overweight young adults were revealed. The interplay and causal relationship between systemic inflammation and bone turnover warrants closer investigation.

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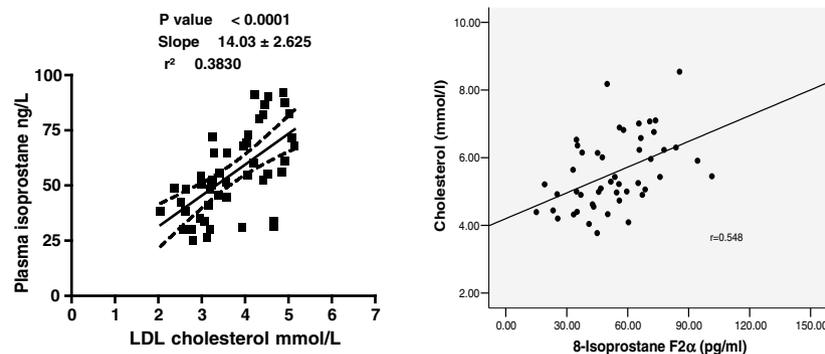
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Plasma 8-isoprostane F2 α concentrations are associated with plasma total cholesterol. By U.Z. MULLA¹, S.E.E. BERRY¹, R. SHERWOOD² and T.A.B SANDERS¹, ¹Nutritional Sciences Research Division, King's College London, 150 Stamford Street, London SE1 9NN, UK and ²Clinical Biochemistry, King's College Hospital, Denmark Hill, London SE5 9RS, UK

Elevated serum cholesterol and hypertension have been associated with increased risk of CVD. Reactive oxygen species (ROS) are believed to play an important part in hypertension, lipid peroxidation and endothelial pathophysiology. F(2)-isoprostanes are a family of metabolites arising from the oxidation of arachidonic acid by ROS. 8-Isoprostane F2 α is currently regarded as the most reliable marker of *in vivo* ROS production and non-enzymic lipid peroxidation¹.

The present study looks at plasma 8-isoprostane F2 α concentration in pre- or mild hypertensive subjects with mildly-elevated serum cholesterol. The subjects were recruited to take part in a randomized controlled trial (DRFRUITVEG ISRCTN50011192). Subjects were asked to follow a low fruit and vegetable intake typical of the national UK average (three portions daily) for 3 weeks. Fasting blood samples were collected at the end of this period for analysis of 8-isoprostane F2 α and cholesterol. Cholesterol was determined by an enzymic method using an automated chemistry analyser. 8-Isoprostane F2 α was determined by immunoaffinity GC–negative chemical ionization MS.

The results for forty-eight subjects are shown in the Figure, adjusted for age and gender (mean cholesterol, mean HDL cholesterol 1.4 (SD 0.35), mean LDL cholesterol 3.71 (0.91) 5.6 (sd 1.08); mean 8-isoprostane F2 α , 53.3 (sd 19.6)).



The present study identifies a significant positive association between plasma 8-isoprostane F2 α and plasma total cholesterol (*r* 0.548, *P*<0.001) after adjustment for age and gender. It also identifies a significant positive association between plasma 8-isoprostane F2 α and plasma LDL cholesterol (*r*=0.38, *P*<0.0001). These results are consistent with the findings of previous studies^{2,3}. There was no significant association between 8-isoprostane F2 α and HDL cholesterol or age.

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A structured patient-education programme for individuals with type 2 diabetes: The X-PERT Programme in Ireland. By Y.M. OBRIEN¹, T.A. DEAKIN², F.M. HORAN¹, J.M. KEARNEY³, S.N. MCCARTHY⁴, M.J. GIBNEY⁴ and K.E. HARRINGTON¹, ¹Community Nutrition & Dietetic Service, Health Promotion Dept, HSE South, Western Road, Cork, Republic of Ireland, ²Nutrition & Dietetic Department, Burnley, Pendle & Rossendale PCT, East Lancashire BB10 2PQ, UK, ³School of Biological Sciences, DIT, Kevin Street, Dublin 8, Republic of Ireland and ⁴UCD School of Agriculture, Food Science and Veterinary Medicine, UCD, Belfield, Dublin 4, Republic of Ireland

The prevalence of type 2 diabetes has increased globally in recent years. Along with the risk of long-term complications, there is a 2–4-fold risk of developing CVD, a concern to health services in terms of service provision, cost implications and patient morbidity. The individual with diabetes is central to looking after their own care¹, and self-management education, in which the individual with diabetes is actively involved, is central to any good diabetes service². Although the importance of patient education is increasingly recognised in Ireland³, limited work has been done in the area.

Structured patient education aims to empower individuals to increase control over their condition by providing the knowledge, skills and confidence to self-manage. In the UK it is recommended that all individuals with type 2 diabetes are offered such education on an ongoing basis. The UK Diabetes X-PERT Programme (X-PERT UK) is an evidence-based structured patient-education programme that meets patient-education guidelines⁴. It is a group programme for individuals with type 2 diabetes facilitated through six weekly sessions. Evaluation via randomised controlled trial showed positive clinical, psychosocial and lifestyle outcomes. The present research aimed to adapt and pilot X-PERT UK in an Irish setting (X-PERT Ireland) and evaluate its impact in terms of similar outcomes at three and 6-month follow up. In addition, X-PERT Ireland provided participants with extra follow-up education sessions.

X-PERT Ireland was piloted with forty-seven individuals with type 2 diabetes in 2006. Patient response (30%), attendance (87% to four or more of six sessions) and interest in the programme were excellent and X-PERT Ireland was found to result in significant improvements in terms of many of the clinical (body weight, waist circumference, glycated Hb, fasting blood glucose, HDL-cholesterol and diastolic blood pressure), lifestyle (diabetes knowledge, fruit and vegetable consumption) and psychosocial (diabetes empowerment score, perceived understanding of diabetes and its treatment) outcomes evaluated.

The findings of this, the first structured patient education programme in Ireland to meet NICE guidance, provide an insight into possible solutions for treating what is a serious, expensive and increasing national problem. The need for and potential of such education is clearly demonstrated by the interest, response and results from this research. Any method of equipping individuals with the skills and confidence to self-manage their condition offers immense benefits, both to the individual and to the health system. This model of care is considered in future service planning, not only for diabetes, but for other chronic conditions. Plans are now underway to deliver an Irish 'Train the Trainers' Course to Community Dietitians to facilitate the delivery of X-PERT Ireland in the primary care setting.

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Cardiovascular risk profiles of women with polycystic ovary syndrome (PCOS) compared with women who are insulin resistant or insulin sensitive and matched for age and BMI. By A. O'CONNOR¹, N. PHELAN², G. BORAN³, J. GIBNEY² and H.M. ROCHE¹, ¹Nutrigenomics Research Group, School of Medicine, Trinity College Dublin Institute of Molecular Medicine, St James's Hospital, Dublin 8, Ireland; ²Departments of Endocrinology and ³Chemical Pathology, Adelaide and Meath Hospital, incorporating the National Children's Hospital, Tallaght, Dublin 24, Ireland

PCOS is a condition affecting 5–10% of women of reproductive age¹, which is characterised by the presence of hyperandrogenaemia and insulin resistance. Women with this condition have been shown to have an increased prevalence of CVD risk factors.

The aim of the present study was to compare the cardiovascular risk profile of women with PCOS with those of subjects who are insulin-resistant (IR) or insulin-sensitive (IS) and matched for BMI and age in order to determine whether or not differences occur between the groups.

A standard 75 g oral glucose tolerance test was carried out with volunteers from each of the three groups, with blood sampled after fasting and after 2 h. Glucose and insulin levels were determined at both time points. Insulin resistance was calculated and subjects were categorised into IR or IS groups using the HOMA model (HOMA-IR). Fasting plasma total cholesterol, HDL-cholesterol, LDL-cholesterol and TAG concentrations were measured.

	IS (n 41)		IR (n 33)		PCOS (n 42)		ANOVA	ANCOVA
	Mean	SD	Mean	SD	Mean	SD	P	P
Age (years)	32.54 ^a	6.27	33.45 ^a	5.04	27.81 ^b	5.23	0.000	
BMI (kg/m ²)	29.08 ^a	3.97	34.69 ^b	5.77	32.89 ^b	7.97	0.000	
Total cholesterol (mmol/l)	4.53	0.89	4.87	0.69	4.42	0.86	0.086	
LDL-cholesterol (mmol/l)	2.43 ^{b,c}	0.84	2.85 ^{b,d}	0.72	2.84 ^{b,d}	0.77	0.040	0.041
HDL-cholesterol (mmol/l)	1.72 ^{a,c}	0.47	1.43 ^{b,d}	0.27	1.39 ^{b,d}	0.29	0.000	0.000
TAG (mmol/l)	0.83 ^{b,c}	0.29	1.48 ^{b,d}	0.82	1.31 ^{b,d}	0.68	0.000	0.000
Insulin (mU/l): Fasting	6.31 ^{b,c}	2.25	16.83 ^{b,d}	6.56	18.19 ^{b,d}	15.37	0.000	0.000
Insulin (mU/l): 2 h	10.29 ^{b,c}	8.05	52.40 ^{b,d}	37.60	56.67 ^{b,d}	41.33	0.000	0.002
Glucose (mmol/l): Fasting	4.51 ^{b,c}	0.42	4.81 ^{b,d}	0.41	4.74 ^{b,d}	0.47	0.001	0.015
Glucose (mmol/l): 2 h	4.26 ^{b,c}	0.88	4.92 ^{b,d}	1.15	4.77 ^{b,d}	1.44	0.002	0.041
HOMA	1.27 ^{b,c}	0.48	3.68 ^{b,d}	1.54	3.95 ^{b,d}	3.73	0.000	0.000

^{a,b} Mean values within rows with different superscript letters were significantly different ($P < 0.05$; ANOVA).

^{c,d} Mean values within rows with different superscript letters were significantly different ($P < 0.05$; ANCOVA controlling for BMI). ANCOVA, analysis of covariance.

ANOVA demonstrated that the BMI of the IS group was significantly lower than those of the IR and PCOS groups ($P = 0.000$). Given the important role of obesity in the development of insulin resistance, further analysis of the CVD risk factors between groups was completed controlling for BMI. After controlling for BMI HDL-cholesterol levels remained lower and plasma TAG concentrations remained higher in the IR and PCOS groups compared with the IS group ($P = 0.000$ and $P = 0.000$ respectively). Fasting and 2 h insulin levels were significantly lower for the IS group compared with IR and PCOS groups ($P = 0.000$ and $P = 0.002$ respectively). The present study demonstrates that women with PCOS and women who are IR have higher TAG, insulin and lower HDL-cholesterol concentrations compared with subjects who are IS. Further analysis is on-going in terms of defining the interaction between obesity, insulin sensitivity and CVD risk factors in women with and without PCOS.

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Modest improvements in weight maintenance on a low-glycaemic index (GI) diet following weight loss in overweight and obese subjects. By E. PHILIPPOU¹, N.M. NEARY², O.B. CHAUDHRI², A.E. BRYNES¹, A. DORNHORST², A.R. LEEDS³, M. HICKSON¹ and G.S. FROST⁴, ¹Nutrition and Diets Research Group, Imperial College London, Hammersmith Hospital Campus, London W12 0HT, UK, ²Department of Metabolic Medicine, Imperial College London, London W12 0NN, UK, ³Department of Nutrition and Dietetics, King's College London, London SE1 9NH, UK and ⁴School of Biomedical and Molecular Sciences, Guildford GU2 7XH, Surrey, UK

GI is a numeric classification of carbohydrate foods based on their glycaemic response, i.e. the rate of carbohydrate absorption¹. Low-GI, i.e. slowly-absorbed, diets may facilitate weight maintenance following weight loss by improving access to stored metabolic fuels between meals and by preventing marked postprandial glucose excursions, thus decreasing hunger². Previous studies suggest that manipulation of GI affects body weight³, lipogenesis⁴ and resting energy expenditure⁵. The present study compared the longer-term effect of altering diet GI on weight maintenance following weight loss in overweight and obese subjects.

The study consisted of two parts: the weight loss phase and the weight maintenance phase. Fifty healthy overweight or obese subjects lost 5% of their body weight on a Slimfast[®] (Unilever UK Foods Ltd, Crawley, West Sussex, UK) diet and were randomised to a high- or low-GI weight maintenance diet for 4 months. Of these, five subjects dropped out (three did not want to follow the diet, one subject had personal problems and one had time issues). Therefore, forty-two subjects who lost a median of 6.1 (interquartile range 5.2–7.1) % body weight completed the study. Following randomisation, subjects were advised to change their main carbohydrate sources and eat to their appetite. They were seen monthly to assess compliance using 3 d food diaries and to take anthropometric measurements. Appetite was assessed bimonthly by 3 d visual analogue scales (VAS) and fasting blood samples were taken bimonthly for measurement of plasma lipids and glucose.

Nineteen subjects in the high-GI group (age, 45.1 (SD 9.4) years; BMI, 31.3 (SD 4.8) kg/m²; two males) and twenty-three in the low-GI group (age 44.6 (SD 11.9) years; BMI 32.5 (SD 4.8) kg/m²; one male) completed the study. At baseline, the groups did not differ in any of the measured variables. During the weight-maintenance phase the mean diet GI increased by 7.1 (SD 10.6) units (v. habitual diet $P < 0.01$) in the high-GI group and decreased by 4.9 (SD 5.1) units (v. habitual diet $P < 0.01$) in the low-GI group. The groups differed both in diet GI ($P < 0.01$) and diet glycaemic load (GL; the product of GI and carbohydrate intake; $P < 0.01$). By 4 months the low-GI group lost a further 0.7 (SD 2.9) kg while the high-GI group gained 0.3 (SD 1.9) kg, although this was not significant ($P = 0.2$). Fourteen of twenty-three subjects (61%) of the low-GI group maintained body weight compared with nine of nineteen subjects (47%) in the high-GI group. Although the groups did not differ in energy intake during the weight-maintenance period, energy intake:energy requirement (EI:E_{req}) was lower in the low-GI group than in the high-GI group (0.62 (SD 0.19) v. 0.72 (SD 0.14); $P = 0.03$). The groups did not differ in appetite (assessed by VAS), cholesterol, TAG or glucose concentrations.

	High GI (n 19)				Low GI (n 23)			
	Baseline		Δ from baseline		Baseline		Δ from baseline	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Diet GI	56.4	3.4	7.1 ^a	10.6	55.5	6.4	-4.9 ^b	5.1
Diet GL	135.1	29.5	1.1 ^c	58.3	122.6	38.6	-30.9 ^d	36.7
Carbohydrate intake (g/d)	239	50	-29.6	70.6	220	63	-40.1	65.2
Energy intake (kJ/d)	8297	1409	-1572	1910	7829	2002	-1655	2103
Weight (kg)	83.6	13.4	0.3	1.9	87.2	15.3	-0.7	2.9
BMI (kg/m ²)	31.3	4.8	0.1	0.7	32.5	4.8	-0.3	1.1
Body fat (%)	37.1	7.9	-0.5	2.0	38.8	5.7	0.1	2.0
Cholesterol (mmol/l)	4.87	0.67	0.46	0.34	4.67	0.93	0.39	0.91
TAG (mmol/l)	1.04	0.34	0.07	0.33	0.93	0.31	-0.19	0.36
Glucose (mmol/l)	4.92	0.47	-0.17	0.45	4.82	0.47	-0.07	0.44

^{a,b,c,d} Means within rows with different subscript letters were significantly different (independent Student's *t* test): ^{a,b} $P < 0.01$, ^{c,d} $P < 0.05$.

These data show that dietary GI can be altered and this change can be maintained over a 4-month period, and suggest a tendency for the low-GI group to achieve better weight maintenance. The study was originally powered for fifty-two subjects, so may be underpowered. The lower EI:E_{req} of the low-GI group may be related to the satiating effects of low-GI foods².

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Effect of flaxseed lignans on biomarkers of breast cancer risk in post-menopausal women. By L.A. WILLIAMSON¹, N.J. MANN¹, A.J. SINCLAIR², D. KILDEA³, D. SMALL¹ and H. ADLERCREUTZ⁴, ¹School of Applied Sciences, RMIT University, Australia, ²Exercise and Nutrition Science, Deakin University, Australia, ³PVC Science Eng & Technology, Math & Geospatial Sciences, RMIT University, Australia and ⁴Institute for Preventive Medicine, Nutrition, and Cancer, University of Helsinki, Finland

Flaxseed is reported to have numerous chemoprotective effects *in vivo* and *in vitro* that may be mediated through its anti-oestrogenic effects and/or its influence on endogenous sex hormone production, metabolism and biological activity^(1–10). The purpose of the present research is to assess the effects of flaxseed lignans on biomarkers of breast-cancer risk in post-menopausal women.

For control of the trial and to eliminate factors thought to increase breast cancer risk or affect metabolism of flaxseed lignans, post-menopausal women were screened with a detailed health and dietary questionnaire. To be considered eligible the women had to be at least one year postmenopausal, non smokers, consume a typical western diet, consume less than 2 alcoholic beverages/day or 5/week, consume less than 3 caffeinated beverages/day, have not been on hormone replacement therapy or antibiotics in the previous 6 months or currently, not regularly consume flaxseed or soy products and medication had to be monitored to be consistent throughout the trial period. Healthy post-menopausal subjects (*n* 35) consumed 50 or 100 mg purified lignans or a placebo daily for a period of 7 weeks in a double-blind placebo-controlled randomized three-way cross-over intervention trial. Levels of flaxseed lignans used are higher than normally encountered in a typical western diet. Blood and urine samples were taken from subjects at the beginning and end of each intervention and analysed for lignan metabolites enterolactone (ENL) and enterodiol (END; time-resolved fluoro immunoassay), sex hormone-binding globulin (SHBG; radio chemiluminescence immunoassay), oestradiol (antibody immunoassay) and oestrogen metabolites 2-hydroxyl oestrone (2-OHE) and 16-hydroxyl oestrone (16αOHE; enzyme immunoassay). Dietary patterns were monitored throughout.

Levels of ENL and END increased in a dose-responsive manner ($P = 0.000$). 2-OHE and 2-OHE:16αOHE-1 showed an increasing trend but did not reach significance. There was no treatment effect or treatment × order effect in levels of SHBG, 16αOHE or oestradiol.

Flaxseed lignans were metabolized to ENL and END in a dose–response manner and were therefore available to exert anti-oestrogenic effects and influence endogenous sex hormone production, metabolism and biological activity. Oestrogen metabolism is forced to the less-oestrogenic 2-hydroxylation pathway as a result of the higher phyto-oestrogen (phenolics) load. Whether this decreases the risk of breast cancer remains to be established.

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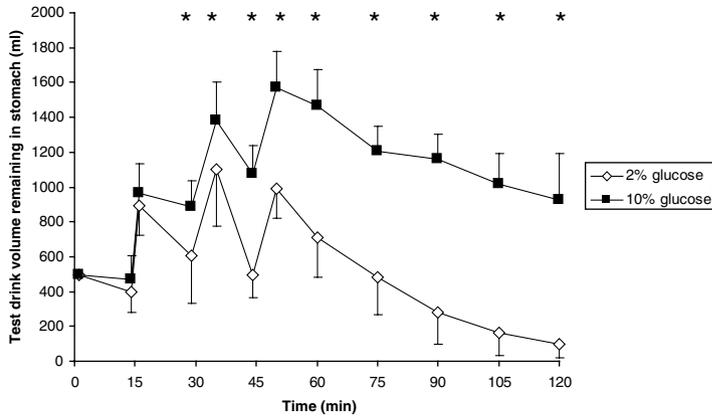
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The rate of gastric emptying and blood ²H accumulation following repeated ingestion of glucose–electrolyte solutions. By G.H. EVANS, S.M. SHIRREFFS and R.J. MAUGHAN, *School of Sport and Exercise Sciences, Loughborough University, Loughborough LE11 3TU, UK*

The effectiveness of a rehydration solution will depend on the rates of gastric emptying and intestinal absorption following ingestion. It has been shown previously that a glucose (10% (w/v))–Na (32 mmol/l) solution was more effective in maintaining whole-body fluid balance than a glucose (2% (w/v))–Na (32 mmol/l) solution¹. It is unclear whether this is primarily a result of the reduced rate of gastric emptying or the reduced rate of intestinal absorption that has been observed following ingestion of high-carbohydrate solutions.

Following Ethics Committee approval, five female and three male volunteers with no history of gastrointestinal disease took part in this study. Subjects completed a familiarisation trial before undertaking two experimental trials that involved repeated ingestion of either a glucose (2% (w/v))–Na (32 mmol/l) solution or a glucose (10% (w/v))–Na (32 mmol/l) solution over a period of 1 h following 13 h of fluid restriction that resulted in a reduction in body mass of 0.9 (SD 0.4) %. The first bolus of test solution amounted to 495 ml and contained 10.0205 (SD 0.0059) g ²H₂O. Three other boli were ingested at intervals of 15 min so that total drink volume amounted to 3% of the subject's initial body mass (i.e. 1986 (SD 274) ml). Gastric emptying was measured at intervals of 15 min for a period of 2 h using a double-sampling gastric-aspiration technique² and arterialised venous blood samples were collected at regular intervals. Urine samples were collected before and after fluid restriction and at the end of the experimental period.



The volume of test drink present in the stomach at the end of the experimental period was lower ($P=0.001$) and the rate of ²H accumulation faster ($P=0.005$) on the 2% (w/v) glucose trial than on the 10% (w/v) glucose trial (Figure). Estimated change in plasma volume tended to increase from baseline values and decrease from baseline values on the 2 and 10% (w/v) glucose trials respectively with significant differences ($P<0.05$) on both trials being observed 75 min after ingestion of the initial bolus. In addition, plasma volume was greater on the 2% (w/v) glucose trial than on the 10% (w/v) glucose trial ($P<0.05$) from 20 min after ingestion of the initial bolus until the end of the experimental period. Urine volume at the end of the experimental period was greater on the 2% (w/v) glucose trial than on the 10% (w/v) glucose trial ($P=0.006$).

These results suggest that reducing the rate of gastric emptying has a positive effect on the maintenance of whole-body fluid balance following dehydration as a result of a reduced rate of total fluid absorption and, consequently, a reduction in the volume of urine excreted.

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Long-term consequences of early fruit-and-vegetable feeding practices in the weaning period. By P. EMMETT¹, H. COULTHARD² and G HARRIS², ¹*Department of Social Medicine, Bristol University, Bristol BS8 1TQ, UK* and ²*School of Psychology, Birmingham University, Birmingham B15 2TT, UK*

Many chronic diseases show an association with poor intake of fruits and vegetables. British adults and children currently consume about half the amount recommended. Increasing intakes of these foods is a public health priority.

Data was collected via self-completion questionnaire from mothers in the Avon Longitudinal Study of Parents and Children¹ about the foods offered to infants during weaning and the frequency with which the child ate fruit and vegetables at age 7 years.

From the original sample of 14541 mothers recruited in 1991–2, 7866 mothers of white singletons provided information about their child's diet at 6 and 15 months and 7 years of age.

Fruits and vegetables fed during weaning were described as 'prepared baby foods (from jar, tin or packet)', 'foods cooked at home by you' or 'raw'. The mother was asked when the child was 6 months if the child had been given items from a list of food, the age in months when starting it and the current frequency of eating². At 7 years the mothers was asked to complete an FFQ about the child. It contained a list of eight types of vegetable and two types of fruit. Fruit and vegetable eating at 7 years was investigated according to the type of fruit and vegetable given before 6 months.

By 6 months 90.1 & 85.5% of infants had been fed ready-prepared vegetables and/or fruit respectively, for home-cooked the proportions were 80.5 & 46.7% and for raw they were 17.9 & 44.7% respectively. Feeding home-cooked or raw fruits or vegetables was positively correlated with eating all eight types of vegetables and both types of fruit at 7 years whereas feeding ready-prepared fruits or vegetables was negatively correlated with the consumption of some of these items.

Type of veg fed by 6 months	Consumption of veg more than once daily at 7 years		Type of fruit fed by 6 months	Consumption of fruit more than once daily at 7 years	
	OR	95% CI		OR	95% CI
Ready-prepd veg: Non-adjusted	0.91	0.77, 1.08	Ready-prepd fruit: Non-adjusted	0.83	0.73, 0.94
Adjusted*	0.88	0.73, 1.07	Adjusted*	0.90	0.79, 1.04
Home-cooked veg: Non-adjusted	1.69	1.50, 1.91	Home-cooked fruit: Non-adjusted	1.43	1.31, 1.57
Adjusted*	1.52	1.32, 1.75	Adjusted*	1.40	1.26, 1.54
Raw veg: Non-adjusted	1.41	1.24, 1.61	Raw fruit: Non-adjusted	1.52	1.39, 1.66
Adjusted*	1.24	1.07, 1.43	Adjusted*	1.43	1.30, 1.58

prepd, prepared; Veg, vegetables. * Adjusted for gender, number of older siblings, educational level and age of the mother, housing tenure, overcrowding and whether breast-fed.

Feeding ready-prepared fruits or vegetables was not related to eating fruit or vegetables more than once daily at 7 years. However, feeding home-cooked or raw fruit or vegetables was associated with a 24–52% increase in the proportion of children eating fruit or vegetables more than once daily after adjustment.

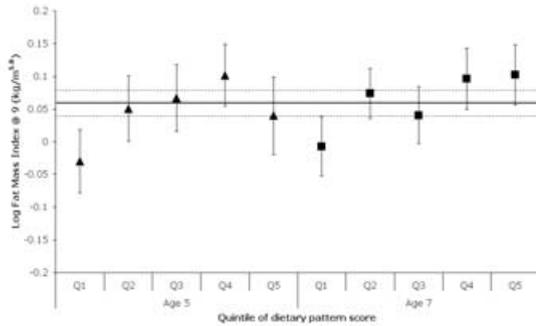
The types of foods introduced at weaning were important determinants of fruit-and-vegetable eating in later childhood. Advice for parents should be to introduce home-cooked and raw fruits and vegetables rather than ready-prepared types.

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Using reduced rank regression to find a dietary pattern related to fat mass in childhood. By L. JOHNSON¹, A. MANDER¹, L.R. JONES², P.M. EMMETT² and S.A. JEBB¹, ¹MRC Human Nutrition Research, Cambridge CB1 9NL, UK and ²ALSPAC, University of Bristol, Bristol BS8 1TQ, UK

Overweight and obesity currently affects 27% of English children under age 11 years¹. Studying the dietary determinants of obesity is complex, as nutrients are not consumed in isolation; they are integrated within foods, which in turn are combined into dietary patterns. Analysis of dietary patterns may provide clearer insights into the causes of obesity than that of nutrients or single-food groups alone. The aim of the present analysis was to identify a dietary pattern characterised by dietary energy density (DED), fibre density (FD) and fat intake (%fat) and to analyse its association with subsequent fat mass and risk of overweight status.

Diet data were collected in a random subsample of children from a prospective cohort study in Avon, England, at age 5 years (*n* 523) and 7 years (*n* 682) using 3 d unweighed-diet diaries. Reduced rank regression was used to derive a dietary pattern score². Food intake was collapsed into forty-six food groups. DED, FD and %fat were selected as intermediate response variables based on evidence of a relationship with obesity from adult studies. Body fat mass was estimated at age 9 years using dual-energy X-ray absorptiometry. Fat mass index (FMI) was calculated by dividing fat mass (kg) by height^{5.8} (m)³. FMI was not normally distributed so it was log transformed. Overweight was defined as the top quintile of logFMI. The association between the dietary pattern and fatness was examined using linear and logistic regression adjusted for potentially-confounding variables.



Bold line represents mean (SE) logFMI at age 9 y for the whole sample. Triangles are mean (SE) logFMI at age 9 y for children in each quintile of dietary pattern score at age 5 y. Squares are mean (SE) logFMI at age 9 y for children in each quintile of dietary pattern score at age 7 y.

The principal pattern score extracted was highly correlated with DED (*r* 0.8), FD (*r* -0.7) and %fat (*r* 0.5). Pattern loadings (*L*) indicated that a high pattern score was characterised by a low consumption of fresh fruit (*L* -0.5) and vegetables (*L* -0.4) and a high consumption of crisps and snacks (*L* 0.2) and chocolate and confectionery (*L* 0.2). Mean logFMI increased from quintile 1 to quintile 5 of dietary pattern score at both age 5 and 7 years (see figure). A difference (of 1 sd) in pattern score at age 5 years and 7 years respectively gave a 0.2 (95% CI -0.1, 0.5) kg and a 0.3 (95% CI 0.1, 0.5) kg difference in fat mass at age 9 years, after controlling for misreporting of energy intake (EI), total EI, maternal education, maternal BMI, overweight status at baseline and TV watching. There was a positive linear trend between risk of overweight at age 9 years and quintile of dietary pattern score at age 5 years (*P*=0.03) and age 7 years (*P*<0.0001). The adjusted odds of excess adiposity at 9 y for children per quintile of dietary pattern score at age 5 y and 7 y respectively was 1.3 (95% CI 1.0 to 1.6) and 1.5 (95% CI 1.2 to 1.8).

This analysis indicates that an energy dense, low fibre, high fat diet is associated with greater fat mass and higher risk of overweight status in childhood. The smaller effect size observed at age 5 years *v.* 7 years may reflect the shorter duration of follow-up or a deterioration in the innate ability of children to match EI to energy needs⁴.

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Increased portion size leads to a sustained increase in energy intake over 4 d in normal-weight and overweight men and women. By M.T. KELLY, A. FLETCHER, S.F. BRENNAN, J.M.W. WALLACE, P.J. ROBSON, R.W. WELCH, M.P. HANNON-FLETCHER and M.B.E. LIVINGSTONE, Northern Ireland Centre for Food & Health, University of Ulster, Coleraine BT52 1SA, UK

Increased food portion sizes may be facilitating excess energy intake (EI) and a higher risk of overweight and obesity. Portion size has been demonstrated to positively influence EI at single meals in adults^{1,2}. However, the effects of increasing portion size on EI over a longer period are unclear. The purpose of the present study was to evaluate the extent to which increasing portion sizes influence food and EI over a 4 d period.

The study used a randomized within-subject cross-over design. Forty-three normal-weight and overweight subjects were fully residential in the Human Intervention Studies Unit for two 4 d periods with a 3-week interval between each study period. Subjects were randomly assigned to two groups. Group 1 received 'standard' portion sizes of foods in the first 4 d period followed by 'large' portion sizes of the same foods in the second 4 d period. The order of presentation was reversed in group 2. The portion sizes selected were driven by commercially-available units, i.e. the smallest preportioned unit was the standard portion and units of the same food designed to serve two or more subjects was the large portion. All foods and beverages were accurately and covertly weighed before being served and any foods leftover were weighed and recorded. Visual analogue scales were used to rate feelings of hunger and satiety immediately before and after meals. Subjects were not informed of the true purpose of the study. Statistical analyses were performed using a general linear model with repeated measures.

There was a significant positive effect of portion size on EI in the total group (*P*=0.020); over the 4 d period when served the larger portion size subjects consumed an additional 14% energy (7 MJ). On a daily basis males and females significantly increased their EI in response to being served the larger portion sizes, and over the 4 d this resulted in a 17% (10 MJ) and 10% (4 MJ) increase in EI in men and women respectively. These increases were sustained over the 4 d period with no compensation being made by subjects for the consumption of larger portions of food. Subjects did not report feeling any fuller as a result of consuming the larger food portions.

	Males (<i>n</i> 21)				<i>P</i>	Females (<i>n</i> 22)				<i>P</i>
	Standard portion		Large portion			Standard portion		Large portion		
	Mean	sd	Mean	sd		Mean	sd	Mean	sd	
Energy (MJ/d)	14.7	4.0	17.1	4.4	–	11.5	2.4	12.6	2.5	0.005
Food weight (kg/d)	4.2	0.9	4.6	1.2	0.051	3.6	0.8	3.9	0.9	0.036
Protein (g/d)	122.2	26.3	140.9	23.6	–	96.2	17.0	103.5	17.8	0.009
Fat (g/d)	147.7	44.4	180.6	52.0	–	123.6	30.4	138.8	33.8	0.003
Carbohydrate (g/d)	442.1	129.3	495.4	140.5	–	329.7	78.2	354.5	77.3	0.033

Subtle increases in the portion sizes of all foods resulted in significant and sustained increases in EI in both men and women. These data suggest that the availability and consumption of larger portions of food may be a major contributing factor in inciting excess EI and adiposity.

This research was commissioned by the Food Standards Agency.

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Snacks v. meals: similarities and differences among children and adolescents. By M.A. KERR¹, T.A. McCAFFREY¹, K.L. RENNIE^{1,2}, J.M. WALLACE¹, M.P. HANNON-FLETCHER¹ and M.B.E. LIVINGSTONE¹, ¹Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine BT52 1SA, UK, ²Present address: Unilever Corporate Research, Colworth House, Sharnbrook, Bedfordshire MK44 1LQ, UK

A decline in the traditional habit of three meals daily has given rise to a secular increase in 'snacking'. This has coincided with the increased prevalence of childhood obesity¹. The contributions of snacks and meals to energy and macronutrient intake were compared in a representative sample of British children and adolescents using the National Diet and Nutrition Survey of young people aged 4–18 years². Of the 1702 participants to complete a 7 d dietary record, children aged 6–8 years (*n* 376) and adolescents aged 13–16 years (*n* 434) were selected for the current analyses. Identification of eating event (meal or snack) was based on time of day. All eating events that took place within three specific 'time windows' of 06.00 hours–9.30 hours, 11.30 hours–14.30 hours and 16.30 hours–19.30 hours were defined as breakfast, lunch and dinner respectively. All eating events taking place outside of these times were considered snacks.

	6–8 y (<i>n</i> =376)				13–16 y (<i>n</i> =434)			
	Snacks		Meals		Snacks		Meals	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
MJ/d	1.4 ^a	0.83, 2.04	5.2 ^b	4.4, 5.9	2.2 ^a	1.4, 3.3	5.4 ^b	4.3-, 6.6
% of TEI [†]	22.2 ^a	13.2, 29.2	77.7 ^b	70.8, 86.6	29.8 ^a	30.3, 39.4	70.1 ^b	60.6, 79.7
Protein (%)*	10.4 ^a	8.1, 13.0	13.2 ^b	12.0, 14.4	11.5 ^a	9.1, 14.2	13.8 ^b	12.3, 15.3
Fat (%)*	35.0	30.7, 39.6	34.7	32.0, 37.5	33.2 ^a	28.5, 38.2	35.3 ^b	31.9, 38.5
CHO(%)*	57.5 ^a	51.4, 63.6	55.4 ^b	52.2, 58.1	58.0 ^a	51.6, 63.4	53.7 ^b	49.8, 57.6
Sugars(%)*	33.3 ^a	12.7, 40.6	24.5 ^b	5.7, 27.9	28.9 ^a	12.0, 36.9	21.0 ^b	17.0, 25.9

Values were compared within age groups using a Wilcoxon *t* test. Values with different superscripts are significantly different ($P < 0.05$). [†]Total energy intake. * Expressed as a percentage of snack or meal energy intake.

The percentage energy derived from fat was significantly lower from snacks than meals among 13–16 year olds only ($P < 0.01$). The percentage energy derived from carbohydrate and total sugars was significantly greater from snacks than from meals among both 6–8 year olds and 13–16 year olds ($P < 0.01$). When those who reported illness (*n* 54, 6–8 y; *n* 91, 13–16 y) or being on a diet (*n* 2, 6–8 y; *n* 35, 13–16 y) during the study period were removed from the analysis these results did not change. Among 6–8 year olds, the five main contributors to snack energy intake (SEI) were milk>biscuits>chocolate>crisps>meat and meat dishes, which collectively contributed 9.8% to TEI (MJ/d). Among adolescents, the five main contributors to SEI (accounting for 13% TEI) also included meat and meat dishes and chocolate, but milk, biscuits and crisps were replaced with potato dishes (inclusive of chips), pasta dishes and bread. Of particular note, the five main contributors to EI from meals among adolescents were identical to those from snacks with the exception of chocolate, which was replaced with breakfast cereals. In conclusion, foods commonly eaten as snacks are also eaten as part of a meal, particularly among adolescents. The overall eating habits of children and adolescents rather than snacking *per se* should be considered in researching dietary predictors of childhood obesity.

This analysis was commissioned by the Food Standards Agency.

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Can the method of calculating energy density influence the interpretation of the relationship between energy density and fat mass in children? By T.A. McCAFFREY¹, K.L. RENNIE^{1,2}, M.A. KERR¹, J.M. WALLACE¹, M.P. HANNON-FLETCHER¹, S.A. JEBB³, W.A. COWARD³ and M.B.E. LIVINGSTONE¹, ¹Northern Ireland Centre For Food and Health (NICHE), University of Ulster, Coleraine BT52 1SA, UK, ²Present address: Unilever Corporate Research, Colworth House, Sharnbrook, Bedfordshire MK44 1LQ, UK and ³MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL, UK

The energy density (ED; kJ/g) of the diet has recently been shown to be positively associated with predictors of obesity in children¹. However, the association between ED of snacks and/or meals and increases in fat mass (FM) remains unclear. Prospective relationships between ED and body fatness were investigated in forty-eight children (thirty boys and eighteen girls), who were initially studied at 6–8 years of age (baseline; BL) and followed up at 13–16 years of age (FU). At BL subjects completed 7 d weighed dietary records concurrently with the doubly-labelled-water method (DLW), from which total body water (TBW) and total energy expenditure were calculated. ED was calculated using two methods: ED of all food and drinks (ED_{all}); ED of food only (including milk as food; ED_{food}). At FU, DLW measurements for body composition were repeated and subjects were requested to complete a self-assessment of pubertal status (*n* 46)². At BL and FU, TBW and fat-free mass, using age- and gender-specific values³ were used to calculate FM. Body size at BL and FU was adjusted for height² (fat mass index; FMI) and gain in FMI was calculated as: FU FMI – BL FMI = FMI_{dif}. As FMI_{dif} was not normally distributed, FMI_{dif} was categorised based on gender-specific tertiles (top tertile v. lower tertiles).

Baseline Food Intake	All eating occasions		Meals		Snacks	
	Mean	(sd)	Mean	(sd)	Mean	(sd)
EI:EE*	0.96	(0.15)	–	–	–	–
Energy (kJ/day)	7434	(1385)	5510 ^a	(1296)	1883 ^b	(654)
Food Weight (g/day)	1422	(302)	1030 ^a	(251)	390 ^b	(154)
ED _{all} (kJ/g/day)	5.40	(0.77)	5.53	(0.78)	5.56	(2.02)
ED _{food} (kJ/g/day)	8.34	(1.28)	7.78 ^a	(1.17)	12.34 ^b	(3.58)

* Energy Intake:Energy Expenditure. ^{a,b} Values with different superscript letters are significantly different ($P < 0.001$).

Mean EI:EE was 96%, indicating low levels of under-reporting at the group level. Although the energy intake and weight of food (g) from self-defined meals was significantly higher than self-defined snacks, the ED_{food} of snacks was significantly higher than that from meals. Overall, ED_{food} (all eating occasions) increased the odds of being in the higher category of FMI_{dif} (OR 2.12 (95% CI 1.08, 4.17); $P = 0.029$) after adjustment for gender, pubertal status and EI:EE. However, the ED_{all} or ED_{food} of meals and snacks were not independently associated with FMI_{dif} categories. In conclusion, ED_{food} of the overall diet was a significant predictor of FM gains between BL and FU. However, different conclusions can be drawn about the associations between ED and increases in FM, depending on the calculation of ED used.

This analysis was commissioned by the Foods Standards Agency.

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The effects of soyabean-phyto-oestrogen-rich diet on lipid profile, endothelial function and menopausal symptoms. By Z. BÜYÜKTUNCER^{1,3}, E. AKGÜL², G. KÖKSAL¹, K. HANNA³, B. ELLAHİ³, H. YARALI⁴ and L. TOKGÖZOĞLU², Hacettepe University, Department of Nutrition and Dietetics 06100 Ankara, TURKEY, ²Hacettepe University, Department of Cardiology, 06100 Ankara, TURKEY, ³University of Chester, Parkgate Road, Chester, CH1 4BJ, ⁴Hacettepe University, Department of Obstetrics and Gynecology, 06100-Ankara, TURKEY

The menopause, one of the important physiological phases of a woman's life, may be associated with numerous symptomatic and asymptomatic manifestations, including vasomotor symptoms, and in the long term may increase the risk of osteoporosis and CVD¹. The effects of phyto-oestrogens on menopausal symptoms have been attracting interest in recent years².

The present study was planned to investigate the effects of a diet rich in soyabean phyto-oestrogens on menopausal symptoms, and was carried out by the Cardiology, Gynaecology and Nutrition and Dietetics Departments of Hacettepe University. Eighteen female volunteers with a natural menopause who had not been receiving hormone-replacement therapy, had no health problems and had a normal blood-lipid profile participated in the 8-week dietary intervention study. Participants consumed a well-balanced diet containing 35 g soyabean protein per day (45 mg isoflavones; Cargill Foods, İstanbul, Turkey). Blood samples were collected on the first day of the study (baseline) and at the end of the 8-week diet period for the analysis of the total cholesterol, TAG, LDL-, HDL- and VLDL-cholesterol, apoA and -B, lipoprotein (a), C-reactive protein (CRP), homocysteine, osteocalcin, insulin-like growth factor-I (IGF-I), oestradiol, follicle-stimulating hormone (FSH), luteinising hormone (LH), endometrial thickness (measured by transvaginal ultrasound) and endothelial function (EDD; evaluated by flow-mediated endothelium-dependent dilation (FMD) using brachial ultrasound). The participants were assessed in terms of their frequency of hot flushes and insomnia with the help of a questionnaire at baseline and at the end of the 8-week period.

There were no significant differences in plasma lipid profile, CRP, homocysteine or osteocalcin. An improvement in EDD of 36% was identified ($P<0.05$) (Table 1).

Table 1: The difference in endothelial function at baseline and after 8-week diet period

	Baseline		8-weeks		P
	X±S	Median	x±S	Median	
Diameter of Brachial Artery (mm)	3.16±0.37	3.19	3.25±0.81	3.22	0.122
Diameter of Hyperemia Artery (mm)	3.45±0.35	3.50	3.66±0.39	3.64	0.028*
FMD (%)*	9.49±4.73	9.11	12.86±6.48	11.92	0.016*

* $P<0.05$, ** (FMD: flow-mediated endothelium-dependent dilation).

The changes in oestradiol, LH and FSH levels were not clinically significant. Endometrial thickness was not affected by the isoflavone-rich diet ($P>0.05$). There was a reduction in the frequency of hot flushes and sleep disturbance during the diet period. The increase observed in IGF-I level, which is a biomarker of osteoporosis, was not significant. The isoflavone-rich diet had no effects on weight, BMI, body composition, skinfolds or waist and hip measurements.

A diet that contains soyabean isoflavones can enhance endothelial function and decrease the frequency of vasomotor symptoms of the menopause, but further long-term studies are necessary to explain the other effects on the menopause.

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The effect of lactulose and inulin on gastric emptying in men. By M. CLEGG¹, A. SHAFAT¹, M. DOBSON² and M. ROWLAND³, ¹Department of Physical Education and Sport Sciences, University of Limerick, Limerick, Republic of Ireland, ²Department of Gastroenterology, Adelaide and Meath Hospital, Tallaght, Dublin 24, Republic of Ireland, ³University College Dublin School of Medicine and Medical Science, The Children's Research Centre, Our Lady's Children's Hospital, Crumlin, Dublin 12, Republic of Ireland

The H₂ breath test provides an indication of mouth to caecum transit time (MCTT). Lactulose, a non-absorbable carbohydrate, has been used widely to measure MCTT, as on reaching the large intestine it is metabolised by colonic bacteria resulting in the production of H₂. The H₂ diffuses into the bloodstream and into the alveoli, where it can be detected in end-exhalation breath. However, this method has been criticised as lactulose, because of its osmotic effects, draws fluid into the intestinal lumen and accelerates small intestinal transit. This acceleration has previously been demonstrated¹. Lactulose has also been shown to have a tendency towards delaying gastric emptying (GE)². Another potential substrate for the H₂ breath test that does not accelerate MCTT is inulin. The aim of the current study was to examine differences in GE when either solid lactulose, solid inulin or lactulose in liquid form were added to a test meal.

Ten healthy male volunteers (age 25.1 (SD 2.7) years, height 1.79 (SD 0.04) m, weight 78.3 (SD 6.0) kg) participated in the study, approved by the University of Limerick Research Ethics Committee. All volunteers recorded their diet for 24 h using a weighed-food diary. Following a 12 h overnight fast they attended the laboratory and consumed, in a randomised order, a pancake meal supplemented with either 12 g solid lactulose or 12 g solid inulin, or a plain pancake and 12 g liquid lactulose dissolved in water. All the pancake meals were also supplemented with 100 mg [¹³C]octanoic acid. Samples of breath were taken every 15 min for 6 h. The test procedure was repeated for the two other substrates with ≥5 d between tests. The food diary from the 24 h before the first test session was repeated for the 24 h before each of the next 2 d. The breath samples were analysed for ¹³CO₂ using isotope-ratio MS. Gastric emptying was analysed using techniques described by Ghooos *et al.*³ and Schommartz *et al.*⁴. Statistical significance ($P<0.05$) was examined with SPSS (version 13.0; SPSS, Chicago, IL, USA) using a repeated-measures ANOVA.

There were significant overall changes in GE half emptying time ($P=0.017$), lag ($P=0.005$) and latency phase ($P=0.004$) but not for ascension time ($P=0.112$) for the three meals (see Table).

Time (min)	Solid inulin		Solid lactulose		Liquid lactulose	
	Mean	SD	Mean	SD	Mean	SD
Half emptying time	107	12	122**	18	123**	20
Lag phase	52	10	57**	10	61**	9
Ascension time	132	13	144*	16	138	19
Latency phase	54	15	59**	14	65***††	12

Solid and liquid lactulose v. inulin: * $P\leq 0.05$, ** $P\leq 0.01$. Solid lactulose v. liquid lactulose: †† $P\leq 0.01$.

A tendency towards slower GE has been reported when foods are supplemented with lactulose². The present data confirm that when foods are supplemented with either solid or liquid lactulose GE is significantly slowed in comparison with solid inulin. One possible explanation is that lactulose, but not inulin, increases the viscosity of the meal. This increased viscosity has a greater effect on GE when liquid meals are used, possibly explaining the trend towards slower emptying when liquid lactulose is compared with solid lactulose. The current data provide further support for the use of inulin in the H₂ breath test.

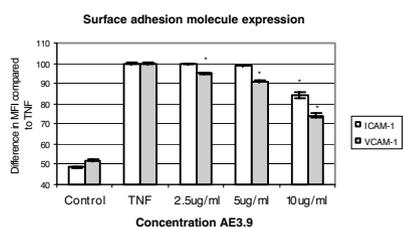
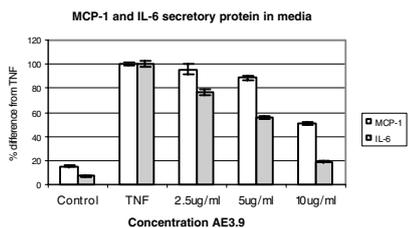
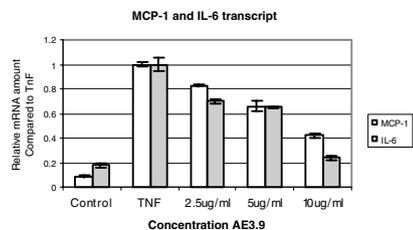
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Apple procyanidins inhibit TNF α -induced pro-inflammatory effects in human umbilical vascular endothelial cells. By C.A. CONNOR, P.A. KROON and D.A. HUGHES, *Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, UK*

CVD is the world's leading cause of death (17×10^6 deaths each year¹). Numerous epidemiological and prospective studies have indicated beneficial effects, in terms of reducing CVD risk, of increased fruit and vegetable consumption. Many polyphenolic compounds found in food and beverages, such as apples and red wine, have been implicated in reducing CVD risk.

Atherosclerosis is now recognised as an inflammatory disease. Reactive oxygen species (ROS) are produced at sites of inflammation and can cause damage to tissues. Polyphenols in apples have been shown to be powerful antioxidants, reducing ROS in human plasma². The major players in this antioxidant activity are epicatechin and procyanidin B2.

IL-6 together with its receptor has been shown to increase E-selectin, intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and the release of monocyte chemoattractant protein-1 (MCP-1), IL-8 and IL-6 from endothelial cells³. Compound mutant mice lacking MCP-1 or its receptor CCR2, which are susceptible to atherosclerosis because of the absence of genes encoding apoE or LDL, show a striking difference in mononuclear cell and local lipid accumulation within sites of atherosclerosis^{4,5}. ICAM-1 and VCAM-1 are also increased at these sites⁶. The adhesion of monocytes to the endothelium and their subsequent migration into the vessel wall are crucial steps in the atherosclerotic process. The ability of low concentrations of an apple extract–procyanidin oligomer mix (with an average extent of polymerization of 3.9; AE3.9) to attenuate the TNF α -induced up-regulated expression of IL-6, MCP-1, ICAM-1 and VCAM-1 in human umbilical-vein endothelial cells (HUVEC) was investigated. Cells were pre-treated with AE3.9 for 45 minutes and TNF- α was added for 6 hours (transcript expression) and 24 hours (protein expression).



IL-6 and MCP-1 gene and protein expression were markedly decreased when the HUVEC were pretreated with AE3.9. This decrease was even notable at concentrations as low as 2.5 μ g/ml. At 2.5, 5 and 10 μ g/ml there were significant decreases in VCAM-1 surface expression but ICAM-1 was only affected by 10 μ g/ml. Decreasing these inflammatory mediators (cytokines, chemokines and adhesion molecules) with flavanols would attenuate the progression of the atherosclerotic plaque.

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The effects of plant sterols on the viability and proliferation of Caco-2 cells. By T.J. DALY, S.A. AHERNE, T.P. O'CONNOR and N.M. O'BRIEN, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Plant sterols are specific phytochemicals that resemble cholesterol in structure¹. The most abundant phytosterols in the diet include β -sitosterol, campesterol and stigmasterol. Plant stanols such as β -sitostanol, which are saturated derivatives of phytosterols, are also present in the diet but in lower quantities². There is a paucity of *in vitro* data in the literature relating to the anti-carcinogenic potential of these phytosterols. The viability of human colonic carcinoma Caco-2 cells following incubation with plant sterols for 24 h has previously been examined³. Thus, in the present study the effects of supplementing Caco-2 cells with a greater range of plant sterol concentrations for longer periods of time were investigated.

Caco-2 cells were cultured in Dubelcco's modified Eagle's medium and maintained in a humidified atmosphere at 37 °C. Cells were supplemented with increasing concentrations of campesterol, β -sitosterol or β -sitostanol for 48 h and 72 h. A wide range of plant sterol concentrations was used to reflect normal dietary intake ($\leq 10 \mu$ M), supplemental intake ($\leq 50 \mu$ M) and high pharmacological doses ($\leq 400 \mu$ M). Cell viability and proliferation were determined by the MTT assay (Roche Diagnostics, Mannheim, Germany).

Sterol concentration (μ M)	% Cell viability (control 100%)											
	Campesterol				β -Sitosterol				β -Sitostanol			
	48 h		72 h		48 h		72 h		48 h		72 h	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
5	95.8	3.3	90.4	4.2	95.0	6.3	90.1	5.4	91.6	4.2	90.1	2.7
10	90.2	2.9	88.5	3.2	96.8	6.7	89.2	4.4	94.5	5.4	92.2	1.9
25	85.5	4.5	84.0*	3.5	88.7	7.9	89.2	3.8	90.2	4.2	90.1	5.0
50	86.8	4.8	83.0*	3.5	87.4	6.9	89.9	4.4	86.3	2.0	84.7*	3.0
100	81.1	7.8	81.1*	3.6	77.7	8.6	83.2*	3.9	82.9	2.5	85.8*	4.0
200	83.1	9.4	69.1*	5.9	71.0*	5.8	76.7*	4.3	76.3*	5.2	80.5*	4.2
400	69.5*	8.1	51.5*	5.8	60.7*	5.6	59.5*	5.5	71.5*	5.5	69.1*	1.8

Means for six independent experiments. Mean values were significantly different from that for the control (one-way ANOVA, followed by Dunnett's test): * $P < 0.05$.

Following 48 h treatment only the higher concentrations of campesterol (400 μ M), β -sitosterol and β -sitostanol (200 μ M, 400 μ M) significantly decreased cell viability and growth. Increasing the incubation time with either plant sterols or plant stanol for a longer period (72 h) resulted in a significant decrease in the viability and growth of Caco-2 cells. To conclude, time-dependent effects of the plant sterols and plant stanol were observed on both cell viability and growth. Although campesterol, β -sitosterol and β -sitostanol had no major effect on cell viability and growth following 48 h supplementation, after a longer period of exposure (72 h) these plant sterols and stanol show potential toxic effects on Caco-2 cells.

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A comparison of the revised restraint scale, the three-factor eating questionnaire and the Dutch eating-behaviour questionnaire in predicting disinhibition. By C. MARTINS, E. TOLHURST and L.M. MORGAN, *Nutrition, Dietetics and Food Sciences Division, School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH, UK*

Dietary restraint has been associated, in the laboratory environment, with counter-regulatory eating in response to preloading. However, the results have been inconsistent and seem to be dependent on the test meal and type of questionnaire used to measure restraint. The present study compares the revised restraint scale (RRS)¹, the three-factor eating questionnaire-18R (TFEQ-18R)² and the Dutch eating-behaviour questionnaire (DEBQ)³ in their ability to predict disinhibition of restraint. Fifteen normal-weight females filled in these questionnaires and were classified as restrained or unrestrained based on a median split. Using a cross-over design, participants were randomly assigned to a high (HEP; 2800 kJ (607 kcal))-energy or low-energy preload (LEP; 1033 kJ (246 kcal)), and energy intake (EI) at an 'ad libitum' buffet with a variety of lunch-type foods (sandwiches, fruit, cake, crisps, yogurt, etc.) was measured 1 h later. Subjective appetite and cognitive and emotional state were also measured, using visual analogue scales, before and immediately after preloading and then at 20, 40 and 60 min after consumption of the preload.

A significant effect of preload, but no effect of restraint ($P < 0.01$), was observed in buffet EI, with a significantly higher intake after the LEP compared with the HEP, independent of the questionnaire (Figure). Unrestrained eaters showed a tendency (NS) towards a better compensatory response compared with restrained eaters. A high significant negative correlation ($r = -0.653$, $n = 15$, $P < 0.01$) was observed between energy compensation and restraint when using the RRS.

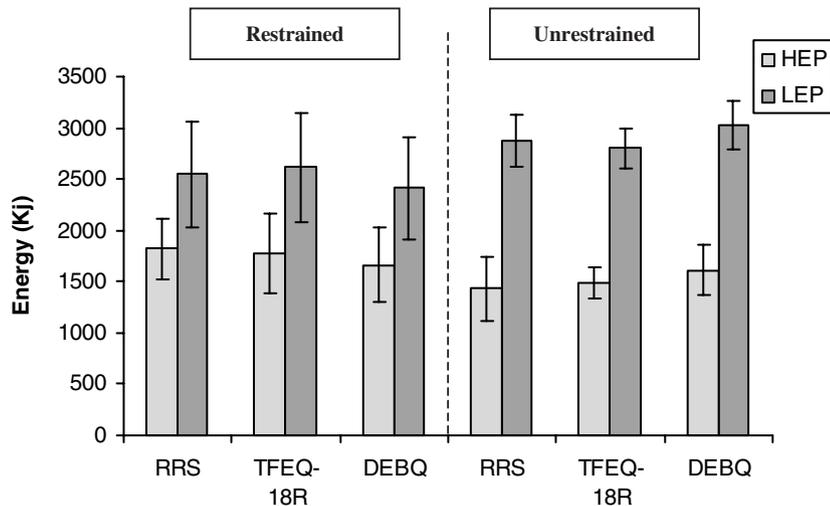


Fig. Energy intake (kJ) at the buffet lunch after the HEP and LEP in restrained (Rest) and unrestrained (Unr) participants classified according to three different questionnaires. Values are means with their standard errors represented by vertical bars. Analysis of variance showed a main effect of preload ($P < 0.01$ for all), but no main effect of restraint or interaction.

It is concluded that restrained females, regardless of the questionnaire used, do not show a disinhibitory eating behaviour, despite a less-accurate compensation than unrestrained eaters. Although the RRS was not able to predict disinhibition, the loss of compensation observed with restraint was best forecast by this scale. These results may be a reflection of the test meal used, with both unhealthy ('diet-breaking') and healthy foods being available.

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Ethnic-specific equations v. a generalised equation in Afro-Caribbeans using the BodPod (self testing). By N. MATHE¹, J. GANDY¹, D. MCCARTHY² and D.A. BRODIE¹, ¹*Research Centre for Society and Health, Buckinghamshire Chilterns University College, Gorelands Lane, Chalfont St Giles, Buckinghamshire HP4 8AD, UK* and ²*Department of Health and Human Sciences, London Metropolitan University, Holloway Road, London N7 8DB, UK*

The proportion of fat-free mass relative to fat is higher in Afro-Caribbean adults than in Caucasian adults¹. Thus, ethnic-specific formulae have been developed to calculate body fat from body density^{1,2}. The aim of the present study was to compare body fat, as estimated by air-displacement plethysmography, in Afro-Caribbean and Caucasian subjects using the default equation and ethnic-specific equations.

The body density of thirty Afro-Caribbean and thirty (age-, BMI- and gender-matched) Caucasian subjects was measured using air-displacement plethysmography (BodPod; Life Measurement Inc., Concord, CA, USA; self testing (S/T)). Body fat was calculated using the BodPod's default equation (Siri)³ and ethnic-specific equations for adult men (Schutte)¹ and women (Ortiz)². Statistical analysis was performed using Student's unpaired or paired *t* test as appropriate. The study was approved by the local ethics committee and all subjects gave written informed consent.

There was no significant difference in body fat between the two ethnic groups when the default equation³ was used for both groups and when ethnicity-specific equations were used for the Afro-Caribbean subjects. However, there was a significant difference ($P < 0.001$) when using the default equation (Siri)³ and the ethnic-specific equations (Schutte¹ and Ortiz²) in Afro-Caribbean adults, as shown in the Table.

	N	Equation used	Ethnicity	Mean	SD
Gender: Male	22				
Female	38				
Age (year)	30		Caucasian	29.4	9.6
	30		Afro-Caribbean	28.5	9.3
BMI (kg/m ²)	30		Caucasian	25.5	4.0
	30		Afro-Caribbean	25.8	3.8
Body fat (%)	30	Siri ³	Caucasian	31.1	8.7
	30	Siri ³	Afro-Caribbean	30.8	8.3
	30	Schutte ¹ /Ortiz ²	Afro-Caribbean	32.2***	8.3

Mean value was significantly different from that of Afro-Caribbean subjects estimated using the default equation of Siri³ (Student's paired *t* test); *** $P < 0.001$.

The default Siri³ equation and the ethnic specific equations of Schutte¹ and Ortiz² did not agree on the estimate for percentage body fat when used in Afro-Caribbean subjects.

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The effects of lignan-rich extracts on human Jurkat T-cells. By M. PHELAN¹, S.A. AHERNE¹, A. WONG² and N.M. O'BRIEN¹, ¹Department of Food and Nutritional Sciences, University College Cork, Republic of Ireland and ²Arbokem Inc., Vancouver, Canada

Recent research has shown that softwood knots (i.e. branch bases inside tree stems) of genus *Pinus* and *Picea* contain exceptionally large amounts of bioactive phenolic compounds such as free aglycones of lignans, oligolignans, stilbenes and flavonoids¹. They constitute a valuable resource with a potential for use as technical antioxidants, biological antioxidants in foodstuffs, functional food ingredients, pharmaceuticals and natural biocides². In the present study the effects of water extracts of Jack pine (*Pinus banksiana* Lamb.) knots and Sitka spruce (*Picea sitchensis*) knots on cell viability, growth and glutathione (GSH) content were investigated in Jurkat T-cells.

Human Jurkat T-cells were cultured in RPMI medium (Sigma Aldrich, Poole, Dorset, UK) and maintained in a humidified atmosphere at 37 °C. Cells were supplemented with increasing concentrations (5–500 µg/ml) of water extracts of Jack pine (Saskatchewan, Canada) knots and Sitka spruce (Co. Roscommon, Republic of Ireland) knots for 24 h. Cell viability and growth were determined by the MTT assay (MTT II Proliferation Kit, Roche Diagnostics, UK). Half maximal inhibitory concentrations (IC₅₀) were calculated and concentrations corresponding to >80% cell viability were selected for subsequent experiments. GSH content was measured in cells supplemented with water extracts of Jack pine knots and Sitka spruce knots (10 and 30 µg/ml) for 24 h at 37°. The content of the samples was analysed by GC.

Concentration (µg/ml)	% Cell viability (control 100%)				Cell growth (MTT reduction index)			
	Jack pine		Sitka spruce		Jack pine		Sitka spruce	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	100.0	0.0	100	0.0	0.62	0.04	0.76	0.04
5	94.2	5.1	90.7	1.7	0.61	0.03	0.67	0.03
10	93.9	5.6	89.3	3.4	0.58	0.04	0.67	0.03
15	88.9	5.1	89.1	4.1	0.54	0.04	0.67	0.04
30	82.0*	5.4	85.0*	3.9	0.46**	0.04	0.64	0.03
60	76.8**	2.4	78.9**	4.0	0.32**	0.04	0.58*	0.03
125	53.0**	3.3	71.1**	3.7	0.13**	0.04	0.53**	0.04
250	24.4**	3.6	61.8**	3.0	0.14**	0.01	0.47**	0.04
500	15.5**	6.9	39.1**	6.7	0.08**	0.02	0.31**	0.06

Means for three or more independent experiments. Mean values were significantly different from the control value (one-way ANOVA, followed by Dunnett's test): * $P < 0.05$, ** $P < 0.01$.

Jurkat cell viability decreased with exposure to increasing concentrations of Jack pine knots and Sitka spruce knots. Cell growth was inhibited with increasing supplementation of both samples. Jack pine knots had a greater inhibitory effect on growth compared with Sitka spruce knots. Jack pine knots were found to be more toxic than Sitka spruce knots, with IC₅₀ values of 153.0 µg/ml and 376.1 µg/ml respectively. Sitka spruce knots exhibited a greater effect on cellular GSH content than Jack pine knots (data not shown). Preliminary GC analysis showed that Sitka spruce knots contained more lignans but less flavonoids and stilbenes than Jack pine knots.

The water extracts were supplied by Arbokem Inc., Vancouver, Canada.

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Adherence to Mediterranean diet across the adult population of Symi aged 18–84 years. By K. JONES¹, B. ELLAHI¹, C. ANDROUHARA² and P.M. LUMB², ¹University of Chester, Biological Sciences, Parkgate Road, Chester, CH1 4BJ UK and ²Symi Health Centre, Yialos, Island of Symi, Greece and ²University of Chester, Department of Mathematics, Parkgate Road, Chester, CH1 4BJ UK

Adherence to a Mediterranean diet (MD) has been related to a lower mortality rate for CHD¹. A cross-sectional survey on a random sample of the adult Symiot population (18–84 years) was undertaken to examine the dietary patterns with a focus on adherence to the MD. A two-part self-reported diet habits and lifestyle questionnaire, incorporating socio-demographic questions and an FFQ, was used to assess adults.

Questionnaires ($n = 113$; 27% ($n = 30$) male, 63% ($n = 83$) female) were coded and analysed using SPSS version 14 (SPSS, Chicago, IL, USA). Mean BMI (self reported) were 27 (sd 3.99) kg/m² for males and 25 (sd 5.19) kg/m² for females. Prevalence of smoking was low (76% ($n = 86$) non-smokers, with two non-reporters), with 13% ($n = 15$) male smokers and 9% ($n = 10$) female smokers. Daily physical activity of 15–30 min was highest amongst females aged 25–44 years. Food habits were compared with the Greek adult population dietary guidelines² and the Mediterranean dietary pyramid³ (see Table).

Food groups	Mean no. of servings (week)		Recommended frequency of consumption and size of serving	Food groups	Mean no. of servings (week)		Recommended frequency of consumption and size of serving
	Mean	sd			Mean	sd	
Fruit	21.19	17.53	Three daily, 60–200 g	Red meat	2.49	1.79	Once daily, 60 g
Fish and seafood	6.03	5.81	Once daily, 60 g	Wholegrain cereals	5.45	5.34	Eight daily, 25–60 g
Olive oil	11.35	5.37	Daily as main lipid	Vegetables	26.63	15.57	Six daily 100 g
Legumes	3.37	3.67	3.5 weekly, 100 g	Sugary fatty foods	8.91	11.23	3.5 weekly, small

The dietary pattern observed is largely in harmony with the recommended servings for the Greek adult population and consistent with an MD. Oily fish, olive oil and fruit consumption were typical of recommendations. However, consumption patterns are characterised by a high frequency of red meat. Significant differences in the consumption of energy-dense foods within the age-groups was observed, with a tendency for the 18–24 and 35–44 year olds to have a preference for these foods. The older generation (65–84 year olds) do not show this tendency. Self reported data, recall bias and small sample size for age categories and gender all present limitations to the study.

Increased intakes of refined carbohydrates and red-meat consumption could result in higher energy intakes if the components are added to the habitual diets, or may cause concern if they displace nutrient-dense foods as a result of the availability of a Western diet. The findings are consistent with those of Sanchez *et al.*⁴, who found a 'Western' and a 'Spanish Mediterranean pattern', and suggested that younger, sedentary, more-educated and single male subjects were more likely to discard the traditional Mediterranean pattern in Spain. Symi is an island undergoing rapid development and, in particular, this raises the need for public health nutritionists to ensure the preventative health agenda is promoted that supports choosing healthier options from the Western diet, particularly with the younger generation.

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Associations between dietary intake of calcium and vitamin D, anthropometry measures and indices of bone health in Caucasian women: preliminary results from the D-FINES study. By P.A. LEE¹, K.Y.K. SIU¹, R. HIPGRAVE¹, D. DAVID¹, W.T.K. LEE¹, D.P. LOVELL¹, M. KIELY², K. CASHMAN², J.L. BERRY³ and S.A. LANHAM-NEW¹, ¹Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7XH, UK, ²Department of Nutritional Sciences, University College Cork, Cork, Republic of Ireland and ³Vitamin D Research Group, University of Manchester, Manchester M13 9WL, UK

Ca and vitamin D are two key nutrients for the optimisation of bone health throughout the lifecycle. Currently, there is no dietary reference value for vitamin D for the age-group 4–64 years and there is a considerable gap in the knowledge of exactly what dietary levels of vitamin D are required for optimisation of vitamin D status. These gaps are particularly evident in ethnic groups. It is known from the findings of the 1958 British Birth Cohort that hypovitaminosis is a problem in UK¹. In the last 10 years some countries have redefined their recommended values for vitamin D, e.g. the USA adequate intake recommendation for the age range 0–50 years is now set at 5 µg/d, rising to 15 µg/d for ≥70 year olds. There have also been data to suggest that a high Ca intake and/or high-dairy product consumption is associated with lower body weight and body fat distribution.

The D-FINES study (Vitamin D, Food Intake, Nutrition and Exposure to Sunlight in Southern England) is currently being undertaken, in which seasonal data have been collected for 242 Caucasian and seventy-two Asian women aged 19–70 years over a period of 12 months. Women were randomly recruited through general practitioners or through Asian community networks in Woking, Kingston and Thornton Health. The data collected include: fasted blood samples for assessment of 25-hydroxyvitamin D status (by HPLC), parathyroid hormone and bone turnover markers; dietary intake; sunlight exposure; physical activity; bone density; skinfold thickness. The aim of the preliminary investigation was to examine the association between dietary intakes of Ca and vitamin D and measures of lumbar-spine and femoral-neck bone mineral density (BMD), body weight and body fat distribution. Diet was assessed using a validated Ca and vitamin D FFQ, BMD was assessed using dual X-ray absorptiometry (DXA) and body fat was measured using skinfold callipers and DXA. Data are presented for forty-five Caucasian women.

Variable	Mean	SD	Variable	Mean	SD
Age (years)	48.8	12.9	Ca (mg/d)	582.4	263.3
Weight (kg)	71.3	12.9	Vitamin D (µg/d)	3.25	2.62
Height (m)	1.65	0.07	BMD (g/cm ²): Whole-body	1.12	0.08
BMI (kg/m ²)	26.4	5.1	Lumbar spine	0.98	0.13
% Body fat: Skinfold calipers	35.2	4.9	Femoral neck	0.79	0.13
DXA method	35.8	6.1	Ward's Triangle	0.66	0.15

As shown in the Table, the age range was 28–70 years and the mean BMI was 26.4 (SD 5.1) kg/m². The mean Ca intake was 582 mg/d with a range of 200–1266 mg/d. The mean vitamin D intake was 3.25 (SD 2.62) µg/d. There was good agreement between the two methods for calculating percentage body fat. Approximately 30% of women were osteopenic at the lumbar-spine and femoral-neck BMD sites (according to WHO criteria²). No associations were found between Ca and vitamin D dietary intakes and indices of bone health, and a high intake of Ca was not associated with lower body weight. Further analysis of the full data-set is currently underway.

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Glycaemic load of breakfast and cognitive function in adolescent children. By R. MÍCHA¹, P.J. ROGERS² and M. NELSON^{1,3}, ¹Nutritional Sciences Research Division, King's College London, 150 Stamford Street, London SE1 9NH, UK, ²Department of Experimental Psychology, University of Bristol, Bristol BS8 1TH, UK and ³School Food Trust, N9 Moorfoot, Sheffield S1 4PQ, UK

Breakfast has traditionally been considered 'the most important meal of the day'. Studies looking at breakfast omission and cognitive function (CF) have provided evidence that supports the view that breakfast omission has an adverse effect on cognition and learning^{1,2}. The findings are less clear on the effects of variation in the macronutrient composition of breakfast on CF after an overnight fast. The brain appears to be sensitive to short-term fluctuations in the glucose supply, and it could be argued that a low-glycaemic-index (GI) breakfast would minimize glycaemic fluctuations, and as such facilitate performance in the period following consumption; the glycaemic load (GL) may also be an influence. A cross-sectional study was carried out to test whether the GI and GL of breakfast affect CF. Sixty children aged 11–14 years were selected on the basis of being regular breakfast eaters. They were categorized into four groups according to the GI and GL of their breakfast reported by recall: low GI–high GL, high GI–high GL, low GI–low GL and high GI–low GL (above or below the median for GI of 60.6 and for GL of 27.5). Consumption of a low GI–high GL breakfast was associated with better performance in a majority of the CF tests 90–120 min later. The effects were significant for the most-cognitively-demanding tasks: speed of information processing ($P=0.001$); serial sevens ($P<0.001$).

In order to establish a causal and more precise association between GI and GL and CF the same hypothesis was tested in an intervention study. Four breakfast meals differing in their GI and GL (see Table) were administered in thirty-two pairs of matched children in a cross-over design (32 males, 32 females). Mean (SEM) age (y), height (cm) and BMI (kg/m²) were 12.7 (0.07), 156.9 (0.85) and 19.6 (0.28), respectively. The GI and GL of the breakfast meals as well as the foods that constituted the meals were modelled on what the children had reported eating in the cross-sectional study. (The glycaemic and insulinaemic responses of these meals were tested in young adults; R Micha, A Leeds and M Nelson, unpublished results.) The children were matched in pairs for gender, year group, age (± 6 months), height (± 0.05 m) and BMI centile. Each matched pair was randomly assigned to a low-GL or high-GL breakfast. Within each GL group children were given high-GI or low-GI breakfasts. All children were seen three times; on their first visit they were interviewed, height and weight was measured and a saliva sample collected to measure cortisol (a marker for stress, a potential confounder of CF). On two further occasions children were given either the low-GI or high-GI meal in a randomised order. The same CF tests used in the cross-sectional study (in two different versions for each breakfast occasion) were administered in randomised order 90 min after breakfast; word generation task, memory recall (immediate), stroop task, matrices, speed of information processing, serial sevens, memory recall (delayed). Mood was also assessed on arrival and immediately before and immediately after the battery of CF tests. Salivary cortisol, blood glucose (BG) levels and Hb were measured at baseline and before and after the CF tests. Capillary BG measurements differed significantly between the high-GL and low-GL meals (repeated measures ANOVA); the high-GL meals had higher glucose responses in comparison with the low-GL meals both before and after the CF tests ($P=0.005$ and $P=0.004$, respectively).

The present study is the first of its kind to address the effect of both GI and GL on CF, and on such numbers of participants. Important confounding factors have been taken into account such as glucoregulation, BG levels, cortisol and iron status, which have not been controlled for in other studies. The anticipated benefits are to determine the importance of the GI and GL of breakfast on the CF and mood of school children.

Breakfast meals...	High GL–Low GI	High GL–High GI	Low GL–Low GI	Low GL–High GI
GI meal	48	61	48	61
GL meal	41	55	21	28
Energy (kJ)	1963	1959	1175	1152
Protein (g)	13.9	13.9	12.5	12.0
Fat (g)	7.0	5.3	6.7	5.1
Total carbohydrate (g)	86.7	90.4	43.2	45.2

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Diet and lifestyle as triggers for heartburn: patient perception. By K.L. OLIVER¹, G.J. DAVIES¹ and P.W. DETTMAR², ¹Nutrition Research Centre, London South Bank University, London, UK, SE1 0AA, UK and ²Technostics, The Deep Business Centre, Tower Street, Hull, East Yorkshire, HU1 4BG UK

Previous studies have determined that diet and lifestyle factors can contribute to the onset of symptoms of gastro-oesophageal reflux disease^{1,2}. It is uncertain which factors are the most influential to the onset of heartburn as an independent gastrointestinal disorder³. Thus, there is a need to define these factors in order to minimise the risk of progression of heartburn to gastro-oesophageal reflux disease, Barrett's oesophagus and oesophageal adenocarcinoma⁴.

The aim of the present study was to investigate the perceived symptom trigger factors from individuals suffering from heartburn. Fifteen Caucasian volunteers, ten females of mean age 51.7 (range 37–62) years and five males of mean age 57.6 (range 41–64) years, participated in focus-group interviews at The Luton and Dunstable Hospital NHS Trust. Subjects were recruited from poster advertisements displayed throughout the hospital and from a local general practitioner clinic in Luton, Bedfordshire. Subjects who were self-diagnosed were screened with the use of a questionnaire, and those subjects who presented with frequent heartburn were invited to participate. The focus-group interviews were tape recorded and transcribed for analysis. Ethical approval was gained from the Bedfordshire Local Research and Ethics Committee.

Multiple factors were perceived by heartburn sufferers as triggering their symptoms. The key dietary factors reported by participants included: eating late (46%), the consumption of: bread (33%); fatty foods (33%); spicy foods (33%); large meals (33%); the high temperature of food and beverages (33%). The key lifestyle factors reported by participants included: alcohol (60%); posture (60%); weight gain (46%); stress (40%).

The findings of the study identify that there are several symptom triggers for heartburn as an independent gastrointestinal disorder, which are in accordance with those previously reported for gastro-oesophageal reflux disease as a whole⁵. This suggests that these diet and lifestyle factors may be contributory to the progression of heartburn to gastro-oesophageal reflux disease. In addition to these findings, new symptom triggers for heartburn have come to light: consumption of bread; hot temperature of food and beverages. The findings for the consumption of bread are in contrast to reports that because of its high fibre content bread may reduce the risk of gastro-oesophageal reflux disease^{1,2}. The consumption of food and beverages at a high temperature has only previously been associated with the risk of oesophageal cancer⁶. Further work is needed to determine whether these perceived trigger factors are valid by using a specifically-designed symptoms and triggers diary focusing on both diet and lifestyle in patients with heartburn.

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Nutritional status of adults in north-west Iran. By M. PAKSERESHT¹, J.E. CADE¹, D. FORMAN¹, R. MALEKZADEH² and A. SADJADI², ¹Centre for Epidemiology and Biostatistics, Leeds University, Leeds LS2 9LN, UK and ²Shariaty Hospital, Tehran, Iran

North-west Iran has been recognized as a high-risk area for gastric cancer, with Ardabil Province having some of the highest rates¹. Stomach cancer is one of the two major cancers, the risk for which may be modified by food and nutrition. However, few studies have described nutritional status in this area and no published studies have explored the link between diet and gastric cancer in Ardabil. A randomly-selected control group from a recent case-control study on the relationship between food and gastric cancer in Ardabil was investigated to explore food and nutrient intake in adults from the province.

A sample of 199 men and eighty-one women aged 35–84 (mean 63 (sd 10.7)) years completed a validated semi-quantitative FFQ to assess diet over the previous year, with additional questions on health and lifestyle. Height and weight were measured and mean BMI was 27 (sd 4.6) kg/m², with 42% overweight and 23% obese. On average women had significantly higher BMI than men (28.8 kg/m² v. 26.4 kg/m², $P < 0.0001$). BMI varied by age-group, peaking at 55–59 years ($P = 0.002$ for trend). Education, income and domicile (urban or rural) had no significant effect on BMI distribution. A regression model adjusting for age, gender, smoking, education and income showed that energy intake (EI) was a strong predictor for BMI ($\beta = 0.3$ for each 100 kcal intake, $P < 0.0001$). Protein and carbohydrate (CHO), but not fat, intakes were also strong predictors of BMI ($\beta = 0.5$ for each 10 g intakes of protein, $P = 0.01$ and $\beta = 0.1$ for each 10 g intakes of CHO, $P = 0.03$). The total weight of food consumed was 3037 g/d on average, with 46%, 19%, 15%, 12% and 8% coming from foods containing fat and sugar; fruits and vegetables; bread, cereals and potatoes; dairy products; and meat, fish and pulses respectively. The mean daily EI was 10 990 kJ (2627 kcal), of which 57%, 27% and 14.5% came from CHO, fat and protein respectively (Table). The average proportion of EI to basal metabolic rate in men and women (1.43) indicated a sedentary lifestyle. Nutrient intakes were higher in men compared with women. There were significant positive relationships between most nutrient intakes and levels of education and income, and a negative relationship with age-group. Food storage and cooking practises were examined. Of the participants 50% kept meat frozen before cooking, 44% used a refrigerator and the remainder did not store meat before cooking. Boiling was the most popular method for cooking food (52%), followed by frying (43%) and barbecuing (5%). The consumption of seeds, which have been highly salted or dry fried for a long time or at high temperature, were greater in men v. women and in urban residents v. rural residents.

		EI (kJ/d)			% EI from		Protein (g/d)	Fibre (g/d)	Vitamin C (mg/d)	Fe (mg/d)	Folate (µg/d)	Ca (mg/d)	Zn (mg/d)
		30–59 years	60–74 years	>74 years	CHO	Fat							
M	Ardabil	12350	11397	10347	56.5	27.4	99	17.6	112	17.4	288	1076	16.8
	DRV	10659	9844	8778	47–50	33–35	54	11.2	40	8.7	200	700	9.5
F	Ardabil	10238	9623	8443	56.7	26.6	85	15.6	87	16.7, 15.3*	249	918	14.6
	DRV	8026	7942	7566	47–50	33–35	46	12.5	40	14.8, 8.7	200	700	7

M, males; F, females; DRV, UK dietary reference values². * Values for ≤50 years and >50 years age-groups respectively.

The prevalence of overweight in north-west Iran is one of the highest in this part of the world. The average macronutrient intake is up to two times more than recommended values and EI is a significant predictor for BMI. The impact of this high BMI and specific dietary habits should be targeted in further studies to explore the relationship between diet and chronic diseases.

This work was funded by a grant from the World Bank and Digestive Diseases Research Centre, Iran.

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Dietary antioxidant intake among young Greek female smokers and non-smokers. By V. COSTARELLI, F. KARYSTINOI, C. NOUTSOI, N. YIANNAKOYRIS and M.G. VAMVAKARI, Department of Home Economics and Ecology, Harokopio University, 70 El. Venizelou Ave, 176 71 Kallithea, Athens, Greece

The prevalence of smoking among young females in Greece is one of the highest in Europe¹. It has been observed that smokers have circulating concentrations of dehydroascorbic acid (a marker of vitamin C depletion) that are >1000 times higher than those of non-smokers, together with mean circulating plasma levels of β -carotene, γ -carotene and cryptoxanthin that are 25% lower^{2,3}. Another reason for the poor antioxidant status of smokers is their well-documented general unhealthy lifestyle patterns and poor dietary habits⁴. The purpose of the current study was to investigate the dietary antioxidant intake and general dietary patterns of young female university student smokers v. non-smokers. Dietary intake was measured using the 4 d weighed-inventory method. Body weight and height measurements were also recorded. The Table summarizes the baseline characteristics of the subjects and the main findings.

	All subjects (n 60)		Smokers (n 30)		Non-smokers (n 30)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	21.88	1.93	22.63	2.02	21.13	1.52
BMI (kg/m ²)	21.08	2.03	21.13	2.33	21.03	1.72
Smoking (no. of cigarettes per d)	–	–	11.83	6.62	–	–
Energy intake (kJ/d)	6466	1396	6329	1596	6609	1170
Vitamin E (mg)	4.44	1.74	4.05	1.41	4.85	1.96
Vitamin A (μ g)	691.2	127.2	640.5	89.4	741.8	115.7
Vitamin C (mg)	76.5	47.5	70.8	39.6	82.14	54.4
Carotenoids (μ g)	915.7	456.7	889.5	386.4	941.9	523.2
Zn (mg)	8.91	2.97	8.68	3.24	9.15	2.71
Se (μ g)	45.9	20.6	41.33	12.10	50.46	26.01
Folic acid (μ g)	159	45	138.9	29.8	179***	49
Vitamin D (μ g)	2.1	2.44	1.47	0.71	2.73*	3.28
Ca (mg)	834	262.6	749.76	210.3	918.2*	285
Fe (mg)	7.20	1.83	6.69	2.06	7.71*	1.42
NSP (gr)	6.53	2.62	5.69	1.80	7.37*	3.04

Mean values were significantly different from those for smokers (independent sample *t* test): * $P < 0.05$, *** $P < 0.001$.

The data suggest that smokers consume less of the key nutrients such as folic acid, Fe and Ca and less dietary fibre than non-smokers; however, there were no significant differences in the consumption of antioxidants nutrients between the two groups. The smokers were also consuming less fruits and vegetables and less dairy products than the non smokers. As a result of the reported susceptibility of smokers to low antioxidant status, it is important to note that the antioxidant intake of young female smokers is not satisfactory and should be increased.

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Comparison of daily nutrient intakes estimated from 7 d food diaries with that estimated from the first 4 d. By F. O'NEILL¹, A. MAVROEIDI¹, D.M. REID¹ and H.M. MACDONALD¹, ¹Osteoporosis Research Unit, School of Medicine, University of Aberdeen, Aberdeen AB25 2ZD, UK

The current gold standard for estimating dietary intakes is generally agreed to be the 7 d weighed-food record¹. Food diaries completed for 7 d with estimated food-portion sizes are considered a good substitute, but they are still burdensome for the participant and the researcher. The 4 d food diary is a less-onerous alternative, but it is advisable to include at least one weekend day². The aim of the present study was to determine the average daily nutrient intake from 7 d food diaries and compare it with the average nutrient intake calculated from the first 4 d of the diary.

A total of 359 post-menopausal women (age 58–65 years) were recruited to participate in a longitudinal study examining the role of diet and sunlight on vitamin D status. At each visit the women were asked to complete a food diary for 7 d. For this investigation seventy-two diaries completed between June and August 2006 were analysed. The food diary (which included photographs for portion-size estimation) was designed for the European Prospective Study into Cancer³ and was analysed for nutrient content using the WinDiets program (The Robert Gordon University, Aberdeen, UK). Most of the nutrients were normally distributed, with the exception of vitamins A, C and D, which were log-transformed before testing for differences by paired *t* test (SPSS version 15; SPSS, Chicago, IL, USA).

The intakes for most nutrients were slightly but not significantly greater for the estimation for the first 4 d compared with the 7 d estimation. There was a significant difference ($P < 0.05$) in the amount of NSP consumed in the first 4 d compared with the full week. There is no obvious explanation for this difference. For 80% of subjects the 4 d included one weekend day and for 56% it included both weekend days. For K and vitamin D the intakes were marginally less if collected over 4 d instead of 7 d, but the difference was not significant.

Average daily intake	4 d				7 d				Difference	<i>P</i> †
	Mean	SD	Min	Max	Mean	SD	Min	Max		
Energy (MJ)	7.1	1.8	3.8	12.1	7.0	1.7	4.1	12.2	-0.1	0.348
Fat (g)	62	19	27	119	62	18	22	126	0	0.874
Protein (g)	69	18	30	107	69	16	35	104	0	0.907
CHO (g)	214	77	91	480	211	70	114	490	-3	0.280
NSP (g)	12.5	4.1	5.4	24.9	12.1	3.6	4.9	25.1	-0.4	0.020
Ca (mg)	808	253	336	1500	796	232	317	1442	-12	0.319
K (mg)*	3228	1020	1532	5773	3262	1053	1654	7799	+34	0.627
Mg (mg)	272	79	128	507	273	85	136	683	+1	0.884
Vit D (μ g)*	3.3	3.1	0.8	23.3	3.4	2.8	0.8	20.9	+0.1	0.335
Vit C (mg)*	190	164	34	837	187	136	33	635	-3	0.713
Folate (μ g)	250	98	95	556	246	83	116	473	-4	0.351
Vit A (μ g)*	1093	1696	166	13300	1057	1146	180	8248	-36	0.728
Zn (mg)	8.2	2.1	3.9	13.9	8.2	1.9	4.3	12.4	0	0.592

CHO, carbohydrate; vit, vitamin; min, minimum; max, maximum. * Log-transformed for *t* test. † Paired *t* test.

In conclusion, for most nutrients there is no advantage to collecting detailed food consumption over one full week. Although 7 d collection may pick up foods that are not eaten every day, it is also likely that subjects are less careful in their recording towards the end of the food-recording exercise.

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Dietary glycated protein modifies detrimentally the human colonic microbiota. By J.M. AMES¹, D.J.S. MILLS², K.M. TUOHY² and G.R. GIBSON², ¹*School of Biological Sciences, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast BT9 5AG, UK* and ²*School of Chemistry, Food Biosciences and Pharmacy, The University of Reading, Whiteknights, Reading RG6 6AP, UK*

The colon is an authentic organ of health and nutrition. As a result of its resident microbiota, it is the most metabolically-active organ in the body. The colon is the site of several acute bacterial infections. Various chronic diseases, including inflammatory bowel diseases, such as ulcerative colitis, are considered to have a bacterial aetiology. Dietary carbohydrate profoundly affects the gut microflora composition but the effect of dietary protein, including glycated forms, is uncertain. The present study was designed to test the hypothesis that glycated protein has detrimental effects on the profile of the human colonic microflora. To test this hypothesis aqueous solutions of BSA (1 mM) were heated with glucose (0.4 M) at 50 °C for 24 h to produce glycated protein (BSA/G), which was subjected to *in vitro* mouth, stomach and small intestine digestion. BSA heated without glucose (BSA/C) and native BSA (BSA/N) were used as controls. Fermentation of undigested material was performed using a validated multi-chamber gut model inoculated with mixed faecal bacteria obtained from patients with ulcerative colitis (*n* 6) or control subjects (*n* 6). Bacteria were enumerated using 16S rRNA sequencing probes.

Compared with healthy subjects, faecal samples from patients with ulcerative colitis contained higher numbers of bacteria considered to be detrimental to health, e.g. sulfate-reducing bacteria and clostridia, but lower numbers of bifidobacteria, which are considered to confer health benefits. Following fermentation in the gut model glycated protein modified the gut flora of patients with ulcerative colitis to a more detrimental profile. Increase/decreases in numbers of cells per mL of fermentation medium (expressed as log₁₀) following treatment with each protein are given in the table. Numbers of sulfate-reducing bacteria (SRB), and clostridia (Clos) were higher ($P < 0.009$) and numbers of putatively beneficial bacteria (bifidobacteria, Bif) were lower ($P < 0.0004$) with glycated protein compared with native BSA. In conclusion, glycated protein adversely affects the profile of the gut flora in an *in vitro* gut model. Animal and human dietary intervention studies are required to investigate whether this effect can be replicated *in vivo*.

This study was funded by the Wellcome Trust.

	BSA/N			BSA/C			BSA/G		
	SRB	Bif	Clos	SRB	Bif	Clos	SRB	Bif	Clos
Median	0.19	-0.33	0.23	0.21	-0.38	0.17	0.32	-0.44	0.47
Interquartile range	0.12	0.13	0.21	0.17	0.14	0.28	0.17	0.12	0.13
Range	0.38	0.37	0.68	0.42	0.48	0.57	0.24	0.50	0.48

Strategy to alter the amount and composition of dietary carbohydrate and fat in free-living individuals participating in the RISCK trial. By C. MOORE¹, R. GITAU², G.S. FROST³, B.A. GRIFFIN⁴, S.A. JEBB¹, T.A. SANDERS⁵ and J.A. LOVEGROVE², ¹*Nutrition and Health Research Group, MRC, Cambridge CB1 9NL, UK*, ²*Department of Food Biosciences, University of Reading, Reading RG6 6AP, UK*, ³*Nutrition and Dietetic Research Group, Imperial College, London W12 0HS, UK*, ⁴*School of Biomedical & Molecular Sciences, University of Surrey, Guildford GU2 7XH, UK* and ⁵*Nutritional Sciences Research Division, King's College London, London SE1 9NH, UK*

The metabolic syndrome (MS) affects a significant proportion of the UK population and confers substantial risk of type 2 diabetes and CVD. There is evidence that the amount and quality of dietary fat can modify some features of MS, including blood lipids, insulin resistance, hypertension and endothelial function¹. A key scientific and public health question is whether reducing intakes of SFA via low-fat high-carbohydrate (CHO) diets or by moderate-fat diets in which SFA are substituted with MUFA have differential effects on risk factors for MS. The debate so far has failed to address the issue of dietary CHO quality, specifically the glycaemic index (GI) of the CHO. The RISCK study is a large-scale multi-centre dietary-intervention study designed to test the relative effects on insulin sensitivity of five isoenergetic diets (Diet A: High SFA/High GI; Diet B: High MUFA/High GI; Diet C High MUFA/Low GI; Diet D: Low Fat/High GI; Diet E: Low Fat/Low GI). The target intake for total fat was 38% energy in Diets A, B and C and 28% energy in Diets D and E, with a higher target intake for total CHO (55% v. 45% energy) in the low fat diets. A key objective of the study was to develop and implement an intervention strategy with minimal disruption to the usual dietary habits in free-living individuals for a 7-month intervention period.

A fundamental tenet of the study was to allow volunteers to eat *ad libitum* but to make appropriate substitutions prescribed for the individual dietary regimens. A food-exchange model was designed based on data from the National Diet and Nutrition Survey². The major 'exchangeable' dietary sources of fat (including added fats: spreads and cooking oils; cheese; milk; biscuits; cakes; buns and pastries) were removed and replaced by study foods with a specific fatty acid profile. Cooking oils, spreads, baking fats and mayonnaises were specially formulated for the study by Unilever Bestfoods (Crawley, West Sussex, UK). Volunteers were also given specifically-chosen commercially-available snacks to replace those normally consumed. The remaining fat replacement was achieved through an appropriate exchange of full-fat or low-fat dairy products. To achieve the isoenergetic targets, changes in the amount of CHO intake were also necessary. An exchange system was used to manipulate GI and volunteers were provided with CHO foods either high (diets A, B and D) or low in GI (diets C and E) to substitute into their habitual diet. CHO foods provided included breakfast cereals, breads, rice, pasta and potatoes; foods estimated to contribute approximately 56% to CHO intakes².

After eligibility into the study was confirmed, volunteers completed a 4 d diet diary to record baseline intakes. The nutritionists at each centre gave one-to-one dietary advice, using information gathered from the baseline diary to tailor advice to individual volunteers. Participants were provided with a booklet to supplement the verbal information and the advice was reinforced during fortnightly food-collection appointments. The 4 d diet diaries were also completed on three further occasions during the course of the study to assess compliance to the dietary interventions. In addition, the measurement of plasma phospholipid-fatty acid status will give biological marker of compliance specific to the dietary fat manipulation.

The RISCK study was funded by the Food Standards Agency, UK.

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The metabolic responses to moderate-intensity walking and subsequent food intake following a high-glycaemic-index or low-glycaemic-index breakfast in sedentary females. By E.J. STEVENSON¹, N.M. ASTBURY², E.J. SIMPSON², M.A. TAYLOR² and I.A. MACDONALD², ¹*School of Psychology and Sports Sciences, Northumbria University, Newcastle Upon Tyne NE1 8ST, UK* and ²*School of Biomedical Sciences, University of Nottingham, Nottingham NG7 2UH, UK*

Previous research has reported that the ingestion of a low-glycaemic-index (LGI) meal before moderate- to high-intensity exercise results in an increased rate of fat oxidation during the exercise period in trained men^{1,2} and women³. It is not known whether this phenomenon occurs during low-intensity exercise and in untrained subjects. Studies have also failed to investigate whether substrate oxidation following the exercise period is altered. The effects of pre-exercise mixed breakfasts containing high-glycaemic-index (HGI) or LGI carbohydrates on substrate utilisation during rest and low-intensity walking exercise were examined in sedentary women. The metabolic responses to a standard lunch consumed after exercise were also investigated.

Eight healthy sedentary eumenorrhic women (mean age 23.8 (SD 7.2) years, BMI 21.3 (SD 1.9) kg/m², V_{O2max} 33.1 (SD 5.1) ml/kg per min) completed two trials in a randomised cross-over design. On each occasion, subjects were provided with a test breakfast 3 h before walking for 60 min at 50% V_{O2max} on a motorised treadmill. Both breakfasts provided 1 g carbohydrate/kg body mass and were isoenergetic. The calculated GI of the meals was 78 (HGI) and 44 (LGI). Following exercise subjects were provided with a standard pasta lunch and remained in the laboratory for a further 2 h. Visual analogue scales were completed before and after each meal and at 30 min intervals during the postprandial periods. Data was analysed by repeated measures ANOVA and *post hoc* Holm-Bonferroni step-wise test.

Plasma glucose and serum insulin responses (incremental area under the curve) were higher during the 3 h postprandial period following the HGI breakfast compared with the LGI breakfast ($P < 0.05$). Following both the HGI and LGI breakfasts fat oxidation was suppressed from fasting values and carbohydrate oxidation increased. Fat oxidation remained below fasting values throughout the 3 h postprandial period but was higher in the LGI trial compared with the HGI trial ($P < 0.05$). During walking exercise the total amount of fat oxidised was greater in the LGI trial compared with the HGI trial ($P < 0.001$), and conversely the total amount of carbohydrate oxidised was higher in the HGI trial compared with the LGI trial ($P < 0.005$). Following the standard lunch fat oxidation remained elevated in both trials despite a large carbohydrate intake. There were no differences in the metabolic responses to lunch between trials. However, subjects reported feeling fuller over the 2 h postprandial period following lunch in the LGI trial compared with the HGI trial ($P < 0.05$).

In conclusion, consumption of a LGI breakfast 3 h before 60 min of low-intensity exercise resulted in a higher rate of fat oxidation both at rest and during exercise in sedentary females compared with when an isoenergetic HGI breakfast was consumed. Consumption of a LGI breakfast also resulted in increased rating of satiety at a subsequent meal. Further investigation is required into the chronic effects of a combination of a LGI diet and exercise on substrate metabolism and appetite regulation.

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The impact of plant-derived flavonoids on mood, memory and motor skills in UK adults. By P.S. HOW², R. COX², J.A. ELLIS¹ and J.P.E. SPENCER², ¹*School of Psychology and Clinical Language Sciences, University of Reading, Whiteknights, Reading RG6 6AL, UK* and ²*School of Chemistry, Food Biosciences and Pharmacy, University of Reading, Whiteknights, Reading RG6 6AP, UK*

Cognitive changes are a normal part of healthy ageing. However, neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease cause rates of change in excess of that considered normal. The number of diagnoses of these diseases is increasing, with 6–8% of >65 year olds diagnosed with Alzheimer's Disease¹.

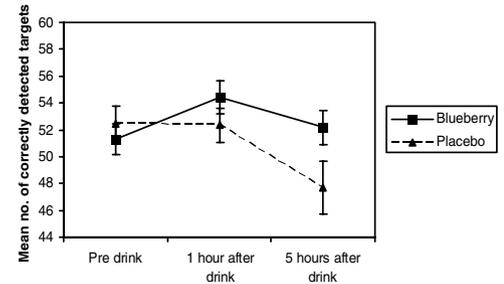
Recent dietary-intervention studies, in particular those using *Vitis vinifera* (grape), *Camellia sinensis* (tea), *Theobroma cacao* (cocoa) and *Vaccinium spp.* (blueberry) in human subjects and animals, have demonstrated potentially-beneficial effects on vascular function and mental performance². Recently, it has been demonstrated that older rats fed a blueberry extract for 12 weeks show significant improvements in spatial working memory and have higher hippocampal levels of brain-derived neurotrophic factor. The present study is the first controlled human investigation into the potential of flavonoid-rich blueberries to influence cognitive function.

Using a double-blind placebo-controlled randomised cross-over design an acute intervention trial was carried out with young adults (18–30 years) using a one-off supplementation of blueberry (200 g blueberries in 150 ml milk) or a placebo control. The effect on mood, memory, executive function and motor skills was measured before the drink and 1 h and 5 h after the drink.

Although most tasks showed no effect of the blueberry supplementation, there was a trend for improvement in attention, using the Go-NoGo task³ as the measure of this executive function (see Figure). The mean number of correctly-detected targets, a measure of attention, showed a trend for an increase in accuracy of detection following blueberry supplementation compared with a decline in attention after the placebo ($P = 0.06$, $n = 12$). Speed of detection was not affected by time or type of drink ($P > 0.05$). In general, measures of spatial and working memory ($P = 0.04$) and motor performance ($P = 0.006$) showed significant improvements over time, irrespective of the type of drink.

It is concluded that acute supplementation of young healthy individuals with blueberry does not significantly improve widespread cognitive performance. However, there was a tendency for an improvement in attention without changes in reaction speeds. Cognitive decline is not expected in young adults, which may explain these findings. The second phase of the study will test older adults (60–75 years) using the same measures and will assess the potential to increase cognitive performance following a chronic supplementation.

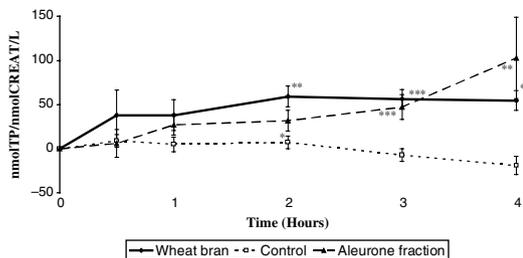
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Assessment of antioxidant biomarkers in human plasma and urine after consumption of wheat bran and aleurone fractions. By L.L. HAMILL, E.M. KEAVENEY, R.K. PRICE, J.M.W. WALLACE, J.J. STRAIN and R.W. WELCH, *Northern Ireland Centre for Food and Health (NICHE), School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, UK*

Epidemiological evidence, suggests that increased consumption of wholegrain foods may help prevent heart disease and diabetes¹. However, the mechanisms underlying these beneficial effects are poorly understood. Apart from being high in cereal fibre, wholegrain products are also rich in bioactive components, including micronutrients and phytochemicals. These components are concentrated in the outer bran layers, with the aleurone fraction being particularly rich in phytochemicals, which have been shown to exhibit antioxidant activity *in vitro*. The aim of the present study was to assess the effects of consumption of wheat bran and a wheat aleurone fraction on postprandial antioxidant potential.

Using a randomised within-subject cross-over design thirteen healthy adults (six male, seven female; age 21–43 years) were studied on three occasions at least 1 week apart. Before each study day subjects consumed a low-phenolic diet for 2 d. On the three study days subjects consumed a meal of 50 g wheat bran (Bühler, Zurich, Switzerland), wheat aleurone fraction (Bühler) or a control product. Blood and urine samples were taken at baseline (0) and at 0.5, 1, 2, 3 h after the meal; with an additional urine sample at 4 h. Samples were analysed for total antioxidant potential by the ferric-reducing ability of plasma (FRAP) assay. Urinary total phenolics (TP) was assessed by the Folin-Ciocalteu method.



Values are means (SE). Data were analysed by repeated measures ANOVA. * denotes statistically different from control ($p < 0.05$), ** denotes statistically different from control ($p < 0.01$), *** denotes statistically different from control ($p < 0.001$).

Fig. 1. Change over time in urinary TP excretion, after meal consumption compared to control.

Compliance was good, with volunteers consuming on average 48.8 g of the three meals. There were no significant differences in plasma ($P = 0.703$) or urinary ($P = 0.515$) FRAP following consumption of wheat bran or wheat aleurone fraction compared with the control. However, urinary TP showed significant postprandial increases following consumption of the bran or aleurone fractions compared with the control.

In contrast to previous research, which reported a significant increase in FRAP following consumption of 100 g wheat bran², no increase in FRAP was found in the current study, perhaps because of the smaller portion of wheat bran provided to volunteers. However, following consumption of the bran and aleurone fractions increases in TP were found in urine. Results suggest that antioxidants may play a role in the beneficial effects of wholegrain consumption on disease processes.

This study was approved by University of Ulster Research Ethical Committee and is financially supported by European Commission in the Communities 6th Framework Programme project HEALTHGRAIN (FP6–514006).

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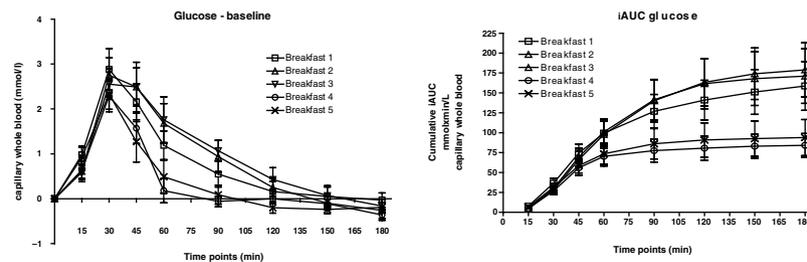
Glycaemic potency of composite breakfast meals. By R. MICHA, A. LEEDS and M. NELSON, *Nutritional Sciences Research Division, King's College London, 150 Stamford Street, London SE1 9NH, UK*

The concept of glycaemic index (GI) for classifying individual carbohydrate (CHO)-containing foods was introduced by Jenkins in 1981¹. The glycaemic load (GL) is a concept that takes into account both the quantity and the quality of CHO. From appropriate FAO equations², it can be shown that a low-GI–high-CHO food and a high-GI–low-CHO food can produce the same GL. Thus, these two concepts should not be used in isolation, but in conjunction, when determining the glycaemic potency (blood glucose-raising potential) of a food or a meal. The aim of the present study was to measure the glycaemic and insulinaemic response of mixed meals and to correlate these measures with the GL and GI values obtained by the FAO equations.

Ten healthy young adults (five males, five females) were recruited to investigate the postprandial responses over a period of 3 h after the ingestion of five different breakfast meals (see Table), and to investigate the validity of methods for calculating GI and GL by measuring the area under the glucose and insulin curve above the fasting level (iAUC). The subjects had a mean age of 24.3 (SD 3.9) years and BMI of 22.2 (SD 1.9) kg/m². The breakfast meals differed in their GI and GL: low-GI–high-GL (1); high-GI–high-GL (2), of similar GL to meal 1; a high-GI–high-GL (3), of similar energy and macronutrient composition to meal 1; low-GI–low-GL (4); high-GI–low-GL (5); the low-GL meals were of similar GL and macronutrient composition. Each subject received all five breakfast meals in a randomized order. Capillary and venous blood samples were collected at baseline (fasting) and 15, 30, 45, 60, 90, 120, 150 and 180 min after breakfast. At each time point measurements were undertaken of capillary (whole blood and plasma) and venous blood glucose and venous insulin and cortisol. A full blood count was also performed on the first and last visit.

Capillary whole-blood glucose measurements and venous insulin responses differed significantly between the high-GL and low-GL meals (repeated measures ANOVA); the high-GL meals had higher glucose and insulin responses in comparison with the low-GL meals. Glucose and insulin iAUC were positively correlated ($r = 0.673$, $P = 0.000$). GL was a strong predictor of glucose and insulin iAUC, both in the early (0–2 h) and the late (2–3 h) postprandial period. GI differences were not significant; however, these were in the expected direction.

Breakfast meals...	Low-GI–High-GL (1)	High-GI–High-GL (2)	High-GI–High-GL (3)	Low-GI–Low-GL (4)	High-GI–Low-GL (5)
GI meal	49	62	61	48	61
GL meal	44	46	57	21	28
Energy (kJ)	2015	1622	2011	1175	1154
Protein (g)	13.9	11.8	14.0	12.5	12.0
Fat (g)	7.1	4.4	5.3	6.4	5.1
Total CHO (g)	89.6	74.5	93.4	43.2	45.2



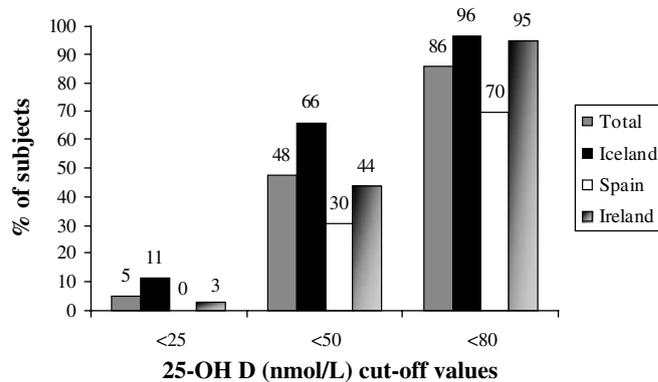
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Wintertime vitamin D status in adults aged 20–40 years from Iceland, Spain and Ireland. By S. MULDOWNY¹, A. LUCEY¹, G. PASCHOS¹, J.A. MARTÍNEZ³, I. THORSDDOTTIR⁴, K.D. CASHMAN^{1,2} and M. KIELY¹, ¹Departments of Food & Nutritional Sciences and ²Medicine, University College Cork, Republic of Ireland, ³Department of Physiology & Nutrition, University of Navarra, Spain and ⁴Unit for Nutrition Research, Landspítali University Hospital, Iceland

While the elderly and very-young subgroups of the population are acknowledged to be at risk of low vitamin D status^{1,2}, the assumption that adolescents and young adults are vitamin D replete should be questioned. The aim of the present study was to investigate wintertime 25-hydroxyvitamin D (25-OH D) status in a sample of young overweight Irish, Spanish and Icelandic men and women and to explore the determinants of vitamin D status in these adults.

Serum 25-OH D and intact PTH levels were measured using ELISA methods (IDS Ltd, Boldon, Tyne and Wear, UK) in baseline samples collected during January–March 2005 from 200 men and women aged 20–40 years who were participants in the SEAFOODplus ‘YOUNG’ dietary intervention study. Dietary intakes of vitamin D and Ca were measured as well as anthropometry, body composition and lifestyle factors such as smoking.

The mean serum 25-OH D was 56.1 (SD 24) nmol/l. As expected, latitude was the most important determinant of serum 25-OH D levels (adjusted R^2 0.200; $P < 0.002$), and Spain had significantly higher ($P < 0.0001$) levels compared with Ireland and Iceland (68.7, 54.6 and 44.8 nmol/l, respectively). Gender and BMI were the only other two determinants of 25-OH D that approached significance ($P < 0.08$). Widespread vitamin D insufficiency was observed (Figure).



The mean intact PTH was 2.8 (SD 1) pmol/l. Spain had significantly higher ($P < 0.0001$) PTH level compared with Ireland and Iceland (3.3, 2.4 and 2.6 pmol/l respectively). An inverse relationship between 25-OH D and PTH was only apparent in Icelandic subjects (r_p -0.247 ; $P = 0.025$) and subjects who entered the study in March (r_p -0.552 ; $P = 0.002$).

These data show that young European adults are at risk of low vitamin D status in wintertime. Furthermore, even in wintertime when skin synthesis of vitamin D is at a minimum, latitude is the most important predictor of serum 25-OH D. Further research on the metabolism of vitamin D is required in the context of diet and physiology.

The YOUNG study (coordinator Professor Inga Thorsdottir) is part of the SEAFOODplus Integrated Project, which is funded by the EC through the 6th Framework Programme contract no. FOOD-CT-2004–506359.

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Bone mineral density in a representative sample of men and women aged 20–40 years. By G. HORIGAN, M.S. BARNES, D. DALRYMPLE, J.J. STRAIN, M.P. BONHAM, E.M. DUFFY and J.M.W. WALLACE, Northern Ireland Centre for Food and Health, University of Ulster, Coleraine BT52 1SA, UK

Osteoporosis, an increasingly common and debilitating bone disease, is estimated to cost the National Health Service £1.8 × 10⁹ annually¹. Approximately 90% of total peak bone mass is attained by the end of early adulthood², and subsequently bone mass decreases with age. The majority of studies to date have focused on the elderly, the group at greatest risk of fractures, but the antecedents of osteoporosis will begin in earlier years. The aim of the present study was to assess bone mineral density (BMD) in apparently-healthy young men and women aged 20–40 years. Healthy men and women aged 20–40 years (n 117) were recruited from local workplaces to the present study. Height and weight were measured and used to calculate BMI. BMD of the lumbar spine (L1–L4), total femur and total body were measured by Lunar Prodigy dual-energy X-ray absorptiometry (DXA) scanner (Lunar Corp., Madison, WI, USA). Lifestyle, physical activity levels and dietary habits were assessed using questionnaires. Data for men and women were analysed separately.

	Males ≤30 years		Males >30 years		Females ≤30 years		Females >30 years	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Height (m)	1.77 ^a	0.04	1.75 ^a	0.06	1.66 ^a	0.06	1.64 ^a	0.047
Weight (kg)	80.4 ^a	11.3	85.7 ^a	12.7	69.1 ^a	15.2	70.7 ^a	16.0
BMI (kg/m ²)	25.4 ^a	2.81	27.9 ^b	3.82	25.0 ^a	4.71	25.9 ^a	5.5
BMD (g/cm ²): Lumbar spine	1.33 ^a	0.15	1.22 ^b	0.16	1.23 ^a	0.13	1.24 ^a	0.14
Total femur	1.21 ^a	0.09	1.10 ^b	0.15	1.06 ^a	0.10	1.02 ^a	0.16
Total body	1.29 ^a	0.08	1.26 ^a	0.10	1.16 ^a	0.07	1.16 ^a	0.09
DXA T-score: Lumbar spine	0.74 ^a	1.30	-0.12 ^b	1.41	0.25 ^a	1.09	0.39 ^a	1.23
Total femur	1.00 ^a	0.72	0.12 ^b	1.19	0.57 ^a	0.90	0.11 ^a	1.17
Total body	0.87 ^a	0.99	0.53 ^a	1.26	0.47 ^a	0.85	0.64 ^a	1.14

^{a,b} Mean values for individuals ≤30 years and for those >30 years with unlike superscript letters were significantly different (independent t test; $P < 0.01$).

BMD at the lumbar spine and total femur was significantly lower for men >30 years compared with those aged ≤30 years. For women there was no significant difference in BMD with age. Of the volunteers aged ≤30 years, one male and three females were classified as osteopenic (defined by a T-score between -1.0 and -2.5 SD at any site) and one female was classified as osteoporotic (defined as a T-score ≤2.5 at any site). Of the volunteers aged >30 years, thirteen males and five females were classified as osteopenic and one male as osteoporotic. Multiple regression analysis including BMI, family history of osteoporosis, social class and past physical activity revealed that family history of osteoporosis was a positive significant predictor of spine BMD for males ≤30 years. BMI was a positive significant predictor of BMD at all three sites for females >30 years and of total body BMD for all males. The incidence of low BMD in young adults aged 20–40 years observed in the current study, if evident in general population, would be of concern and would have marked consequences for public health. Research examining BMD in younger age-groups, not traditionally studied, may help to identify individuals in the early stages of bone loss and thereby improve treatment.

We would like to acknowledge the Food Standards Agency for their support.

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An investigation of a possible nutritional interaction between vitamin D and K status in Danish girls. By E.M. OCONNOR¹, C. MØLGAARD³, K.F. MICHAELSEN³, J. JAKOBSEN⁴, and K.D. CASHMAN^{1,2}, ¹Department of Food and Nutritional Sciences and ²Department of Medicine, University College Cork, Republic of Ireland, ³Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, Denmark and ⁴Danish Institute for Food and Veterinary Research, Søborg, Denmark

There is some evidence for a nutritional interaction between vitamin D and vitamin K status. It has recently been reported that serum % undercarboxylated osteocalcin (ucOC; a marker of vitamin K status¹) is inversely correlated with serum 25 hydroxyvitamin D (S25OHD) concentration (the most-widely-used marker of vitamin D status) in healthy Danish girls (aged 11–12 years)². This is in line with a similar relationship reported in elderly women¹. Whether the relationship between vitamin D status and serum %ucOC is causal has not been investigated. The objective of the present study was to test the hypothesis that improving vitamin D status significantly lowers serum % ucOC.

Serum samples from sixty-seven healthy Danish girls (aged 11–12 years) who participated in a 12-month double-blind placebo-controlled vitamin D-intervention trial (as part of the OPTIFORD project (www.optiford.org) were used for the present study. These girls were a subset of subjects who began and finished the intervention during wintertime, thus avoiding the influence of seasonality on vitamin D status. Thirty-three and thirty-four of the girls had been randomised to treatment with 10 µg cholecalciferol/d and placebo for 12 months respectively. Compliance was evaluated by tablet counting. The median compliance was 92% with no significant difference between the two groups ($P=0.29$). Total osteocalcin concentration and the fraction of ucOC in serum as well as S25OHD concentration were assessed by ELISA. Repeated measures ANOVA was used to investigate the effect of vitamin D intervention on serum % ucOC.

Vitamin D intervention significantly ($P<0.001$) increased S25OHD but had no effect on serum % ucOC ($P>0.8$).

	Pre-intervention				Post-intervention			
	Placebo		Cholecalciferol		Placebo		Cholecalciferol	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
S25OHD (nmol/l)	47.8	18.4	47.6	15.8	39.7	17.7	57.9	14.3
Serum %ucOC	24.0	5.2	22.5	3.5	27.7	5.8	26.9	7.1

In conclusion, the findings of this intervention study in young girls do not support the concept of a nutritional interaction between vitamin D and vitamin K status.

The study was supported with funding from the European Commission Fifth Framework Programme (OPTIFORD contract no. QLK1-CT-2000-00623) and the National Development Plan (2000–2006) Dublin, Republic of Ireland.

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Percutaneous endoscopic gastrostomy in patients with Cystic Fibrosis. By M. O'REILLY¹, O. TULLY², G. HOULIHAN², S. SUGRUE¹ and C. GALLAGHER², ¹Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland and ²National Adult Referral Centre for Cystic Fibrosis in Ireland, St Vincent's University Hospital, Elm Park, Dublin 4, Republic of Ireland

Nutritional status has long been acknowledged as an important prognostic indicator in cystic fibrosis (CF). The hope is that restoration of nutritional status may ameliorate the rate of decline in lung function (FEV₁)¹. Percutaneous endoscopic gastrostomy (PEG) feeding is the preferred option for supplemental feeding, with guidelines advising that it be initiated in patients who consistently achieve <85% ideal body weight (IBW)².

By means of a clinical audit, the patient records of twenty-seven adult patients with CF who had a PEG inserted between 1999 and 2006 were examined. Data collected were used to look at the effect of supplemental overnight PEG feeding on the weight status and lung function of these patients. A separate interviewer-assisted questionnaire was used to assess acceptance of and compliance with PEG feeding in nineteen adult patients with CF currently receiving PEG feeding.

The audit sample experienced significant increases ($P=0.001$) in mean % IBW and an improvement in FEV₁ after 3 months. In the surviving sample a trend towards improvement in mean % IBW was seen after 2 years (see Table).

	n	% IBW	P*	N	FEV ₁ (%predicted)	P*
Insertion	27	75.7		26	33.3	
3 month	26	84	0.001	17	37.4	NS
6 month	14	82	NS	8	22.9	NS
9 month	11	82.5	NS	6	29.9	NS
1 year	15	83.4	NS	13	27	NS
2 years	7	83.7	NS	9	33.4	NS

% IBW is calculated using a BMI of 22 kg/m².

* Significance of difference between groups.

When first proposed, 89% of those in the questionnaire sample resisted PEG insertion. Length of time of resistance varied, with 37% resisting PEG for ≤1 year, 42% resisting for ≤2 years and 11% resisting PEG for >2 years. Reasons for resistance included social stigma, vanity and inconvenience. Of the patients 74% reported situations that would impinge on their PEG compliance. These situations tended to be social in nature and included holidaying, swimming, sharing a bed and night time socialising.

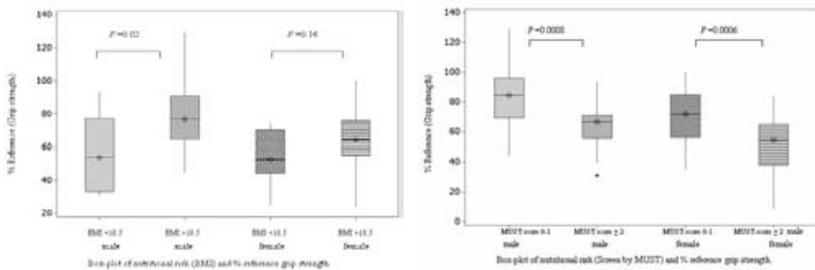
Supplemental PEG feeding appears to result in an improvement in % IBW after 3 months and a trend towards improvement in % IBW over 2 years. Initiating patients onto PEG feeding remains a challenge, and social situations appear to affect their acceptance of it and compliance with it. Given the benefits of supplementary feeding in patients with CF, the results of the questionnaire would suggest more time needs to be spent developing resources that encourage the patients to accept PEG when first suggested by minimising their negative associations with it.

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TQ1

Nutritional status of elderly patients: assessment of handgrip strength and malnutrition universal screening tool. By S.S. WONG^{1,2}, J.J. REILLY¹ and C.Y. YAU³, ¹Human Nutrition Section, Division of Developmental Medicine, University of Glasgow, Yorkhill Hospitals, Glasgow, G3 8SJ, UK, ²Department of Nutrition and Dietetics, Stoke Mandeville Hospital, Mandeville Road, Aylesbury, Bucks. . HP21 8AL, UK and ³Medicine for Older People, Stoke Mandeville Hospital, Aylesbury HP21 8AL, UK

Malnutrition (undernutrition) is a major UK public health problem that often goes undetected and unmanaged. There is an increasing emphasis on nutritional screening of all patients; however, there are limited validation studies on the wide variety of nutritional assessment tools in use. The aim of the present study were: to carry out a functional assessment of nutritional status in the elderly using handgrip (HG)¹ to assess the practical utility of malnutrition universal screening tool (MUST)² and HG: to assess agreements between HG and MUST in the elderly. A validation study was carried out on 120 elderly patients aged ≥65 years admitted to Stoke Mandeville Hospital, Aylesbury, Bucks. Anthropometric measures of mid-upper arm circumference (MUCA) and functional assessment (HG) and nutritional screening using MUST were performed. The present study found that the prevalence of undernutrition was 47%. Patients with impaired HG (<85% median) were found to have a significantly higher MUST score (≥2) for both genders, and for men a lower BMI (<18.5 kg/m²) and lower MUAC (<5th percentile) was found. When compared with MUST as a reference method, HG had a sensitivity of 58.5% and a specificity of 50%. Agreement in the assessment of undernutrition between the two methods using λ -statistics was moderate to substantial (κ 0.478 (95% CI 0.343, 0.613)). Undernutrition is still common in UK hospitals. HG dynamometry may be a valid and practical tool for nutritional screening and it merits further research.

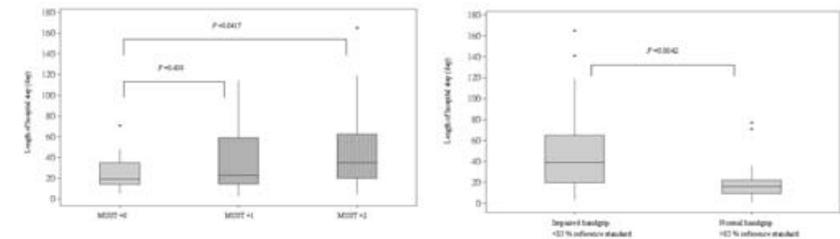


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2. Elia M (2003) *Screening for Malnutrition: A multidisciplinary Responsibility. Development and Use of the Malnutrition Universal Screening Tool (MUST) for adults*. BAPEN.

TQ1: Please check fig quality OK?

Nutritional status predicts hospital length of stay and mortality in older adults: assessment of handgrip and malnutrition universal screening tool. By S.S. WONG¹, C.Y. YAU² & J.J. REILLY³, ¹Department of Nutrition and Dietetics, ²Medicine for Older People, Stoke Mandeville Hospital, Aylesbury HP21 8AL, UK and ³Human Nutrition Section, Division of Developmental Medicine, University of Glasgow, Yorkhill Hospitals, Glasgow, G3 8SJ

Malnutrition (undernutrition) is frequently reported in older adults and it is associated with adverse hospital outcomes. Data on functional assessment by handgrip (HG)¹ (Klidjian *et al.* 1980) and malnutrition universal screening tool (MUST)² (Elia, 2003) on hospital outcomes in older adults are lacking. The aim of the present study was to investigate the hospital outcomes of patients admitted to a district general hospital. A prospective observational study was carried out on 108 patients aged ≥65 years who were admitted to Stoke Mandeville Hospital, Aylesbury, Bucks. Nutritional state was assessed by MUST and functional assessment of HG. The impact of nutritional status on prevalence and hospital outcomes were analysed for these individuals. The outcomes were: length of stay in hospital (LOS); mortality rate in 180 d. It was found that the prevalence of undernutrition was high (MUST score ≥2, 52.7% (fifty-seven of 108); HG <85%, 74.1% (eighty of 108)). Undernourished individuals had a significantly longer LOS (MUST 2, 31 d v. MUST 0, 16 d, $P=0.0004$; HG<85%, 30.5 d v. HG >85%, 14 d, $P=0.0002$) and a greater mortality in 180 d (MUST 2, 16 (28%) v. MUST 0, 1 (4%); OR 8.87 (95% CI 1.40, 10.56), $P=0.009$; HG<85%, 16 (20%) v. HG >85%, 1 (3.57%); OR 5.85 (95% CI 1.004, 8.805), $P=0.049$) than those at lower risk. Both MUST and HG can predict clinical outcomes in hospitalised elderly adults in a rehabilitation unit. HG appears to be a better tool for predicting LOS than screening by MUST alone. HG dynamometry is fast, portable, low cost and easy to administer. It may be a valid tool for first-line nutritional screening in hospital admissions.



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2. Elia M (2003) *Screening for Malnutrition: A multidisciplinary Responsibility. Development and Use of the Malnutrition Universal Screening Tool (MUST) for adults*. BAPEN.

TQ1: Please check fig quality OK?

Plasma long-chain *n*-3 PUFA status in patients receiving long-term home parenteral nutrition.

By B.A. GRIFFIN¹, S.E. PAYNTON¹, D.A.J. LLOYD², S.M. GABE², A. RODRIQUEZ MATEOS³, and J.A. LOVEGROVE³, ¹*School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK*, ²*Lennard-Jones Intestinal Failure Unit, St Mark's Hospital, Harrow, UK* and ³*Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading, UK*

Home parenteral nutrition (HPN) is lifesaving for individuals with severe intestinal failure. Parenteral feeds may contain lipid but do not contain the long-chain (LC) *n*-3 PUFA, EPA and DHA. The aim of the present study was to assess LC *n*-3 PUFA status in patients receiving HPN compared with matched controls and to investigate possible relationships with adverse clinical events.

Fasting plasma samples were obtained from forty-four patients receiving HPN. Eighteen patients were receiving regular infusions of Intralipid 20% (75% soybean oil, Pharmacia & Upjohn, Stockholm, Sweden) and two patients Clinoleic (80% olive oil, Clintec Parenterals, Cedex, France). Plasma phospholipids were isolated by solid-phase extraction and fatty acids were quantified by GC. The fatty acid profiles of patients receiving HPN were compared with those of fifty-three age-, gender- and BMI-matched controls.

Plasma EPA and DHA were significantly lower in patients receiving HPN compared with controls (% total fatty acids (TFA); 1.0 v. 2.0 and 2.8 v. 5.3 respectively, $P < 0.001$), and were unaffected by the administration of regular lipid infusion. Plasma DHA was inversely related to the duration of HPN ($P = 0.006$) and weakly associated with intestinal length ($P = 0.06$). Plasma α -linolenic acid was higher in patients receiving HPN (0.36% TFA v. 0.24% TFA, $P = 0.01$), although not in patients with > 1000 mm small intestine. Plasma linoleic acid was lower (12.3% TFA v. 19.8% TFA, $P < 0.001$) and plasma oleic acid was higher (16.3% TFA v. 10.3% TFA, $P < 0.001$) in patients receiving HPN v. controls. There was no significant difference in plasma arachidonic acid between patients and controls, or any evidence of associations between plasma fatty acids and biochemical abnormalities, skin problems or hepatic or catheter-related complications.

The present study reveals a relative deficiency of plasma phospholipid-EPA and -DHA in patients receiving HPN. This could reflect reduced dietary provision and/or impaired *n*-3 fatty acid desaturation and elongation. The clinical relevance of this deficiency in the longer term is still unclear.

The effect of increasing fruit and vegetable intake on endothelial function.

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Fruit and vegetable (F&V) consumption is associated with a decreased risk of CVD, but the dose-response relationships with established risk factors is uncertain. It has been hypothesised that F&V have a beneficial effect on vascular function because of their high levels of bioactive compounds, such as antioxidant vitamins and flavonoids. DRFRUITNVEG is a randomized dose-response cross-over trial (ISRCTN50011192) designed to test the hypothesis that an increased intake of F&V improves arterial compliance and endothelial function in subjects with moderately-elevated blood pressure (BP; $> 120/80$ and $< 160/100$ mmHg). This report compares three diets containing low, medium and high intakes of F&V in forty-eight subjects (twenty-three males, twenty-five females; mean age 45 years, mean BP 137/89 mmHg). Following a 3-week run-in on a low intake of F&V (three portions F&V daily) subjects were randomly allocated to the interventions. Each intervention lasted 6 weeks and was separated by a 3-week wash-out period. Endothelial function was determined by brachial artery flow-mediated dilatation (FMD) using ultrasound, arterial compliance by carotid to femoral pulse-wave velocity (PWV) and peripheral vascular resistance by measurement of peripheral augmentation index (PAIx) derived from the radial pulse wave, using the SphygmoCorTM system. Measurements were made at the end of each treatment period. Results are shown in the Table.

F&V level	F&V intake (g/d; <i>n</i> 36)		FMD (%; <i>n</i> 48) [§]		PWV (m/s; <i>n</i> 48) [§]		PAIx (%; <i>n</i> 48) [§]	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Low	285 ^{††‡‡}	270, 300	6.7	6.2, 7.3	7.9	7.5, 8.2	76.8	72.9, 80.7
Medium	527 ^{***}	479, 575	6.8	6.2, 7.4	7.9	7.6, 8.3	76.0	72.1, 79.9
High	642 ^{**††}	573, 711	7.0	6.3, 7.6	7.9	7.6, 8.2	76.9	72.5, 81.2

PAIx, peripheral augmentation index.

Mean values were significantly different from that for low F&V (Bonferonni multiple comparison test): ** $P < 0.01$.

Mean values were significantly different from that for medium F&V (Bonferonni multiple comparison test): †† $P < 0.01$.

Mean values were significantly different from that for high F&V (Bonferonni multiple comparison test): ‡‡ $P < 0.01$.

§ There were no significant differences between levels of F&V intake (Bonferonni multiple comparison test).

Self reported intake of F&V on the low level of intake (285 g/d) was similar to the UK average (260 g/d¹), and increased significantly ($P < 0.01$ for all) from low to medium intakes (by 242 (95% CI 193, 290) g/d) and medium to high intakes (by 115 (95% CI 41, 190) g/d). However, there were no changes in endothelial function, PWV and PAIx between levels of F&V intakes.

These results suggest that in subjects with a level of F&V intake similar to the average UK intake additional F&V is without effect on endothelial function and arterial compliance.

This study (N02030) was funded by the Food Standards Agency.

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Obesity and risk of pancreatic cancer: a systematic literature review. By V.J. BURLEY, D.C. GREENWOOD, J.E. CADE, J. MORETON, D. CHAN, Y.-K. TU, J.D. THOMAS and D. FORMAN, *Centre for Epidemiology and Biostatistics, University of Leeds, 30–32 Hyde Terrace, Leeds LS2 9LN, UK*

Other than smoking, which increases risk, few modifiable risk factors have been identified for cancer of the pancreas. In preparation for the second report from the World Cancer Research Fund/American Institute for Cancer Research (*Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective*)¹ a systematic review of the literature relating to pancreatic cancer has been undertaken. As part of this review, one objective was to assess the relationship between BMI and risk of pancreatic cancer.

All original aetiological peer-reviewed studies were considered, with no exclusions on the basis of quality, language or publication date. Separate random-effects meta-analyses were performed for cohort and case-control studies based on derived linear dose-response curves. Heterogeneity was explored by meta-regression on each of the following factors: age; gender; ethnicity; nationality; dietary-assessment methodology; control-group source; number of exposure categories; covariate adjustments including smoking and alcohol consumption.

Data on obesity were extracted from twenty-four cohort and fifteen case-control studies, with seventeen and thirteen of these studies respectively providing sufficient information for meta-analysis. Studies conducted in North America, Scandinavia and Canada predominated, and many were substantial in size, the largest having more than 1900 cases. The pooled estimate of relative risk per 5 kg/m² was 1.14 (95% CI 1.07, 1.22; $P=0.001$) from the cohort studies and 1.00 (95% CI 0.87, 1.15; $P=0.96$) from the case-control studies. No significant gender effects were observed, although this analysis was conducted on 10 cohort studies and 7 case-control studies only. In the cohort studies males had similar pooled estimates to females. The pooled estimate of relative risk for males was 1.24 (95% CI: 1.06 to 1.46, $P=0.009$) and for females it was 1.08 (95% CI: 0.97 to 1.20, $P<0.001$). The difference was not statistically significant ($P=0.8$). Similarly, in the case-control studies males had very similar pooled estimates to females. The pooled estimate of relative risk for males was 1.17 (95% CI: 1.00 to 1.36, $P=0.04$) and for females it was 1.18 (95% CI: 1.11 to 1.25, $P=0.2$). The difference was not statistically significant ($P=0.3$).

There was a moderate amount of heterogeneity within the cohort studies (I^2 51%, Q 45.3, df 22, $P=0.002$) but more so within the case-control studies (I^2 80%, Q 96.7, df 19, $P<0.001$). Although the cohort studies had a much higher pooled estimate, the difference between the study types was not significant (Q 2.0, df 1, $P=0.2$). Small-study biases such as publication bias were explored using Egger's test. This was not significant for either cohort ($P=0.5$) or case-control studies ($P=0.2$), and neither funnel plot showed any asymmetry. Exploring heterogeneity through meta-regression within each study type did not identify any characteristics significantly associated with different-sized estimates.

In the case-control studies, the weight loss commonly observed prior to pancreatic cancer diagnosis may have biased weight recall. Additionally, it is recognised that the short post diagnosis survival time associated with pancreatic cancer creates a reliance on proxy collected exposure data and/or a bias in use of cases with unusually prolonged survival. These factors suggest that case-control studies may be less reliable than cohort studies with regard to pancreatic cancer. Based on the more robust cohort analyses, the data from this review indicate a significant elevation in risk of pancreatic cancer associated with increasing levels of obesity as assessed by BMI. In moving from a normal category to an obese category the pooled data from this analysis suggest that the risk of developing pancreatic cancer is increased by 14%. However, the small size of the effect does not rule out the possibility that the association is a result of uncontrolled confounding or bias.

The World Cancer Research Fund provided support for this analysis.

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Dietary patterns and risk of breast cancer: analysis from the UK Women's Cohort Study. By J.E. CADE¹, E.F. TAYLOR¹, V.J. BURLEY¹ and D.C. GREENWOOD², ¹*Nutritional Epidemiology Group and* ²*Biostatistics Unit, Centre for Epidemiology and Biostatistics, University of Leeds, 30–32 Hyde Terrace, Leeds LS2 9LN, UK*

Evidence linking dietary patterns to risk of developing breast cancer is limited and results vary according to population studied and dietary assessment method used^{1,2}. The UK Women's Cohort Study (UKWCS) described previously³ forms the basis for this analysis of commonly-recognised food patterns in relation to risk of breast cancer.

The UKWCS includes 35 372 women aged 35–69 years at recruitment, which was between 1995 and 1998. All women completed a 217-item postal FFQ. The cohort was designed to have a range of different dietary intakes, with 28% being self-reported vegetarians. Subjects were flagged on the National Health Service Central Register and incident cancers and causes of death were coded according to the International Classification of Diseases 9⁴ and 10⁵. The investigation censor date was 1 April 2005, with a median follow up of 8 years. Incident breast cancer was recorded from 320 premenopausal women and 439 post-menopausal women. The dietary patterns assessed in this analysis were meat eating, poultry eating, fish eating and vegetarian. Meat eaters were women who consumed meat types other than poultry more than once weekly (poultry may or may not have been eaten by this group); poultry eaters ate poultry more than once weekly (but not other meat); fish eaters ate fish more than once weekly (but not poultry or other meat); vegetarians ate meat, poultry and fish less than once weekly. A multiple logistic regression analysis was undertaken to assess the impact of these dietary patterns on risk of developing breast cancer. Two models were created: a simple model that adjusted for age and total energy only; a complex model adjusting for a range of potential confounders: age; total energy intake; energy adjusted fat; BMI; physical activity; oral contraceptive use; hormone-replacement therapy use; smoking; parity; age at menarche; ethanol intake; length of breast-feeding; socio-economic class; level of education.

Amongst post-menopausal women only there was a strong significant inverse association between fish-eating dietary pattern and vegetarianism and risk of breast cancer in the simple model. In the fully-adjusted model this difference remained for the fish eaters but became borderline non-significant for the vegetarians. Hazard ratios for the fully-adjusted model comparing fish eaters with meat eaters was 0.59 (95% CI 0.37, 0.97) and for vegetarians compared with meat eaters was 0.77 (95% CI 0.34, 1.75).

Dietary patterns that include fish rather than meat or eating a vegetarian diet are associated with a lower risk of developing breast cancer, particularly in older women.

Thanks to the UK Women's Cohort Study Steering Group and the women who participated in the study. The World Cancer Research Fund provided support for the cohort and this analysis.

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Prevalence of obesity in preschool Greek children using two different classification methods. By V. COSTARELLI, M. KOLOTOUROU, K. KONDAKIS, C. TZAVARA, G. MOSCHONIS and Y. MANIOS, *Department of Nutrition & Dietetics, Harokopio University, 70 El. Venizelou Ave, 176 71 Kallithea, Athens, Greece*

Overweight and obesity among preschool children is of great concern, because it may lead to long-term health consequences¹. Some recent studies have suggested that childhood obesity in most cases tracks into adulthood² and increases the risk of degenerative diseases later in life³. A total of 2374 children aged 1–5 years from 105 nurseries in five counties were examined from April 2003 to July 2004. Weight (kg) and height (m) were obtained and BMI (kg/m²) was calculated. Both the US Centers for Disease Control (CDC)⁴ and the International Obesity Task Force (IOTF)⁵ methods were used to classify each child as 'normal', 'at risk of overweight' and 'overweight'. The prevalence (%) of obesity by gender and age is shown in the Table.

	Weight status of children					
	CDC		IOTF		Overweight	
	Normal weight*		At risk for overweight			
Males (n 1218)						
Age group (years)						
1–2 (n 100)	83.5				16.5	–
2–3 (n 274)	66.1	82.3‡	20.1	13.8	13.8	3.9‡
3–4 (n 488)	65.1	78.0‡	18.4	15.3	16.5	6.7‡
4–5 (n 356)	68.5	77.9	14.8	12.9	16.7	9.2
All ages	67.8	80.8‡	16.3	12.9	16.0	6.2‡
Females (n 1156)						
Age group (years)						
1–2 (n 107)	88.6				11.4	–
2–3 (n 226)	67.1	77.5	18.3	15.8	14.6	6.7
3–4 (n 434)	68.5	74.2	15.5	17.8	16.0	8.0‡
4–5 (n 389)	64.0	72.3	19.6	16.9	16.4	10.9
All ages	68.3	76.4‡	16.2	15.5	15.5	8.1‡

*For CDC classification normal weight or underweight.

‡ $P < 0.001$ for the comparison of proportions by the two methods.

US Centers for Disease Control (CDC).

International Obesity Task Force (IOTF).

The overall estimates of at risk of overweight and overweight using the CDC method was 31.9%, which was 10.6 percentage points higher than the IOTF estimate of 21.3 ($P < 0.001$). Both boys and girls were 1.72 times more likely to be classified as at risk of overweight or overweight using the CDC method as compared with the IOTF method. Furthermore, both boys and girls were 2.42 times more likely to be classified as overweight using the CDC method as compared with the IOTF method. A boy was 2.86 times more likely to be classified as overweight by the CDC method than by the IOTF method, while a girl was 2.08 times more likely to be classified as overweight by the CDC method than by the IOTF method. Both methods used to assess prevalence of obesity have demonstrated that a high percentage of the preschool children in this sample were overweight; however, there are significant differences between the two methods of obesity classification.

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Maternal and neonatal plasma selenium and risk of early childhood wheeze. By L.C.A. CRAIG¹, G. DEVEREUX¹, G. McNEILL¹, G. NEWMAN², S.W. TURNER³, S. MARTINDALE¹, P.J. HELMS³ and A. SEATON¹, ¹Department of Environmental and Occupational Medicine, University of Aberdeen, Aberdeen AB25 2ZP, UK, ²Analytical Group, The Macaulay Institute, Aberdeen AB15 8HQ, UK and ³Department of Child Health, University of Aberdeen, Aberdeen AB25 2ZG, UK

In the UK 1.1 million children (one in ten) are being treated for asthma, and it is one of the commonest causes of hospital admission and long-term medication use¹. It has been proposed that decreasing the dietary intake of antioxidants has contributed to the recent increases in asthma prevalence, and an association between maternal vitamin E intake during pregnancy and risk of asthma has previously been shown in children². Several studies have reported associations between asthma and Se status in adults and children, and the aim of the present study was to investigate whether childhood wheeze and asthma up to the age of 5 years are associated with maternal and neonatal plasma Se concentrations.

Pregnant women ($n = 2000$) were recruited and plasma Se concentrations measured during early pregnancy (12 weeks of gestation) and in neonatal cord blood. Information on the children's respiratory symptoms at 1, 2 and 5 years of age was collected by questionnaire. Questionnaire data and maternal plasma Se measurements were available for 1412 children at 1 year, 1282 children at 2 years and 1167 children at 5 years. Logistic regression analysis was carried out to determine the relationship between maternal and neonatal plasma Se and childhood respiratory symptoms with adjustment for covariates.

Median maternal plasma Se was 79.1 (5th–95th centile 57.2–107.9) µg/kg. Maternal and cord plasma Se concentrations were not associated with any respiratory symptoms during the first year of life. As shown in the Table below, maternal and cord plasma Se concentrations were inversely associated with wheezing and seeing a doctor because of wheezing in the second year of life, such that children born to women with plasma Se concentrations in the 5th centile would be 2.36 (95% CI 2.33, 2.38) times more likely to wheeze than children born to women in the 95th centile. However, by age 5 years there were no associations between maternal or cord plasma Se and respiratory symptoms.

Outcome during second year	OR/10 µg/kg increment in plasma Se					
	Unadjusted			Adjusted*		
	OR	95% CI	P	OR	95% CI	P
Maternal plasma Se (n 1282)						
Wheeze in last 12 months	0.85	0.77, 0.95	0.003	0.86	0.76, 0.97	0.011
Wheeze without cold in last 12 months	0.88	0.75, 1.03	0.110	0.91	0.76, 1.09	0.320
Seen doctor with wheeze in last 12 months	0.80	0.71, 0.90	<0.001	0.79	0.69, 0.93	0.001
Doctor confirmed asthma	0.88	0.74, 1.04	0.140	0.92	0.76, 1.12	0.410
Cord plasma Se (n 489)						
Wheeze in last 12 months	0.71	0.55, 0.93	0.012	0.67	0.47, 0.96	0.030
Wheeze without cold in last 12 months	1.02	0.78, 1.33	0.900	0.98	0.47, 1.30	0.350
Seen doctor with wheeze in last 12 months	0.71	0.51, 0.99	0.043	0.62	0.41, 0.93	0.022
Doctor confirmed asthma	0.97	0.70, 1.34	0.840	0.95	0.57, 1.58	0.850

* Adjusted for infant gender, maternal atopy, maternal smoking, maternal age at recruitment, maternal parity, mode of delivery, birth weight, birth crown–heel length, birth occipito–frontal head circumference, (Scottish Index of Multiple Deprivation (SIMD)), use of antibiotics during first year of child's life and maternal or cord plasma ascorbate.

The Se status of mothers during early pregnancy and of neonates is associated with early childhood wheezing but not asthma, furthermore this association is absent by the age of 5 years. This transient association may be related to an effect of Se on early childhood viral-associated wheeze rather than atopic asthma.

This research was funded by the Scottish Executive's Chief Scientist Office and Asthma UK.

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Prevalence of chronic diseases amongst Bahraini older adults. By G. AL-RAEES¹, J. EARLAND¹ and A.O. MUSAIGER², ¹Faculty of Health and Life Sciences, Coventry University, Coventry CV1 5FB, UK and ²Bahrain Centre for Studies and Research, PO Box 496, Manama, Bahrain

The percentage of the population in Bahrain aged ≥60 years increased from 4 in 1980 to 6.4 in 2001¹. Life expectancy also increased from 68.2 years during 1980–5 to 73.8 years during 2000–5¹. Due to the lack of data on this age group, the first comprehensive survey in Bahrain of the health and nutritional status of older adults was conducted in 2002. The information collected will be used when developing future programmes and policies for social services and health care. Findings on the nutritional status and health status in relation to chronic diseases are presented here.

A multi-stage area sampling method was used, with the sampling frame being all free-living adults aged ≥60 years, taken from the most recent national census (CIO, 2000)². A questionnaire, designed to collect demographic, health and lifestyle data, was administered during home visits by nutritionists or trained health workers. A list of twenty-three common conditions was developed from previous studies on adults and the results of the pilot study. The elderly were asked to report whether the doctor had informed them that they had any of these or other conditions. A range of anthropometric measurements was taken. BMI was calculated from weight and height measurements, and waist and hip circumferences were measured in order to calculate the waist:hip ratio (WHR). For cultural reasons, whenever possible measurements were taken by fieldworkers of the same sex as the participants. Blood pressure (BP) was taken using an auto-deflate digital electronic blood pressure monitor (Omron Healthcare, Bannockburn, Illinois, USA).

The sample comprised 400 subjects aged 60–95 (median 69.0) years of which 53% were male. The majority of the sample was illiterate (73.1% of males and 86.1% of females). Only 4.8% of the subjects were living alone, with 82% living with their children or children and spouse.

It was found that 20% of subjects were obese (BMI ≥30 kg/m²), with a further 32% being overweight (BMI 25.0–29.9 kg/m²) and 10% being underweight (BMI <18.5 kg/m²). Although the risk of complications for central obesity may vary according to ethnicity³, approximately three-quarters of subjects (73%) were in the high-risk category (WHR ≥0.95 in males and ≥0.80 in females). Using the WHO criteria⁴ of systolic BP ≥140 mm Hg and/or diastolic BP ≥90 mm Hg, 73% of the sample was found to be hypertensive, with no significant difference between genders.

When asked about diagnosed chronic diseases only 6.8% reported having no condition, with almost one-third having three to five conditions. The most-commonly-reported conditions were joint diseases such as arthritis (56.3%), followed by hypertension (40%), osteoporosis (34.8%), stomach problems (31.0%) and diabetes (29.3%). One-fifth of the sample had been diagnosed with heart disease and 29% suffered from a respiratory condition. Females were significantly more likely to report a number of health problems, including hypertension ($P < 0.001$), osteoporosis ($P < 0.001$) and diabetes ($P < 0.01$). Only 9.8% of the subjects reported being on a special diet such as a diabetic, low-fat or low-salt diet.

Although there may have been inaccuracies in reported levels of chronic diseases in the present study, it has served to highlight the serious health problems affecting Bahraini older adults. Strategies for the provision of health services for older adults are required to address the needs of this population group.

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Riboflavin lowers blood pressure in patients with premature CVD homozygous for the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism. By G. HORIGAN¹, M. WARD¹, H. McNULTY¹, J. PURVIS², J.J. STRAIN¹ and J.M. SCOTT³, ¹Northern Ireland Centre for Food and Health, University of Ulster, Coleraine BT47 1SA, UK, ²Cardiac Unit, Altnagelvin Hospital, Londonderry BT47 6SB, UK and ³Department of Immunology and Biochemistry, Trinity College, Dublin, Republic of Ireland

The common C677T polymorphism in MTHFR is associated with elevated homocysteine. Among those homozygous (TT genotype) for this polymorphism, an increased risk of CVD, in particular stroke, has been reported in some but not all studies¹. This polymorphism has also recently been reported to be associated with a higher risk of hypertension². Riboflavin is required as a cofactor for the MTHFR enzyme, and while it has been shown that riboflavin can significantly lower homocysteine, specifically among healthy individuals with the TT genotype³, its effect on blood pressure has not previously been examined. The aim of the present study was to determine the effect of riboflavin on blood pressure in patients with CVD with different MTHFR genotypes. From a cohort of patients with premature CVD ($n = 404$) pre-screened (on the basis of a buccal swab) for MTHFR genotype, forty-nine were identified with the TT genotype and a similar number of age–gender-matched individuals with the CT ($n = 68$) and CC ($n = 64$) genotype. Patients, within each genotype group, were stratified on the basis of their baseline homocysteine levels and randomized to receive either riboflavin 1.6 mg/d or placebo for a 16-week period.

At baseline both systolic and diastolic blood pressure were found to be significantly higher in the TT group compared with the CC or CT genotype groups ($P = 0.002$ and $P = 0.038$ respectively; data not shown). Intervention with riboflavin resulted in a significant improvement in riboflavin status in all three genotype groups, as indicated by a decrease in the biomarker erythrocyte glutathione reductase activation coefficient (EGRac). A corresponding significant decrease in both systolic and diastolic blood pressure was, however, only observed in the TT group (Table).

	TT placebo ($n = 24$)					TT riboflavin ($n = 25$)					P
	Pretreatment		Post-treatment		P	Pretreatment		Post-treatment		P	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		
EGRac	1.32	0.12	1.34	0.11	0.298	1.41	0.20	1.27	0.09	<0.001	
Systolic blood pressure (mmHg)	143.8	18.1	141.6	22.5	0.566	142.8	22.1	131.6	20.9	0.005	
Diastolic blood pressure (mmHg)	84.5	10.7	85.4	12.7	0.739	88.1	14.2	80.2	14.2	0.002	

Results show, for the first time, a significant and clinically-important reduction in both systolic and diastolic blood pressure in response to riboflavin, specifically among patients with the TT genotype. The magnitude of the reduction observed can be estimated from meta-analysis to be associated with a lowering of the risk of heart disease by 29% and stroke by 46%. Although the precise mechanisms for these effects are unclear, the findings have important implications for the prevention and treatment of a major primary risk factor for CVD and stroke.

We would like to acknowledge support from the Northern Ireland Chest Heart and Stroke Association.

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Fish oil fatty acids improve postprandial vascular reactivity. By K.G. JACKSON, C.K. ARMAH, F. CHEGHANI, L. JAMES, I. DOMAN and A.M. MINIHANE, *Hugh Sinclair Unit of Human Nutrition, Department of Food Biosciences, University of Reading, Reading RG6 6AP, UK*

With increasing recognition of the pivotal role of vascular dysfunction in the progression of atherosclerosis, the vasculature has emerged as an important target for dietary therapies. Although high-fat meals have often been associated with a loss of postprandial vascular reactivity, recent findings from this research group have shown an improvement in postprandial vascular tone after the addition of fish oil fatty acids to a standard test meal¹. This research has been extended to determine the underlying molecular mechanisms for these positive effects of EPA and DHA on postprandial vascular reactivity.

Twenty-five healthy males (mean age 46 (SD 18) years and BMI 25.5 (SD 4) kg/m²) attended the investigation unit on two separate occasions to consume in random order a standard test meal containing either 40 g mixed fat (palm olein–soybean oil (80:20, w/w); placebo oil meal) with a fatty acid composition representative of a typical UK diet or 31 g mixed fat and 9 g fish oil providing 5.4 g DHA+EPA (fish oil meal). Vascular reactivity was measured at baseline (0 h) and 4 h after the meals by laser Doppler iontophoresis, and blood samples were taken for the measurement of plasma TAG and total nitrite. The Svedberg flotation rate (S_r)>400 chylomicron-rich fraction was isolated from the 4 h plasma samples by density-gradient ultracentrifugation and incubated with human umbilical vein endothelial cells. Their impact on endothelial NO synthase (eNOS), NADPH oxidase subunit NOX-4 and β-actin gene expression was determined using real-time RT-PCR.

Compared with baseline, significant increases in sodium nitroprusside (endothelial-independent vasodilator)-induced reactivity ($P=0.03$) and total nitrite were observed after the fish oil meal ($P=0.001$). In addition, postprandial (4 h) nitrite levels were significantly greater following the fish oil compared with the placebo oil meal ($P=0.046$), whereas postprandial TAG concentrations were similar following both meals. In cell-culture studies there was a significant increase in eNOS and decrease in NOX-4 mRNA gene expression following incubation with the postprandial S_r>400 chylomicron-rich fraction isolated after the fish oil compared with the placebo oil meal ($P\leq 0.03$).

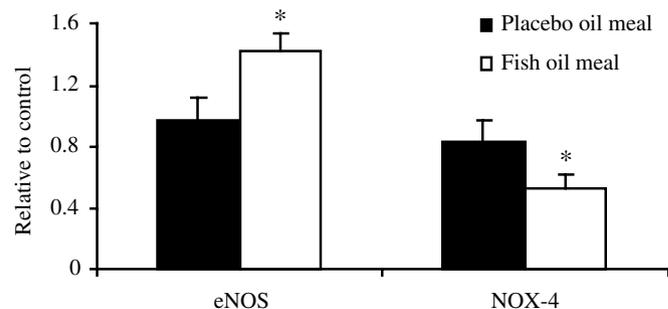


Fig. eNOS and NOX-4 gene expression. Mean values were significantly different from those for placebo oil meal: * $P\leq 0.03$.

The present study provides novel findings that suggest that inclusion of EPA and DHA in a meal can improve postprandial vascular reactivity. The increase in the postprandial endothelial-independent vasodilation induced by sodium nitroprusside with fish oil indicates an impact of treatment on the maintenance of NO. This potential mechanism is further supported by the cell-culture studies that indicate that fish oil fatty acids may reduce superoxide formation via down-regulation of NADPH oxidase, which would lead to an increase in the amount of bioavailable NO in the vascular endothelium.

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Processing of oats: effect on antioxidants and induction of gene expression in vascular endothelial cells. By L. STENHOUSE, A.M. SALTER, D.A. GRAY and G.A. TUCKER, *School of Biosciences, Sutton Bonington Campus, The University of Nottingham, Leicestershire LE12 5RD*

Increased whole-grain intake is associated with a reduced risk of several chronic diseases such as CVD¹, type 2 diabetes² and various cancers.

Grains contain components that may have several health benefits and that may act individually or synergistically with other nutrients³. A beneficial component is the antioxidants. Cereals often need to be processed to make them consumer friendly and a crucial question that still remains to be fully answered is whether processing of whole grain has any effect on the content of and the beneficial effect of antioxidants.

The products of the different stages of processing for oats are: raw oat grains (OG) and oat flakes (OF). The aim of the present work was to investigate whether processing of oats from raw oat grain to oat flakes (primary processing) and heating of oat flakes (HOF; secondary processing) had any effect on antioxidant content and solubility and ability of extracts to induce beneficial gene expression in human umbilical vein endothelial cells (HUVEC).

Samples (obtained from a commercial oat-producing company; 1 g) were extracted in 50 ml methanol. The same methanolic extract was used to determine antioxidant content using the oxygen radical absorbance capacity (ORAC) assay (measured as Trolox equivalence (TE)) and phenolic content using the Folin-Ciocalteu assay (measured as gallic acid equivalence (GAE)). To measure gene expression HUVEC were treated with oat extracts with a total phenolic content of 0.1 μM (GAE) and quantitative RT-PCR was used to monitor expression of selected genes.

During primary processing extractable antioxidant level was significantly reduced as was phenolic content. Secondary processing increased these values to a level that was higher than that originally recorded from the raw product (Table 1). Treatment of HUVEC with OG, OF and HOF extracts with a total phenolic content of 0.1 μM significantly increased expression of endothelial NO synthase (eNOS), the enzyme responsible for the production of the vasodilator, NO ($P<0.001$; Table 2). Vascular endothelial growth factor (VEGF) is involved in atherosclerosis, so a reduction in VEGF may have the potential to prevent CVD. Endothelin 1 (ET-1) is a vasoconstrictor involved in vascular homeostasis. A significant increase in ET-1 is only apparent when cells were treated with OG. But GATA2, a positive transcription factor for ET-1, is significantly decreased with all treatments, suggesting that a decrease in ET-1 expression may occur.

Table 1

Oat product	Antioxidant content (TE; μmol/100 g)		Phenolic content (GAE; mg/100 g)	
	Mean	SE	Mean	SE
OG	1579	34	33	0.80
OF	800**	45	27*	0.48
HOF	1900**	58	75**	2.10

n 4–8. Mean values were significantly different from those for oat grains (one-way ANOVA): * $P<0.05$, ** $P<0.01$.

Table 2

Gene name	OG (%)	OF (%)	HOF (%)	ANOVA: SED	P<
VEGF	58*	55*	53*	0.1500	0.002
GATA2	83*	56*	46*	0.1158	0.001
ET-1	160*	117*	119*	0.1307	0.004

All treatments were expressed as % control and were normalised to glyceraldehyde 3-phosphate dehydrogenase. *Significant effect.

In conclusion, primary processing resulted in a significant reduction in antioxidant and phenolic extractability in oats. However, this level could be regained following secondary processing by the consumer. These oat extracts had a significant effect on the expression of several genes, which suggests the potential for these oat products to reduce the risk of CVD.

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The interstitial glucose profile preceding symptoms attributed to hypoglycaemia, in otherwise healthy women. By E.J. SIMPSON¹, M. HOLDSWORTH² and I.A. MACDONALD¹, *Schools of ¹Biomedical Sciences and ²Biosciences University of Nottingham, Nottingham NG7 2UH, UK*

The reporting of postprandial symptoms attributed to 'hypoglycaemia' by otherwise healthy individuals appears to be a relatively common phenomenon in the UK¹. Whether these symptoms, which occur 2–4 h after eating, are related to blood glucose has long been a contentious issue, which periodic ambulatory blood glucose measurement has failed to resolve². The aim of the present study was to investigate, using continuous glucose-monitoring technology (Medtronic Minimed, Northridge, CA, USA; CGMS), whether symptoms attributed to low blood glucose were associated with hypoglycaemia or a previous fall in interstitial glucose (IG).

Thirty healthy non-obese women (age 20–48 years, BMI 15.7–26.2 kg/m²) who self-reported these symptoms more than once weekly wore a subcutaneous CGMS probe in abdominal fat for 4–7 (median 5) d. Volunteers were free-living and were asked not to change their usual diet and activity routines during the recording period. An event marker was entered into the CGMS monitor each time they ate and when symptomatic. Volunteers were blinded to their IG concentration during the study. IG profiles preceding symptoms were analysed using one-way ANOVA, with time of symptoms being defined as *t* 0 min.

Clear symptoms <4 h after eating were reported by twenty volunteers, with thirty-six symptomatic episodes recorded during the study period (twenty-one accompanied by neurogenic symptoms and fifteen by adrenergic symptoms), with a mean 'symptomatic' IG of 4.38 (SE 0.11) mmol/l, occurring 2.3 (SE 0.15) h after eating. In 11% of cases symptoms were associated with an IG concentration of ≤3.5 mmol/l, and in 25% it was ≤4.0 mmol/l. In 39% of cases symptoms were associated with a fall in IG of ≥0.5 mmol/l over the previous 30 min, with 56% being associated with either a fall in IG or an IG concentration of ≤4.0 mmol/l. Episodes associated with adrenergic symptoms were almost twice as likely to be preceded by a fall in IG when compared with those associated with neurogenic symptoms (53% and 29% respectively). Symptoms were preceded by a rise in IG on only one occasion and the event marker in this case was 10 min after a nadir of 2.8 mmol/l.

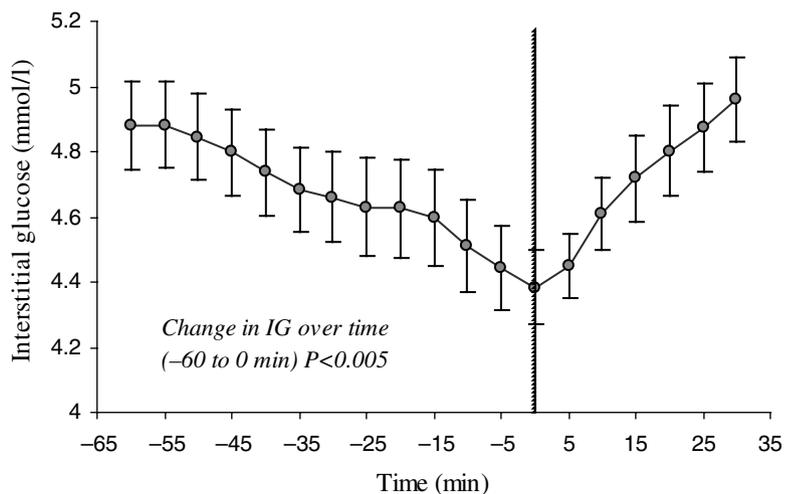


Fig. 1. IG profile preceding symptoms of hypoglycaemia.

Although the majority of symptoms attributed to 'low blood glucose' occurred at a higher IG concentration than traditionally defined as 'hypoglycaemia', there is some suggestion that a fall in IG may be implicated in the development of symptoms, especially in those symptomatic events associated with adrenergic symptoms.

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The ability of dietary polyphenols to protect against endogenously-formed neurotoxins. By D. VAUZOUR, K. VAFEIADOU and J.P.E. SPENCER, *Molecular Nutrition Group, School of Chemistry, Food and Pharmacy, The University of Reading, Reading RG6 6AP, UK*

Parkinson disease is characterized by a progressive and selective loss of dopaminergic neurons in the *substantia nigra*. Although the mechanisms by which these neurons degenerate is unclear, accumulating evidence suggests that endogenously-formed 5-S-cysteinyldopamine (CysDA) conjugates, formed during the oxidation of dopamine in the presence of cysteine (or other cellular thiols), may contribute to nigral death¹. Recent investigations have shown that CysDA possesses strong neurotoxicity and may initiate a sustained increase in intracellular reactive oxygen species in neurons leading to DNA oxidation, caspase-3 activation and delayed neuronal death². In addition, CysDA may undergo further oxidation to yield new species, such as dihydrobenzothiazine, which have been reported to be potent mitochondrial respiratory complex I inhibitors³. Recently there has been intense interest in the effects of dietary antioxidants and polyphenolic compounds, present in fruits and vegetables, to protect against neuronal damage and cognitive decline⁴. Whilst flavonoids may exert their biological effects via their antioxidant capacity, there is accumulating evidence suggesting that they might exert neuromodulatory activities through the modulation of cellular signalling pathways, in particular the mitogen-activated protein kinase pathway⁵.

The present study focused on the ability of dietary-derived polyphenols to protect against neurotoxicity exerted by endogenously-formed CysDA and derived species. To achieve this, primary cultures of mouse cortical neurons were prepared from 14–16 day-old Swiss mouse embryos and were cultured at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. After 5–6 days *in vitro*, cortical neurons were exposed to CysDA (100 μM, 24 h) or to the peroxynitrite generator SIN-1 (500 μM, 2h). To assess the protective effects of polyphenols, neurons were pre-treated with the individual compounds for 18h prior to the addition of CysDA or SIN-1. Neuronal damage and protective effects elicited by the treatments were evaluated by the Alamar Blue assay and by morphological examination under light microscope.

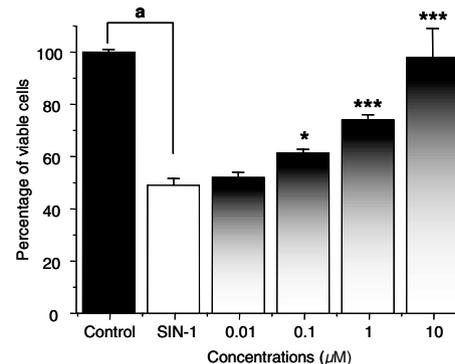


Fig. Effect of treatment with tyrosol against toxicity induced by SIN-1 (peroxynitrite source; 500 μM). *Decrease in viability compared with the control ($P < 0.001$). Mean values were significantly different from those for the cultures treated with SIN-1: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

In vitro experiments demonstrated that CysDA may be formed during the oxidation of dopamine by tyrosinase or peroxynitrite. However, in presence of polyphenols (resveratrol, hesperetin, caffeic acid and (+)-catechin) a small but significant decrease in CysDA formation was observed. Moreover, these reactions led to the formation of various polyphenol–cysteinyldopamine adducts, which may represent novel metabolic forms present *in vivo*. Caffeic acid, gallic acid and tyrosol also exerted strong protection against peroxynitrite-induced injury to primary cortical neurons (Figure), whilst hesperetin and pelargonidin were observed to protect against CysDA neurotoxicity. The mechanism by which polyphenols inhibited neuronal death was found to be linked to their ability to induce the activation of both Akt/PKB signalling and the ERK1/2 pathways. The protective effects of polyphenols against neurotoxins-induced toxicity will help shed light on their mechanism of neuroprotection.

This work is funded by the Medical Research Council (G0400278).

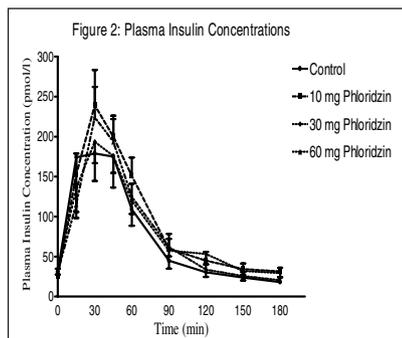
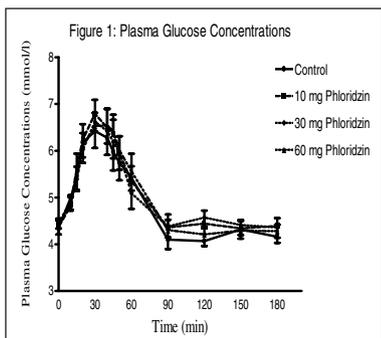
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The effect of consuming an apple-derived product on glucose tolerance and insulin *in vivo*. By M.S. AL OLAN, M.N. CLIFFORD, K.L. JOHNSTON and L.M. MORGAN, *School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7HX, UK*

Phloridzin is a naturally-occurring dihydrochalcone (flavonoid), which is mainly found in apples and apple-derived products¹. Thus, it occurs in the human diet and has been safely administered intravenously to human subjects². In the 1950s the cellular and molecular actions of phloridzin were investigated. It was noted that concentrations of 10^{-4} – 10^{-6} M inhibited sugar transport in both the kidney and the small intestine³. Phloridzin is a potent inhibitor of Na-dependent glucose transporter SGLT1 and SGLT2⁴. Johnston *et al.*⁵ have found that consuming a commercial apple juice containing approximately 10 mg phloridzin has significant effects on improving glucose tolerance and lowering insulin concentrations in volunteers. The aim of the present study was to determine whether phloridzin present in an apple-based food product of higher phloridzin content had any further beneficial physiological effects on plasma glucose and insulin when consumed as a glucose-containing drink.

Eleven healthy volunteers (four men and seven women) of mean age 27 (SD 3.8) years participated in a four-way single-blind randomised cross-over study in which they consumed 25 g glucose in a 200 ml beverage containing phloridzin in three different concentrations (10, 30 and 60 mg) or the placebo. Venous blood was sampled for 3 h post consumption of the drink. Plasma glucose and NEFA concentrations were determined using enzymic colorimetric methods (ILab analyser; Instrumentation Laboratory (UK) Ltd, Warrington, Cheshire, UK), and plasma insulin was analysed using ELISA (MLT, Cardiff, UK).

Results showed that consuming apple-extract products had no effect on glucose tolerance in human subjects, as no significant differences in plasma glucose and insulin concentrations were found between the treatments and the control. Postprandial NEFA levels were also similar throughout. This suggests that the effect of apple on reducing blood glucose levels in the Johnston *et al.*⁵ study may relate primarily to compounds other than phloridzin.



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Determinants of food choice in adults of low-socio-economic status: a qualitative investigation By M. BARTON¹, B. STEWART-KNOX¹ and J. KEARNEY², ¹*Northern Ireland Centre for Food & Health (NICHE), University of Ulster, Coleraine BT52 1SA, UK and* ²*Dublin Institute of Technology (DIT), Dublin, Republic of Ireland*

The incidence of chronic diseases are increasing in certain societal segments¹ indicating a need to explore perceptions of food and health in ‘at risk’ groups. The aim of the present qualitative study was to gain an in-depth understanding of factors determining food choice from the perspective of residents of socio-economically-deprived areas of Northern Ireland. Socio-economically deprived areas were identified using the Northern Ireland Multiple Deprivation Measure (NI MDM). Seven focus groups were conducted involving forty-two adults (three males and thirty-nine females) recruited through community groups. Data were collected and analysed according to grounded theory principles. Topics were drawn from previous research in the area and used to guide and stimulate discussion. The data were transcribed verbatim and analysed for themes by two experienced researchers. Data were catalogued using QSR-NUD*IST[®]. Several themes emerged spontaneously throughout discussions. For example, healthy foods were perceived to be those that had not undergone any processing and foods deemed ‘traditional’ comprising a ‘proper meal with vegetables’ such as stews and casseroles. Food choices, including notions of food quality, were strongly influenced by knowledge of food production methods and food processing techniques ‘it is the left over chicken that goes into chicken burgers’. Financial restraints were not considered a barrier to healthy eating, but the associated lifestyle characterized by unemployment, lack of physical activity, smoking and drinking was considered to be ‘toxic’. Although discussants conveyed knowledge of nutritional concepts, food choices were not driven by nutritional concern, but were a reflection of cultural beliefs about health, food and food production. Health-promotion messages should be delivered in terms that take culture into account.

This research was funded by Safefood Ireland.

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Differences in the absorption and excretion of soya-milk isoflavones in Oriental and Caucasian men. By J.E. BROWN, K. CHANA and C. MAHENDE, *Division of Nutrition, Dietetics and Food Sciences, School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH, Surrey, UK*

Isoflavones are a group of polyphenolic compounds present naturally in soyabeans. Experimental and epidemiological studies have highlighted the health beneficial effects of consuming soyabeans and soya products that may relate to the isoflavones present. Isoflavones are generally present as glucosides in soya foods and when ingested they are hydrolysed by intestinal β -glucosidases to release isoflavone aglycones, which are then absorbed. Subsequent aglycone glucuronidation and/or sulfation *in vivo* facilitate the renal excretion of these compounds. Previous studies have shown that urinary isoflavone excretion is influenced by food matrix, chemical composition and gender¹ and these variables also influence plasma isoflavone pharmacokinetics². Yet, there are little data comparing the absorption and excretion of isoflavones in different ethnic groups. In the present study ten healthy male subjects (five Oriental and five Caucasian) were recruited. After collecting the 24 h baseline urine, subjects ingested a standardised single oral bolus dose of soya milk (0.44 mg isoflavone aglycones/kg body weight). Three further consecutive 24 h urine collections were taken. Urine samples were treated with β -glucuronidase and sulphatase (37 °C, 18 h), extracted with diethyl ether and analysed by HPLC.

	Urinary isoflavone excretion							
	Day 1		Day 2		Day 3		Total	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Daidzein ($\mu\text{mol/d}$): Caucasian	8.0*	2.3	2.6*	0.7	2.4†	0.8	13*	3.1
Oriental	1.5	0.8	0.3	0.2	0.3	0.1	2.1	1.1
Genistein ($\mu\text{mol/d}$): Caucasian	4.3†	1.2	1.1	0.4	1.1	0.4	6.6*	0.9
Oriental	1.3	0.8	0.3	0.2	0.3	0.1	1.9	1.0

Mean values were significantly different from those for the Oriental group (unpaired *t* test): **P*<0.05. No significant differences were observed between the groups in terms of body weight, height and age. †Trend observed (*P*<0.08).

As expected, and consistent with other studies¹, the amount of daidzein excreted exceeded that of genistein and the majority of both isoflavones were recovered within the first 24 h period. Urinary excretion of daidzein was consistently higher for Caucasians than Orientals, with the total daidzein excretion being >5-fold higher (*P*<0.05). Similar differences in genistein excretion were evident, in particular for the total amount of genistein excreted (*P*<0.05). These results show that clear ethnic differences exist in the absorption and excretion of isoflavones. This may relate to differences in β -glucosidase activity (e.g. lactase phloridzin hydrolase) or gut transit time. These findings may provide a better understanding of the physiological effects of soyabean isoflavones between different population groups and may have implications for the recommendations for soyabeans and soya products in the diet.

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The effect of intermittent v. chronic energy restriction on weight loss in premenopausal women: preliminary results from a randomised pilot trial to reduce breast cancer risk. By M. CHAPMAN¹, A. HOWELL¹, J. CUZICK², A. FLYVBJERG³, P. HOPWOOD¹, S. JEBB⁴, G. PARFITT⁵ and M. HARVIE¹, ¹South Manchester University Hospitals Trust, Manchester M20 2LR, UK, ²CRUK Department of Epidemiology and Statistics, Wolfson Institute, London EC1M 6BQ, UK ³Medical Research Laboratories, Aarhus University, Denmark, ⁴MRC Human Nutrition Research Group, Cambridge CB1 9NL, UK and ⁵School of Sport and Health Science, University of Exeter, Exeter EX1 2LU, UK

Post-menopausal breast cancer risk increases twofold in women who gain significant amounts of weight, and there is evidence that energy restriction may reduce risk¹. Animal studies indicate intermittent energy restriction (IER) reduces risk and may be superior to continuous energy restriction (CER)². It has been shown that chronic energy restriction reduces biomarkers of breast cancer in women at risk but is hard to maintain. It is hypothesised that IER may be superior to CER in reducing biomarkers of breast cancer risk and may also be more acceptable to women, and a 6-month randomised trial is being undertaken to compare the two approaches.

A total of 108 premenopausal women (mean age 40.1 (sd 3.9) years, mean adult weight gain 20.7 (sd 11.2) kg) have been randomised to either CER (75% estimated energy requirements: approximately 6270 kJ (1500 kcal) for 7 d/week) or IER (75% estimated energy requirements: 2665 (650 kcal) for 2 d and approximately 7524 kJ (1800 kcal) for 5 d/week) over 6 months. Study end points are weight and body composition (waist and hip circumference, fat-free mass and total fat mass by bioelectrical impedance), measures of insulin sensitivity (HOMA, sex hormone-binding globulin and testosterone), potential breast cancer growth factors (insulin-like growth factor axis, leptin and adiponectin), inflammatory markers (C-reactive protein and sialic acid) and oxidative stress markers (protein carbonyls, nitrotyrosine, isoprostanes and 4-hydroxynonenal adducts). To date fourteen participants have withdrawn from the study (IER eight, CER six; reasons: stress four, pregnancy three, change in employment two, couldn't follow diet two, other three). The preliminary results for weight and body composition for the first seventy participants for baseline to 3 months are reported here.

	<i>n</i>	Percentage weight loss			Fat loss (kg)			Fat-free mass loss (kg)			Waist circumference loss (cm)		
		Mean	SD	<i>P</i>	Mean	SD	<i>P</i>	Median	Range	<i>P</i>	Mean	SD	<i>P</i>
IER	44	7.4	4.0	0.04	4.3	2.8	0.21	1.7	-0.9–5.7	0.23	5.9	4.3	0.05
CER	46	5.7	3.4		3.7	3.0		1.5	-4.4–3.8		4.3	4.4	

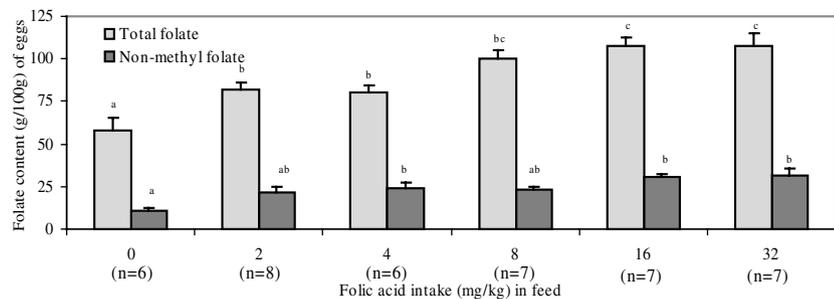
n 90 because 4 women missed their 3 month appointment.

These preliminary results show that significant weight loss occurred in both groups over 3 months, however decreases in weight and waist circumference are significantly greater in the intermittent diet group.

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Unmetabolized folic acid content of eggs produced by hens fed diets with added folic acid. By L. HOEY¹, H. McNULTY¹, M.E.E. McCANN^{2,3}, K.J. McCracken³, J.M. SCOTT⁴, B. BLAZNIK MARC⁵, A.M. MOLLOY⁵, C. GRAHAM¹ and K. PENTIEVA¹, ¹University of Ulster, Coleraine BT52 ISA, UK, ²Agricultural, Food and Environmental Science Division, Agri-Food and Biosciences Institute, ³The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX, UK, ⁴Department of Immunology and Biochemistry, Trinity College, Dublin 2, Republic of Ireland and ⁵Department of Clinical Medicine, Trinity College, Dublin 2, Republic of Ireland

Previous work has shown that egg folate can be substantially improved by the addition of folic acid (FA; the synthetic form of the vitamin) to the diet of hens. However, studies have not considered whether the additional intake exceeds the capacity of the hen to convert FA into natural forms of the vitamin. This is of importance as the presence of unmetabolized FA at high levels could potentially cause adverse effects on human health. The aim of the present study was to identify whether exposing the hen to different levels of FA for 12 weeks leads to unmetabolized FA in the egg. Forty-eight 30-week-old laying hens were randomised to receive the basal diet to which had been added one of the following FA levels (0, 2, 4, 8, 16 or 32 mg/kg feed). The total folate (TF) content of eggs was measured by thermal extraction with tri-enzyme treatment followed by microbiological assay with *Lactobacillus casei*¹. The unmetabolized FA content of eggs was estimated by two approaches. Using a microbiological assay with *Streptococcus faecalis* (B Blaznik Marc, JM Scott, AM Molloy, unpublished results) to specifically determine non-methyl folate (NMF), the FA content of enriched eggs was estimated by assuming that any increase in NMF relative to TF (compared with that in regular eggs) was the maximum attributable to FA. Second, a combined HPLC-microbiological assay^{2,1} was used to directly measure the FA content in a subset of egg samples.



Means with standard errors shown. Within each assay, values with unlike letters are significantly different ($P < 0.05$).

Results show that in regular eggs NMF comprised 21% of the TF content compared with 23–31% in enriched eggs (Figure). Thus, $\leq 10\%$ of the TF content of enriched eggs could potentially be unmetabolized FA. When FA was measured directly in eggs produced by hens fed the two highest levels of added FA (i.e. optimal egg TF), unmetabolized FA was found to account for 2.3% and 5.1% TF respectively (not shown). The combined results show that FA would represent $\leq 10\%$ of the TF content of enriched eggs.

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Irish mothers' perception of their own and their child's weight status. By A.F. McGLOIN¹ and L. DELANEY², ¹National Nutrition Surveillance Centre, School of Public Health and Population Sciences, University College, Dublin, Republic of Ireland and ²Geary Institute, University College, Dublin, Republic of Ireland

Addressing the obesity crisis is a key public health issue in Ireland. However, there is growing evidence that a large proportion of the population may fail to identify themselves¹ or their children^{2,3} as overweight or obese. The aim of the present study was to identify whether perception of body-weight status is a barrier to weight reduction in mothers and children in Ireland.

Results were obtained from a nationally representative, cross-sectional survey of school-aged children (5–12 years) and their mothers ($n = 556$) conducted between 2003 and 2004 by the Irish University Nutrition Alliance⁴. Body weights and heights of mothers and children were measured in duplicate by field workers and perception of mother's own weight status and that of her child was assessed by self-administered questionnaire. A total of 516 mothers answered the question 'Do you think your weight is fine for your age' and 549 mothers answered the question 'Do you think your child's weight is fine for his or her age'. Obesity in adults is defined according to WHO classifications⁵. In children it is defined according to UK 1990 cut-offs⁶. Using these criteria 'overweight' is defined as having a BMI between the 91st and 98th percentile and 'obesity' is a BMI on or above the 98th percentile.

For most mothers the reported definition of whether their weight was fine for their age was aligned with WHO categories for weight status. Of obese mothers 4%, compared with 36% of overweight mothers and 85% of normal-weight agreed that their weight was 'fine' for their age. In contrast, the majority of mothers of obese or overweight children thought that their child's weight was 'fine'. Of mothers of obese children 52% and 86% of mothers of overweight children either tended to agree, or strongly agreed, that their child's weight was 'fine' for their age.

Child's weight is fine for his/her age	Weight status of child							
	Normal weight (n 422)		Overweight (n 65)		Obese (n 62)		Total (n 549)	
	n	%	n	%	n	%	n	%
Strongly agree	211	50.0	26	40.0	12	19.4	249	45.4
Tend to agree	164	38.9	30	46.2	20	32.3	214	39.0
Tend to disagree	25	5.9	7	10.8	14	22.6	46	8.4
Strongly disagree	2	0.5	2	3.1	13	21.0	17	3.1
Don't know	20	4.7	0	0	3	4.8	23	4.2

While Irish mothers appear to be aware of their own weight status, a worrying proportion did not accurately perceive that their children were overweight or obese. For all behaviour-change models, awareness of the need to change is a prerequisite for success. Since parental participation is vital in effective obesity treatment in children, these findings have important implications for obesity interventions and for social marketing campaigns targeted at children. Parents who are unaware that their child is an unhealthy weight are unlikely to proactively seek out treatment, or to understand that health information relating to obesity is relevant for their child.

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Nutrient intakes at lunchtime of primary-school children in Cornwall: a comparison of school meals and packed lunches. By G.A. REES, C.J. RICHARDS and J. GREGORY, *School of Biological Sciences, The University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK*

With the rising prevalence of obesity in children causing concern in the UK, media attention has recently focused on the nutritional composition of school meals. The government has responded by the publication of new standards for the composition of school meals¹. However, some schools report that children disliking the new healthy menus are consuming packed lunches instead. The aim of the present study was to assess the nutrient intake of children at lunchtime, comparing those consuming meals provided by schools with children consuming packed lunches.

Four primary schools were asked to participate and all agreed. The percentage of children receiving free school meals at the schools ranged from 1.8% to 17%. All parents were provided with an information leaflet and consent form. Ethical approval was granted by the Ethics Committee of the Faculty of Science, University of Plymouth. Children were chosen at random from class lists of those children for whom informed consent had been granted. Approximately equal numbers of girls and boys, packed lunch and school lunch were chosen. The age of the children ranged from 6 years to 11 years. Participants were observed at a meal time and the food items consumed were recorded. Wastage was also recorded. Nutrient analysis was performed using CompEat nutrient analysis program (Nutrition Systems, Banbury, Oxon, UK) using additional information from packaging and catering staff. Statistical analysis was performed using SPSS version 11.5 (SPSS, Chicago, IL, USA). The differences in means between groups were determined by independent samples *t* tests.

	School meals (n 62)		Packed lunch (n 58)		P
	Mean	SD	Mean	SD	
Energy (kJ)	1856	677	2058	681	0.108
Protein (g)	18	9	18	7	0.951
Carbohydrate (g)	52	21	71	27	<0.001*
Fat (g)	20	10	16	9	0.03*
Sugar (g)	13	0	28	18	<0.001*
Saturated fat (g)	5	4	7	4	0.021*
% Energy as: Protein	18	12	15	5	0.059
CHO	44	12	55	10	<0.001*
Fat	38	11	29	11	<0.001*
Sugar	11	9	21	11	<0.001*
NSP(g)	3	1	4	2	0.002*
Na (mg)	542	323	834	340	<0.001*
Ca (mg)	124	72	295	176	<0.001*
Fe (mg)	1.8	0.6	2.2	0.9	<0.016*
Vitamin C (mg)	17	15	24	25	0.076

CHO, carbohydrate. *The difference between groups was significant.

The study demonstrates that whilst energy intakes were similar, there were significantly higher intakes of carbohydrate, sugar, saturated fat, NSP, Na, Ca and Fe in children consuming a packed lunch. Children consuming school meals had a higher percentage of their energy derived from fat compared with those having packed lunch, but consumed less saturated fat.

It is perhaps worrying that on average children taking a packed lunch to school are consuming approximately double the amount of sugar and 50% more Na and saturated fat in their midday meal compared with those having a school lunch. On average, food consumed from the school meals did not meet the new standards for Fe (40% reference nutrient intake² (RNI), i.e. 3.5 mg for a child aged 7–10 years) and Ca (40% RNI, i.e. 220 mg).

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Effects of a synbiotic on biomarkers of oxidative stress and faecal microbiota in healthy adults: results of a cross-over double-blind placebo-controlled trial. By D.M.A. SAULNIER¹, P. HÜTT², M. MIKELSAAR², D. BOSSCHER³, G.R. GIBSON¹ and S. KOLIDA¹, ¹*Food Microbial Sciences Unit, University of Reading, Reading RG6 6AP, UK*, ²*Department of Microbiology, University of Tartu, Estonia* and ³*Orafiti, Tienen, Belgium*

A synbiotic is a combination of a live microbial food supplement (probiotic) and a prebiotic; the latter should be a non-digestible food ingredient that can be used by the probiotic to increase its survival or activity in the host gastrointestinal tract, while selectively stimulating the indigenous beneficial bacteria in the gut¹.

The activity of probiotics to scavenge reactive species has been reported². Ingestion of probiotics with antioxidative activity could help to maintain a physiological acceptable redox status in intestinal mucosa cells as well as in the other body cells of the host, the antioxidative network being affected by intestinal events³.

A synbiotic formulation (10⁹ cells of the probiotics *Lactobacillus fermentum* ME-3, *Lactobacillus paracasei* 8700:2 and *Bifidobacterium longum* 46 with 6.6 g Beneo™ oligofructose, ingested daily for 3 weeks) was evaluated in a randomized double-blind cross-over placebo-controlled trial in fifty-three healthy Estonian adults. Oligofructose has been shown to enhance probiotics growth *in vitro*⁴. Probiotics were selected because of their strong antioxidative activity^{5,6}. Oxidative-stress biomarkers were monitored in blood: Total antioxidative activity (TAA) was assessed by the linolenic acid test³ and oxidation of LDL by the quantification of the baseline diene conjugates of LDL (BCD-LDL)⁷. Predominant groups of the faecal microbiota were enumerated using fluorescent *in situ* hybridisation and SCFA were measured by GC. A paired *t*-test (or Wilcoxon signed rank test, when data were not normally distributed) were used to compare results obtained with the synbiotic and placebo.

After synbiotic ingestion biomarkers of antioxidative stress improved compared with the placebo (maltodextrin); TAA was higher with the synbiotic compared with the placebo, with means of 42.4 (SD 1.9) % and 41.9 (SD 2.1) % (*P*=0.04) respectively. Moreover, oxidation of LDL decreased with the synbiotic (12.6 (SD 4.0) μM compared with 14.6 (SD 7.2) μM for the placebo; *P*=0.01). In faecal samples results showed significantly higher numbers of bifidobacteria with the synbiotic (9.7 (SD 0.2) log(10) cells/g v. 9.5 (SD 0.4) log(10) cells/g for the placebo; *P*=0.002) and, to a lower extent, of the *Atopobium* group (9.7 (SD 0.2) log(10) cells/g with the synbiotic v. 9.6 (SD 0.2) log(10) cells/g for the placebo; *P*<0.001). Increases in these groups were more pronounced when baseline levels were low before synbiotic ingestion. Significantly higher faecal butyrate concentrations were observed with the synbiotic (10.0 (SD 7.9) mM v. 8.1 (SD 6.5) mM for the placebo; *P*=0.009).

The improvement in antioxidative-stress biomarkers and the increase in both bifidobacteria and butyrate on ingestion of the test synbiotic in this prophylactic study may warrant further research in diseases in which oxidative stress plays a role (such as CVD) or in populations in which the gut microbiota composition has been disturbed.

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The feasibility of a school-based intervention (Tees Consumption and Activity in Kids Experience; TeesCAKE) for the prevention of childhood obesity in a socially-deprived area of the UK. By S.A. SMITH¹, C.L. O'MALLEY¹, L. AVERY¹, R. LANG², D. SIMPSON¹, F. HILLIER¹, V.J. WHITTAKER¹ and C.D. SUMMERBELL¹, ¹*School of Health and Social Care, University of Teesside, Middlesbrough TS1 3BA, UK* and ²*Association for the Study of Obesity, 20 Brook Meadow Close, Woodford Green, Essex, IG8 9NR.*

The TeesCAKE project is a simple, cheap, novel and sustainable school-based health promotion intervention that aims to improve dietary intake and physical activity levels. TeesCAKE consists of: (1) a 26-week activity programme delivered by Middlesbrough Football Club Community Project (MFCCPT); (2) a 12-week dance programme delivered by Middlesbrough Borough Council's Sports Development Team (MBCSPT); (3) an 8-week food preparation and tasting programme delivered by researchers at the University of Teesside. The project was piloted in two primary schools in an area of Middlesbrough ranked in the top 10% of deprived areas in England, matched in terms of total number and ethnicity of pupils. By a simple toss of a coin, one school was allocated intervention and the other control. A sample of eighty-four children aged 9–10 years (response rate 97%) started the project; four dropped out.

A number of variables (height, weight, waist circumference, physical activity and dietary intake (24h recall), and taste preference scores for fruit using a five-point hedonic rating scale) were measured at baseline (September 2005) and post intervention (May 2006). Qualitative methods were used to obtain perceptions of TeesCAKE from pupils, school staff and MFCCPT and MBCSPT.

The TeesCAKE pilot was not powered to test differences in changes over time, so the following results should be interpreted with caution. Significant increases in mean body weight ($P \leq 0.0001$ for both schools), waist circumference ($P \leq 0.01$ for intervention group) and BMI ($P \leq 0.01$ in both schools) were seen over time, but no differences between schools were identified. A significant reduction in reported total fat intake ($P = 0.02$) over time was seen in both schools, but again no differences between schools were identified.

In terms of feasibility, all three components of the intervention were successfully delivered within the normal school day, and the costs for each were minimal. The children (girls and boys) enjoyed all components of the intervention, and 'loved' the dance sessions. A key aim of the intervention was to involve parents and families wherever possible, but their involvement was low. However, the teachers were enthusiastic about the intervention, felt that they would like it to run again next year and commented on how well it linked into other areas of the curriculum. MFCCPT and MBCSPT were also enthusiastic about the intervention and the successful partnership working.

The TeesCAKE intervention was delivered successfully within the school day at relatively low cost. The key elements to the successful delivery of the TeesCAKE intervention are: (1) a simple, cheap and novel intervention; (2) effective inter-agency partnership working; (3) making sure that the intervention is made up of components that the children will enjoy; (4) ensuring that the intervention is not perceived as an additional burden to the school, and preferably that it is perceived as adding value to the school curriculum.

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Increasing fruit and vegetable intake does not favourably affect plasma lipids and folate status. By S.E.E. BERRY¹, R. SHERWOOD², P.J. CHOWIENCZYK³, Z. MULLA¹ and T.A.B. SANDERS¹, ¹*Nutritional Sciences Research Division, King's College London, 150 Stamford Street, London SE1 9NN, UK*, ²*Clinical Biochemistry, King's College Hospital, Denmark Hill, London SE5 9RS, UK* and ³*Cardiovascular Division, King's College London School of Medicine, St Thomas' Hospital, London SE1 7EH, UK*

Fruit and vegetable (F&V) consumption is associated with decreased risk of CVD. Current dietary advice in the UK is targeted at increasing intakes of F&V from the average intake¹ of three portions daily (260 g/d) to five portions daily. Previous research suggests that dietary advice to increase F&V intake has a favourable effect on blood pressure (BP), but does not affect plasma lipids, and the effect on folate status is unclear. DRFRUITNVEG is a randomized dose-response cross-over trial (ISRCTN50011192) designed to test the hypothesis that an increased intake of potassium-rich F&V improves BP in subjects with moderately-elevated BP (>120/80 and <160/100 mmHg). Four treatments compared diets containing low, medium and high intakes of F&V, in forty-eight subjects (twenty-three males, twenty-five females, mean age 45 years, mean BP 137/89 mmHg). Following a 3-week run-in on a low intake of F&V (three portions F&V daily) subjects were randomly allocated to the interventions. Each intervention lasted 6 weeks and was separated by a 3-week wash-out period. At the end of each intervention measurements were undertaken (for BP and vascular function) and a fasting blood sample was collected for determination of inflammatory markers, plasma lipids and folate status. Results for self-reported F&V intake, plasma lipids, folate and homocysteine are presented in the Tables.

F&V level	F&V intake (g/d)		Fruit intake (g/d)		Vegetable intake (g/d)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Low	285	270, 300 ^{a,b,c,e}	162	142, 180 ^{a,c}	123	113, 134 ^{a,c}
Medium	527	479, 575 ^{b,d,e}	340	296, 383 ^{b,d}	187	157, 217 ^{b,d}
High	642	573, 711 ^{b,c,f}	416	350, 482 ^{b,d}	226	192, 260 ^{b,d}

n 36. ^{a,b,c,d,e,f} Mean values with unlike superscript letters were significantly different (Bonferonni multiple comparison test; $P < 0.01$).

F&V level	Total C (mmol/l)		HDL-C (mmol/l)		LDL-C (mmol/l)		TAG (mmol/l)		Homocysteine (μmol/l)		Folate (μmol/l)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Low	5.8	5.5, 6.1	1.4	1.3, 1.5	3.8	3.5, 4.1	1.3	1.1, 1.5	12.0	10.9, 13.1	9.6	8.2, 11.1
Medium	5.6	5.2, 6.0	1.4	1.3, 1.5	3.7	3.4, 4.0	1.1	0.9, 1.3	11.1	10.2, 12.0	9.1	7.8, 10.3
High	5.7	5.4, 6.0	1.4	1.3, 1.5	3.8	3.5, 4.0	1.2	1.1, 1.4	11.3	10.4, 12.2	9.3	8.0, 10.5

n 48. C, cholesterol. There were no significant differences between levels of F&V intake (Bonferonni multiple comparison test).

The self reported F&V intake on the low, medium and high levels was significantly different between all levels of intake ($P < 0.01$ in all cases), but did not result in any changes in total cholesterol, LDL- and HDL-cholesterol and TAG concentrations. Furthermore, increasing F&V intake did not affect folate status.

These results suggest that in subjects with a level of F&V intake similar to average UK intake additional F&V does not affect plasma lipids and folate status.

This study (N02030) was funded by the Food Standards Agency.

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The effects of fish oil and DHA on soluble epoxide hydrolase in *apoE*^{-/-} mice. By Y. MAVROMMATIS¹, F. THIES¹, K. ROSS¹, M.J. GORDON¹, E. McLEOD¹, A. SNEDDON¹, G. RUCKLIDGE¹, M. REID¹, G. DUNCAN¹, C. MAYER², G. HORGAN², J. ARTHUR¹ and B. DE ROOS¹, ¹*Division of Vascular Health, Rowett Research Institute, Aberdeen AB21 9SB, UK and* ²*Biomathematics and Statistics Scotland at the Rowett Research Institute, Aberdeen AB21 9SB, UK*

Epoxyeicosatrienoic acids (EET) are cytochrome P450 products of arachidonic acid that exert beneficial effects on the cardiovascular system. They are associated with decreased inflammatory response, reduced blood pressure and reduced blood coagulation¹. Soluble epoxide hydrolase (sEH) converts EET to the less-active dihydroxyeicosatrienoic acids. Preliminary studies have shown that fish oil possibly affects hepatic sEH levels². Here, the *in vivo* effects of DHA and fish oil (DHA+EPA) consumption on hepatic sEH protein and gene levels and on the hepatic proteome in time are reported.

ApoE^{-/-} mice were randomised to three dietary groups and received a high-fat high-cholesterol diet supplemented with high-oleic acid sunflower oil (HOSF; 2%, w/w), DHA (2%, w/w) or fish oil (DHA+EPA; 2%, w/w). Tissues were collected from five mice per group on day 2 and weeks 1, 2, 4 and 10 of the intervention. Relative levels of hepatic sEH were measured using Western blots and relative levels of sEH mRNA using RT-PCR. Both protein and mRNA results were analysed using two-way ANOVA. Total hepatic proteins were separated by two-dimensional gel electrophoresis. Proteins that differed significantly between treatments (two-way ANOVA for time-course data) have been identified by MS.

DHA and fish oil had no effects on hepatic sEH levels over time (Fig. 1(A)). Similarly, hepatic sEH mRNA levels were not affected by DHA or fish oil, supporting the findings of hepatic sEH protein levels. Atherosclerosis was developed in all groups by week 10. Atherosclerotic plaque size in the aortic root increased in time but did not differ between the control and the treatment groups (Fig. 1(B)).

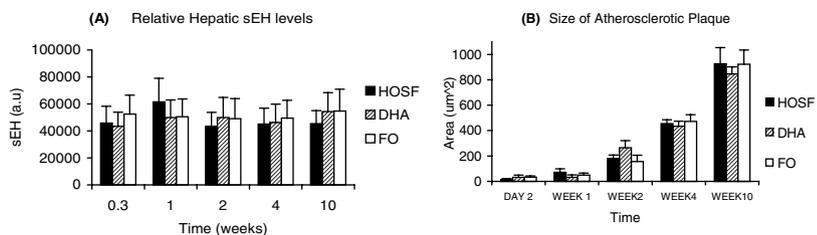


Fig. 1. Relative sEH protein (A) levels and atherosclerotic plaque size (B) in the HOSF, DHA and fish oil groups. Values are means with their standard errors represented by vertical bars.

Intervention with DHA or fish oil caused differentiated regulation of 40 hepatic proteins. These proteins are involved in various pathways including lipid, protein, carbohydrate, and homocysteine metabolism.

In conclusion fish oil (2%, w/w) or DHA (2%, w/w) alone do not affect *in vivo* hepatic sEH protein levels, hepatic sEH gene expression or atherosclerotic plaque size over time in the *apoE*^{-/-} mouse model. A total of 40 hepatic proteins were affected by the fish oil or DHA intervention. Further analyses are necessary in order to investigate possible effects of DHA and/or fish oil on the activity of sEH.

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Assessment of the nutritional status of a sample of adult patients with asthma. By M.S. MOHAMED, *Nutrition Dept, Faculty of Home Economics, Minufiya University, Egypt*

The present study was carried out on 120 adult patients (sixty male and sixty female; mean age 42.6 (SD 1.8) years) who were selected from outpatient clinics of Tanta University Hospital, Gharbia Governorate, Egypt. All selected patients were diagnosed according to standard medical criteria as being asthmatic. Food intakes were assessed by 24 h recall for 3 d. In addition, blood samples were collected for the determination of Hb, packed cell volume, erythrocyte, leucocyte, eosinophil and lymphocyte counts and IgE.

The results revealed that the majority of females were of low or intermediate socio-economic status (45.0% and 45.0% respectively), while the majority of males (65.0%) were of intermediate socio-economic status. The majority of the male and female patients were diagnosed as having asthma because of a chest allergy (50.0% and 60.0% respectively) and suffered daily asthma attacks (75.0% and 70.0% respectively), and almost all had an asthma attack while sleeping. A large percentage of the patients suffered from diabetes, hypertension or CHD. Most of the female and male patients had a food allergy (65.0% and 75.0% respectively), particularly to eggs and fish. BMI values showed that the majority of females and males were obese (55.0% and 60.0% respectively). Although mean Hb levels for the males were significantly ($P < 0.05$) higher than those for the females, the levels for both were very low (9.3 (SD 1.14) g/l v. 8.7 (SD 1.23) g/l respectively). However, the IgE concentrations for the females were significantly ($P < 0.01$) higher than those for the males (3.2 (SD 0.58) v. 2.9 (SD 0.65) g/l). The results revealed that the intakes of essential nutrients were lower than requirements for both males and females.

In conclusion, in the present study the patients with asthma suffered from obesity that may be a result of inactivity accompanying the disease, and they also showed signs of Fe-deficiency anaemia and nutritional deficiency. These findings emphasise that these patients with asthma had a poor nutritional status and that there is a need for nutritional programmes to improve their nutritional status and recovery.

Table. Nutrients intakes of patients with asthma

Nutrient†	Females (n 60)			Males (n 60)		
	Mean	SD	% WHO*	Mean	SD	% WHO*
Energy (kJ/d)	6858	1521		7226	1657	
Total protein (g/d)	61.3	16.2		62.6	18.0	
Ca (mg/d)	524.6	171.4	52.5	483.2	181.6	48.3
Total Fe (mg/d)	15.8	4.7	54.5	15.3	4.3	109.3
Zn (mg/d)	14.4	7.0	146.9	16.3	5.2	116.4
Mg (mg/d)	160.3	44.6	72.9	177.8	58.5	68.5
Na (g/d)	1.93	0.65		2.01	0.76	
K (g/d)	2.23	0.52		2.61	0.83	
TQ1 Vitamin A (µ/d)	402.6	219	80.5	292.9	227.9	48.8
Vitamin C (mg/d)	200.4	101.4	445.3	120.5	85.4	267.8

* Percentage of World Health Organization⁽¹⁾ recommended intake.

† Nutrients intakes calculated using Egyptian Food Composition Tables⁽²⁾.

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TQ1: Please check unit is OK?

Daily consumption of marjoram (*Origanum majorana* L.) and lettuce (*Lactuca sativa* L.) oils improves the health status of patients with asthma. By M.S. MOHAMED, H.H. SAAD and M.A. EL KHALEK, *Nutrition Dept, Faculty of Home Economics, Minufiya University, Egypt*

Folk medicine claims that some foods and ingredients can improve the health status of patients with asthma, examples being marjoram and lettuce oils. The aim of the present study was to investigate the effect of these oils on asthma outcome.

A sample of thirty patients with asthma (fifteen females and fifteen males; aged 42.6 (SD 1.84) years) were chosen from outpatient clinics of Tanta University Hospital, Gharbia Governorate, Egypt. All selected patients were diagnosed according to lung function, blood variables and clinical signs as being asthmatic. The patients, who were receiving standard medical therapy, were divided into three equal groups: control group (CG); marjoram group (MG), who received two drops of marjoram oil daily; lettuce group (LG), who received 5 ml lettuce oil daily. The intervention continued for three consecutive months. Lung function (forced vital capacity (FVC), maximum voluntary ventilation (MVV) and peak expiratory flow rate (PEFR)) was measured at baseline and after the intervention. Blood eosinophilic cell counts and IgE concentrations were also determined at baseline and after 3 months of intervention.

The results revealed that the majority of subjects were of intermediate socio-economic status (53.3%) and had suffered from asthma for 14.6 (SD 6.3) years. FVC increased significantly ($P < 0.01$) after the intervention for all groups, particularly for MG. On the other hand, MVV increased significantly ($P < 0.001$) for MG (by 7.34%) and LG (by 2.63%), while it decreased by 4.3% for CG. Moreover, PEFR increased significantly for all groups, the highest increment being for CG (9.4%). Eosinophilic cell counts decreased significantly for all subjects, particularly for MG (-42.7%) as compared with CG and LG (-19.7% and -21.9% respectively). Finally, the IgE concentration decreased significantly ($P < 0.01$) to normal levels for MG and LG.

In conclusion, data suggest that the administration of marjoram oils at a safe dose (two drops daily) is effective in improving the health and lung function of adult patients with asthma.

Is there a relationship between B-vitamin status and bone mineral density in young adults? Evidence from the Young Hearts Project, Northern Ireland. By A.M. GALLAGHER¹, C.E. NEVILLE², L.J. MURRAY², J.J. STRAIN¹, C.A.G. BOREHAM³, I. YOUNG² and H. McNULTY¹, ¹Northern Ireland Centre for Food and Health, University of Ulster, Coleraine BT52 1SA, UK, ²Centre for Clinical and Population Sciences, Queen's University of Belfast, Belfast BT12 6BJ, UK and ³Institute for Sport and Health, University College Dublin, Dublin, Republic of Ireland

Evidence from large observational studies suggests that increased homocysteine (tHcy) is associated with decreased bone mineral density (BMD)¹ and increased risk of osteoporotic fractures². In addition, treatment with vitamin B₁₂ and folate results in significant reduction in fractures v. placebo after 2 years follow-up³, supporting the hypothesis that reducing tHcy and/or improving the status of metabolically-related B-vitamins may delay progression of osteoporosis. To date, studies in this area have focused on older populations at risk of osteoporosis and little cognisance has been given to younger populations. The present study investigated whether tHcy and related B-vitamins were associated with BMD in 454 young adult participants of the Young Hearts Project (mean age 22.6 (SD 1.64) years)⁴.

BMD was determined by dual-energy X-ray absorptiometry at the lumbar spine (L2-L4) and femoral neck (hip). No significant association was found between tHcy and BMD (either site). However, BMD (hip) was found to be significantly, albeit weakly, correlated with status of both vitamin B₁₂ (r 0.243, $P < 0.001$) and folate (r 0.121, $P = 0.023$). BMD (lumbar) was significantly correlated with vitamin B₁₂ only (r 0.128, $P = 0.018$). When expressed as quartiles of BMD, a higher BMD was found for those with a higher status of vitamin B₁₂ (both sites) and folate (hip only; Table).

	Quartile 1		Quartile 2		Quartile 3		Quartile 4		P
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
BMD lumbar (g/cm ²)	1.06	0.065	1.17	0.022	1.25	0.023	1.39	0.085	
Erythrocyte folate (µg/l)	129.6	60.01	137.4	67.2	132.5	70.47	139.6	53.81	0.679
Serum vitamin B ₁₂ (ng/l)	260.2**	104.06	296.5	116.75	249.1***	106.6	311.6	144.2	0.003
Serum tHcy (µmol/l)	10.27	5.17	9.48	3.79	10.77	6.73	9.57	3.37	0.205
BMD hip (g/cm ²)	0.90	0.050	1.04	0.033	1.15	0.035	1.32	0.100	
Erythrocyte folate (µg/l)	119.3*	58.15	134.7	60.59	143.6	76.76	141.5	52.04	0.031
Serum vitamin B ₁₂ (ng/l)	226.0***	86.15	280.8	116.71	299.6	122.05	304.3	139.3	<0.001
Serum tHcy (µmol/l)	10.73	6.02	9.82	5.14	9.93	4.58	9.55	3.59	0.369

Mean values were significantly different from those for quartile 4 (one-way ANOVA): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Vitamin B₁₂ is associated with markers of osteoblastic activity and bone formation *in vitro*; the current study suggests that low vitamin B₁₂ status may adversely affect bone formation *in vivo*. These data support the need for further exploration of the relationship between B-vitamin status and bone health in younger populations for whom peak bone mass is not yet complete, and highlight the possible need for public health strategies aimed at improving the future health of this group.

The Young Hearts Project, Northern Ireland was supported by the Wellcome Trust and the British Heart Foundation.

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Depression and erythrocyte folate levels among women in the Southampton Women's Survey: cross-sectional and longitudinal analyses. By H. INSKIP¹, N. DUNN², S. ROBINSON¹, A. OESTMANN³, J. BARNETT³, K. GODFREY¹, C. COOPER¹, T. KENDRICK³ and the SWS Study Group, ¹MRC Epidemiology Resource Centre, ²Division of Medical Education and ³Primary Medical Care Group, University of Southampton, Southampton SO16 6YD, UK

Lower blood folate levels have been associated with depression in several cross-sectional surveys, but longitudinal studies are needed to assess whether depression is a consequence or a cause of folate deficiency.

The Southampton Women's Survey (SWS) comprises a cohort of 12 500 non-pregnant women recruited from the general population between 1998 and 2002 who are being followed through subsequent pregnancies. The SWS has been shown to be broadly representative of the general population¹. From March 2000 all 7210 women recruited into the study were asked to complete a GHQ12 questionnaire to assess depression and anxiety². All women were asked to provide venous blood samples from which erythrocyte folate (RCF) was assayed using an Abbott microparticle enzyme immunoassay IMx-folate kit and an IMx analyzer. Consent was obtained from the women to access their general practitioner (GP) records to obtain evidence of incident depression over the 2-year period following the baseline interview.

Complete GHQ12 data were provided by 7020 women (97%) and RCF measurements were obtained for 5051 (72%) of them. Among the 5051 women 1588 (31%) were identified as depressed at the baseline survey. Using Poisson regression modelling with RCF as a continuous variable it was found that RCF was inversely associated with the risk of depression. The prevalence ratio associated with an increase in RCF of 100 nmol/l was 0.985 (95% CI 0.974, 0.995; $P=0.005$); those with RCF levels <960 nmol/l were 14% more likely to be depressed than those with higher levels. The association was attenuated after adjustment for confounding factors ($P=0.05$) but still indicated that lower RCF levels were linked to depression (prevalence ratio 0.98 (95% CI 0.97, 1.00; $P=0.05$).

Follow-up data were available for 3996 women whose RCF levels had been measured (79%). The 1264 women who were identified as depressed at baseline either from the GP notes or from the GHQ12 questionnaire were excluded. Of the remaining 2732 women 307 (11%) had an incident episode of depression recorded by their GP in the 2 years following baseline interview. In a Cox regression model no relationship between RCF and incident depression was identified; the unadjusted hazard ratio per 100 nmol/l increase in RCF was 0.99 (95% CI 0.96, 1.02; $P=0.4$) and the adjusted hazard ratio was 1.00 (95% CI 0.97, 1.03; $P=0.9$).

The finding of an association between RCF and prevalence of depression in a cross-sectional analysis but not with incident depression during follow-up of the cohort indicates that lower RCF levels may be more a consequence than a cause of depression. However, a positive association with incident depression cannot be excluded as smaller numbers of women contributed to the follow-up than to the cross-sectional analysis. Nonetheless, the 95% CI associated with the hazard ratio indicates that even a 100 nmol/l increase in RCF would be unlikely to reduce the risk of depression by more than about 3%. Thus, the contribution of low folate status to depression appears modest, and folate supplementation to prevent depression does not seem warranted on the basis of these findings.

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An investigation of Irish consumers' use and understanding of nutrition labels on pre-packaged foods. By S. KEOGH¹, M. CULLEN² and A. McKEVITT³, ¹Virtual School, School of Biomedical Science, University of Ulster, Coleraine, Co. Londonderry BT52 1SA, UK, ²Food Safety Authority of Ireland, Lower Abbey St, Dublin, Republic of Ireland and ³School of Biomedical Science, University of Ulster, Coleraine, Co. Londonderry BT52 1SA, UK

Diet plays an important role in chronic disease, accounting for one-third of cases of CVD and 30–40% of cancers¹. Consumer education on diet and nutrition is important in helping to prevent chronic disease². In conjunction with nutrition education, the nutrition label has the potential to act as a source of nutrition information, promote healthy eating and assist consumers in making healthy food choices, thus supporting public health objectives³.

A thirty-one-item structured interview-assisted questionnaire was developed, piloted and revised. A total of 536 consumers were interviewed in supermarkets and data collection took place throughout the day and week in supermarkets in city, mid-size town and rural locations.

Of the consumers 53.5% reported reading the nutrition label before purchase. Statistical analysis found that gender, age and education significantly influenced label use. Younger consumers, women and those with tertiary education were most likely to read the label.

Table. Do you read the nutrition label before purchase?

	Always (% total)	Sometimes (% total)	Never (% total)	New food (% total)
Total population (n 536)	20.0	33.5	45.1	1.7
Gender***: Male	11.7	24.1	60.6	3.6
Female	22.8	36.6	39.8	1.0
Age* (years): 18–35	22.5	39.4	37.3	0.7
36–50	20.6	32.9	41.9	4.5
51–65	18.4	28.9	52.0	0.7
>65	17.5	31.0	51.7	0.0
Education***: Primary	17.1	22.0	60.2	0.8
Secondary	15.4	32.4	50.5	1.6
Third-level	25.6	41.9	30.8	1.7
Postgraduate	24.5	34.0	37.7	3.8

* $P<0.05$, *** $P<0.001$.

However, understanding of the label was limited, with only 32.3% of the population knowing that there is a difference between salt and Na and 10% understanding the difference between energy and calories. Consumers also initially confused the term 'nutrition label' with the ingredient list, best-before date and other aspects of the food label. Lack of interest (43.1%) and time (18.7%) were the main reasons consumers gave for never using the label. Label print size was an issue for consumers, especially those >65 years. The media (31.6%) was found to be the main source of nutrition information for the study population.

If the nutrition label is to play a role in helping consumers to choose healthier food and helping to prevent chronic disease, then education in use and understanding of the label is required. Consumers particularly need education about common nutrition terms. Men, older consumers and those with lower levels of education need to be targeted. Issues such as small print need to be addressed. Before initiating programmes to encourage label use, research is needed to determine whether consumers who use labels actually have healthier diets.

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Soft drinks and fruit juice: impact on energy and nutrient intakes and adiposity of British adults. By E.M.B. VAN HEIJNINGEN, C.W. THANE and A.M. STEPHEN, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL, UK*

In the USA sweetened beverage consumption, or high intake of added sugars, has been associated with poorer dietary quality¹ and obesity² among adults. Similar findings were not observed in the UK in relation to intake of non-milk extrinsic sugars (NMES)³⁻⁵. The aim of the present study was to examine the impact of non-diet sweetened soft drinks plus fruit juice on energy and nutrient intakes and measures of adiposity in British adults.

Diet and adiposity were examined using data from a nationally-representative sample of 1724 British adults aged 19–64 years who participated in the 2000–1 National Diet and Nutrition Survey⁶. Non-diet soft drinks plus fruit juice were categorised using 7 d weighed dietary records. Beverages included non-diet carbonated, dilutable and ready-to-drink types and fruit juices. Beverages were included if the NMES content was ≥ 1 g/100 g. NMES contents of foods were provided by an electronic version of the NDNS Nutrient Databank. Beverages (g/d) were divided into four categories: non-consumption and approximate thirds. Adiposity was assessed by BMI and waist circumference (WC), each measured by trained fieldworkers. Associations between beverage consumption and energy, fibre and nutrient intakes, and measures of adiposity were determined using ANOVA.

Associations between soft drinks and fruit juice consumption (g/d) and intakes of energy (EI) and macronutrients are shown in the Table.

Intake	Men (n 766)				Women (n 958)			
	None (n 188)	Low (>0–105; n 198)	Medium (>105–265; n 187)	High (>265; n 193)	None (n 261)	Low (>0–85; n 232)	Medium (>85–205; n 229)	High (>205; n 236)
Energy (MJ/d)	9.17 ^a	9.29 ^a	9.70 ^a	10.51 ^b	6.19 ^a	6.54 ^a	6.97 ^b	7.75 ^c
Protein (% EI)	16.6 ^a	15.6 ^b	15.1 ^{b,c}	14.3 ^c	16.9 ^a	16.5 ^a	15.6 ^b	14.5 ^c
Fat (% EI)	33.1 ^a	33.9 ^a	33.1 ^a	33.3 ^a	33.6 ^a	33.3 ^a	33.9 ^a	33.1 ^a
SFA (% EI)	12.4 ^a	12.8 ^a	12.4 ^a	12.5 ^a	12.7 ^a	12.5 ^a	12.8 ^a	12.7 ^a
CHO (% EI)	43.0 ^a	43.6 ^{a,b}	45.1 ^{b,c}	46.9 ^c	45.9 ^a	46.2 ^a	46.1 ^a	48.7 ^b
NMES (% EI)	10.4 ^a	11.0 ^{a,b}	12.1 ^b	16.4 ^c	8.7 ^a	9.8 ^a	11.8 ^b	15.8 ^c

CHO, carbohydrates. ^{a,b,c} Within gender mean values within rows with unlike superscripts were significantly different (Bonferroni test following ANOVA; $P < 0.05$).

No differences in micronutrient intake were observed by level of beverage consumption, except for a positive association with vitamin C. High beverage consumption contributed 40% of vitamin C intake. Beverage consumption was not associated with the percentage of individuals with low micronutrient intakes (less than the lower reference nutrient intakes). BMI, WC and physical activity level did not vary significantly across different categories of beverages, for both genders.

The present study shows no negative impact of non-diet soft drinks plus fruit juice on nutrient intakes, BMI or WC. Results were similar when adjusted for age and misreporting, with EI varying less across categories, and did not lead to different conclusions.

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An examination of socio-economic variation in food and nutrient intake patterns among young women in Dublin using novel diet scores. By J.M. WALSH, M.T. O'NEILL, D.M.A. McCARTNEY, K.M. YOUNGER and J. KEARNEY, ¹*School of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland*

Socio-economic status has been identified as an important factor in determining dietary quality. Shahar *et al.*¹ have found a poorer quality of diet in those who are of low socio-economic status, while Robinson *et al.*², using diet scores, have shown an association between poor educational attainment and poor dietary quality in a sample of young UK women. The present study aims to explore socio-economic differences in the consumption of breakfast cereals, fruit and vegetables, red meat (including processed meat), confectionery, fizzy drinks, fibre and selected micronutrients among a population of young Dublin women (n 73). Socio-economic variation in the overall quality of these women's diets is then described using novel diet scores based on the intake of these food groups and nutrients.

Subjects' demographic details, health- and diet-related views and attitudes, health status and behaviours, local environment and other social factors were recorded. Food and nutrient intakes were assessed using a 7 d diet history. To create the novel diet scores, intakes of fruit and vegetables, breakfast cereals, red meat, confectionery, fizzy drinks, Ca, Fe, folate, vitamin C and fibre were dichotomised and each subject identified as having a low or high intake of each, based on splitting this group 50:50. Subjects were given a score of one for each of the following: high intake of fruit and vegetables, high intake of breakfast cereals, low intake of red meat, low intake of confectionery, low intake of fizzy drinks and high intake of Ca, Fe, folate, vitamin C and fibre. Higher scores indicated better overall dietary quality.

Using these novel diet scores, disadvantaged women were found to have poorer overall dietary quality. Mann-Whitney and Kruskal-Wallis analyses revealed that women of lower social class, low socio-economic group and low educational attainment had significantly lower diet scores, as did those who were unemployed or who left school early. The present study provides new data relating to the dietary intakes of young disadvantaged women in Dublin. The findings of low micronutrient intake and over-consumption of certain food groups among disadvantaged young women highlight the need for continued targeted public health strategies aimed at improving the quality of these women's diets.

Table. Association between variables of disadvantage and quality of diet using novel diet scores

Variable	Status	Diet score	P
Social class ⁺	High	5.44	0.038
	Low	4.26	
Education	High	6.58	<0.001
	Intermediate	4.96	
	Low	3.76	
Early school leaver	No	5.69	<0.001
	Yes	3.56	
Socio-economic group ⁺	High	5.62	0.006
	Low	4.15	
Employment	Employed	5.61	0.037
	Unemployed	4.42	

⁺ Subjects were defined as high or low Social Class and Socio-economic group by comparing their occupation with the UK Standard Occupational Classification³.

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Nutritional quality of school-day diets in Irish children (5–12 years). By J. WALTON, E.M. HANNON and A. FLYNN, *Department of Food and Nutritional Science, University College Cork, Cork, Republic of Ireland*

The objective of present study was to estimate the nutritional quality of school-day diets in Irish children. Analysis was based on The National Children’s Food Survey (NCFS), which was carried out between April 2003 and April 2004 to establish a database of habitual food and drink consumption in a representative sample of Irish children aged 5–12 years. A 7 d weighed food record was used to collect food intake data from 594 children (293 boys, 301 girls). Analysis of dietary intake data was carried out using WISP© (Tinuviel Software, Llanfechell, Anglesey, UK), which is based on *McCance and Widdowson’s The Composition of Foods Sixth Edition*¹. A dataset of food and drink consumption on school days only was established.

Mean daily energy intakes in boys and girls were 7.6 and 6.8 MJ respectively. Mean daily fat, saturated fat and carbohydrate intakes (% total energy; %TE) were 33.2, 14.2 and 52.6 respectively. The percentage of individuals not meeting macronutrient and dietary fibre recommendations and also those with mean daily intakes (MDI) less than average requirements (AR) for micronutrients are estimated in the Table.

Age-group (years) ... n ...	Boys			Girls		
	All	5–8	9–12	All	5–8	9–12
Percentage of individuals not meeting recommendations	261	130	131	268	137	131
Fat (%TE ≤35)	32	32	31	36	32	40
Saturated fat (%TE ≤11)	84	82	87	90	91	88
Monounsaturated fat (%TE ≥13)	90	89	91	90	91	89
Polyunsaturated fat (%TE ≥6.5)	92	95	90	88	90	87
Carbohydrate (%TE ≥50)	29	32	26	33	32	33
Dietary fibre ≥age of child +5	51	39	63	60	47	73
Percentage of individuals with MDI < AR						
Total vitamin A	20	18	22	25	23	28
Riboflavin	8	8	9	12	10	15
Folate	18	13	24	32	21	43
Ca	22	17	27	30	17	44
Fe	13	9	17	30	15	44

Mean daily salt intakes (Na × 2.5; g) from food sources only (5–6 years, 4.7; 7–10 years, 5.3; 11–12 years, 6.0) exceeded target levels² for total salt (g) for 5–6 year olds of 3 and 7–10 year olds of 5 and were at the target level for 11–12 year olds of 6.

The study shows that there is a need to reduce fat intake and improve the unsaturated fatty acid: SFA in school-day diets. Furthermore, intakes of dietary fibre and a number of micronutrients need to be increased.

The project was funded by *safe*food and the Health Service Executive.

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Disadvantaged groups of women’s perceptions of their nutrition service needs: a qualitative study. By O. HAUGHEY, S. KLEEMANN and K. YOUNGER, *Dublin Institute of Technology, Kevin Street, Dublin 2, Republic of Ireland*

Numerous studies have found a general trend of lower compliance with dietary recommendations amongst the lower socio-economic groups in Ireland¹, with the highest prevalence of obesity amongst this sector of society². As a result, this population needs to be targeted with effective nutrition interventions. However, studies have found that there are numerous obstacles in accessing this target population³. As public health education funds are limited, it is important to determine which methods of nutrition intervention are most effective for promoting a healthy diet to this target population. Evaluation research has proved that nutrition interventions are more effective when they are culturally acceptable and age appropriate, and incorporate the relevant health concerns of the population they are intended to benefit⁴.

The present study used qualitative research methods, focus groups being the chosen qualitative component, in order to investigate the perceptions of disadvantaged groups of women in relation to food knowledge, motivators to a healthy diet and perceived nutrition service needs.

Four focus groups of women (Total n=23), from four different disadvantaged areas in Dublin, were recruited. Three of the groups shared varying levels of social deprivation and limited levels of educational attainment. However, these three groups differed significantly in other respects, most notably in age. The fourth group was a stakeholder group who worked with groups of disadvantaged women. Despite the different backgrounds, certain themes were consistent across all groups. Five key discussion themes were identified: nutrition knowledge; motivators to dietary change; sources of nutrition information; barriers in acquiring nutrition information; and perceived service needs.

The majority of participants felt that they had adequate knowledge of the basics of nutrition. Regardless of age and nutrition knowledge all participants admitted to their unhealthy eating habits. Previous research has shown that the relationship between nutrition knowledge and dietary behaviour is not sufficient to motivate dietary change⁵. The importance of how food relates to weight was the prime motivator for dietary change in all focus groups. The older adults portrayed health concerns to be a significant motivator to dietary change, with nutrition-related health issues becoming a concern. For the younger adults their children were a strong motivator to dietary change. Mothers perceived themselves as role models for their children. The media was the most common source of information cited by all groups. However, the general consensus was that nutrition information supplied through the media was biased. All groups cited health professionals as a source of nutrition information. However, cost was a barrier to accessing this professional advice. The majority of participants were familiar with the symbol of the Food Pyramid. However, the consensus was that it was difficult to understand and that new and innovative measures are needed to promote healthy eating.

The majority of participants expressed the view that the best way to structure nutrition education was through group classes, as the support of a group would increase motivation levels. The most popular format identified for group education was practical classes with hands-on activities such as cooking classes. A majority of participants felt that community involvement was essential in nutrition programme design.

Focus-group themes suggest that an intervention with disadvantaged groups of women should address practical information needs through hands-on activities, which should be tailored to specific sub-populations. However, because the focus groups consisted of single groups of four different sub-populations, the sample is not representative of all disadvantaged groups of women in Dublin. Nonetheless, the present study conveyed that clients from disadvantaged groups have valuable opinions and insights that programme developers targeting this hard-to-reach group should hear.

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Tracking the prevalence of obesity and overweight in Liverpool schoolchildren. By L.M. BODDY^{1,2}, A.F. HACKETT^{1,2} and G. STRATTON^{1,2}, ¹*Research into Activity and Children's Health Group, Liverpool John Moores University, Henry Cotton Campus, 15-21 Webster Street, Liverpool L3 2ET, UK* and ²*School of Tourism, Consumer and Food Studies, Liverpool John Moores University, IM Marsh Campus, Barkhill Road, Liverpool L17 6BD, UK*

The increasing prevalence in overweight and obesity is well documented. The Liverpool-based *SportsLinx*¹ project annually conducts field-based fitness and health testing on approximately 4000 9–10-year-old (year 5; Y5) Liverpool schoolchildren as part of a wider health, fitness and nutritional programme. In 2006 height and weight data were collected from year 6 (Y6) children (10–11 year olds) across the Liverpool Local Education Authority. Of this cohort 2362 participants (1227 boys and 1135 girls) were matched by name and date of birth to participants field tested by *SportsLinx* when in Y5, providing a unique data-set for a substantial number of pre-pubescent children. Original Y5 stature and body mass data from 2004–5 were used to calculate BMI in Y5, and the data collected 1 year later in 2005–6 were used to calculate BMI in Y6. BMI values were classified using international age- and gender-specific cut-off points². Mean age (years) when tested in Y5 was 9.93 (SD 1.26) for boys and 9.81 (SD 0.38) for girls and corresponding ages in Y6 were 11.26 (SD 0.33) and 11.26 (SD 0.32). The mean difference in age (years) between Y5 and Y6 was 1.45 (SD 0.24) for boys and 1.45 (SD 0.23) for girls.

Results describe substantial increases in the percentage of children classified as obese and overweight. In Y5 22.9% of girls were classified as overweight; in Y6 the percentage for the same cohort was 26.9, an increase of 17.5%. For boys the percentage of participants who were classified as overweight had risen from 17.5 in Y5 to 19.6 in Y6, an increase of 12%. When examining changes in the prevalence of obesity within the matched participants, the percentage of girls classified as obese remained constant at 8.1. For boys the prevalence of obesity had risen from 5.9% to 8.1%, an increase of 37.3%. When taken together, the percentage of girls classified as either overweight or obese increased from 31 in Y5 to 34.9 in Y6, a 12.5% increase in 1 year. For boys the pattern is similar, an increase in the percentage of overweight and obese subjects from 23.5 in Y5 to 27.6 in Y6, a rise of 17.4.

These findings are particularly worrying as Liverpool school children are exposed to many interventions and initiatives to improve dietary habits, increase levels of physical activity and inform children and adults of the constituents of a healthy lifestyle. The current target of halting the year-on-year rise in obesity in children by 2010 would seem to be remote.

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Excretion of isoflavonoids in Australian women who do and do not report obvious consumption of soya foods. By K. HANNA¹, P. LYONS-WALL², G. EAGLESHAM³ and S. O'NEILL⁴, ¹*Dept of Biological Sciences, University of Chester, Chester CH1 4BJ, UK*, ²*School of Public Health, Queensland University of Technology, Australia*, ³*Public Health Sciences, Queensland Health, Australia* and ⁴*Royal Brisbane and Women's Hospital and the University of Queensland, Australia*

Studies have evaluated the usual intake of isoflavonoids in Asian populations consuming a traditional soya-based diet and populations consuming a Western diet. Intake of isoflavonoids in Caucasian populations compared with Asian populations is typically lower and qualitatively different, with inclusion of processed soya foods and soya or isoflavonoid supplements². However, no study has evaluated a tool for the assessment of isoflavonoid status within Australian populations. The current aim was to investigate exposure to isoflavonoids by examining the association between usual intake and urinary excretion in a group of Australian women.

A sample of 133 women aged 40–59 years was recruited from a larger cohort of women participating in the Brisbane Longitudinal Assessment of Ageing in Women. Three 24 h urine samples were collected and used to form a composite sample. Analyses were conducted for nine isoflavonoids using HPLC. Intake of isoflavonoids from food and supplements over the previous month was assessed using a specially-designed frequency questionnaire containing forty-six items selected to include major sources in the Australian market. Values for the isoflavonoid content of items were sourced from published scientific data. Analyses were conducted separately for the total group and for soya consumers (*n* 53), defined as individuals who reported consumption of at least one serving of soya foods over the previous month¹.

Reported intake and excretion of isoflavonoids were higher in soya consumers compared with non-consumers (Mann-Whitney; $P < 0.001$ and $P < 0.009$ respectively); however, small amounts of isoflavonoids were detected in the urine of 100% of participants who reported no intake of soya.

	<i>n</i>	Isoflavonoid intake (mg/d)		Isoflavonoid excretion (μmol/d)	
		Median	Range	Median	Range
Soya consumers	53	7.67	0.28–153	0.76	0.048–42
Soya non-consumers	80	0.0075	0–0.16	0.331	0.015–6.9
All participants	133	0.02	0–153	0.427	0.015–42

There was a significant association between intake and excretion of isoflavonoids in the total group (p 0.215, $P < 0.05$), with a stronger association in soya consumers (p 0.355, $P < 0.01$). Excretion of isoflavonoids in non-consumers suggests that women may be consuming small amounts from hidden sources within the food supply. Products containing soya were not included in the questionnaire if their isoflavonoid content was expected to be low; for example, breads in which soya flour is used to whiten the grain and processed meats in which soya flour acts as a binder. There was a significant association between isoflavonoid intake and excretion within soya consumers; however, the ability to evaluate the questionnaire using excretion was limited because of consumption of small quantities of isoflavonoids from unidentified food sources not listed in the questionnaire.

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Dietary polyphenols regulate lipolysis in cultured pig fat explants. By K.R. HEADLAND, L. ERRARD, G.A. TUCKER and J.M. BRAMELD, *Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK*

Polyphenols are common constituents of foods of plant origin, and one of the major antioxidants in the human diet. Some foods and beverages containing high levels of dietary polyphenols have been linked with body-weight loss. Supplementation of the diet with the polyphenol epigallocatechin gallate (EGCG) has been shown to abolish diet-induced obesity in both rodents^{1,2}. The aim of the present study was to determine whether any anti-obesogenic properties of polyphenols might be attributable to lipolytic effects on adipocytes.

Pig fat explants from subcutaneous (SC) and peri-renal (PR) depots (three pigs) were cultured, as described by Budd *et al.*³, with or without increasing concentrations of individual polyphenols (EGCG, gallic acid, phloridzin, quercetin or resveratrol) for 21 h, followed by a 3 h culture in Krebs-Ringer bicarbonate buffer (plus adenosine deaminase (ADA); 0.75 U/ml) in the presence of the same polyphenols with or without isoprenaline (IP; 10 μM). Glycerol released into the media during the 3 h culture was determined using a colorimetric assay (Sigma, Poole, Dorset, UK) as a measure of lipolysis and corrected for lipid content.

As expected, IP stimulated lipolysis ($P < 0.001$) in both depots, with the PR depot being more responsive³. There were significant dose-dependent effects of polyphenol treatment ($P < 0.001$) in both depots, but no interaction with IP ($P > 0.15$). In the SC depot (Fig. 1) EGCG (100 μM), gallic acid (100 μM) and quercetin (100 μM) all reduced lipolysis compared with the control ($P < 0.05$), whereas phloridzin (25 μM) increased lipolysis ($P < 0.05$). In the PR depot (Fig. 2) EGCG (50 and 100 μM) and quercetin (100 μM) significantly ($P < 0.05$) reduced lipolysis compared with the control.

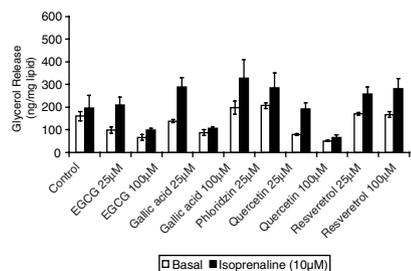


Fig. 1. Polyphenol effects in SC fat explants.

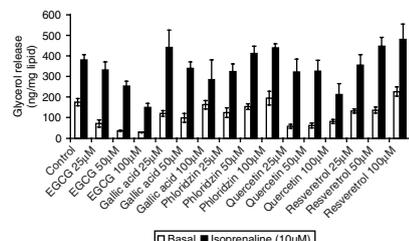


Fig. 2. Polyphenol effects in PR fat explants.

In conclusion, both stimulatory (phloridzin) and inhibitory (EGCG, gallic acid and quercetin) effects of individual polyphenols were observed, independent of IP. One possible mechanism for these effects is that polyphenols may act as adenosine receptor agonists or antagonists. If so, the inclusion of ADA in the media may limit the potency of any antagonists (possibly phloridzin and resveratrol) to increase lipolysis, because of the lack of adenosine in the cultures. There appears to be some relationship to polyphenol structure in terms of the number of hydroxyl groups present on the benzene rings and their effects on lipolysis, with one hydroxyl group tending to increase lipolysis, while two or more inhibit lipolysis.

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An exploration of food provision, and commitment to the introduction of a nutrition incentive scheme, in the preschool setting. By C. JOHNSTON MOLLOY¹, M. MURTAGH¹, C. CORISH², J. KEARNEY² and C. GLENNON¹, ¹Community Nutrition & Dietetic Service, Health Service Executive Dublin Mid-Leinster, Marlinstown Office Park, Mullingar, Co. Westmeath, Republic of Ireland and ²Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland

Preschools play a valuable role in the lives of young children, as food offered can affect nutritional status, food habits and future health^{1,2}. In Ireland food and nutrition guidelines for preschool services³ exist, but methods to encourage provision of nutritious food in this setting are lacking and must be pursued to ensure guidelines are followed. A preliminary study has demonstrated that preschools are in favour of introducing the model of a nutrition incentive scheme⁴. The purpose of the present study was to obtain baseline data on the meal and snack categories being provided by preschools and parents, and to determine preschool commitment to enrolling in a future nutrition incentive scheme. A structured telephone questionnaire was used to obtain the views of preschool providers based in the Midlands region of Ireland, who are those directly involved with the daily care of the children in their establishment ($n = 89$).

Seventy-three of eighty-nine preschools registered with the Health Service Executive (HSE) took part in the investigation. Sixty-seven preschools reported that they provided food: breakfast ($n = 37$); mid-morning snack ($n = 47$); main meal ($n = 65$); mid-afternoon snack ($n = 46$); light meal ($n = 23$). Forty-eight preschools stated that parents provide some food: breakfast ($n = 3$); mid-morning snack ($n = 45$); main meal ($n = 3$); mid-afternoon snack ($n = 19$); light meal ($n = 1$). Whilst sixty-three noted that they had a healthy food policy, only forty-two said they had created it in conjunction with the Food and Nutrition Guidelines for Preschool Services³ and only forty-three had a policy on food brought in by parents.

When asked to rate the idea of a nutrition incentive scheme on a Likert scale (1, poor; 10, excellent), the majority of participants gave scores of 8–10. Most preschool providers ($n = 64$) said that they would sign up for a nutrition incentive scheme and cited many benefits. The Table shows benefits suggested by the preschool providers.

Benefits cited	<i>n</i>
Provide training for members	19
Facilitate children to eat more healthily	16
Information for parents	8
Raise profile amongst parents	7
Improve practice	6
Deliver more information on healthy food	6
Improve menus	5
Obtain recognition from HSE	3

Although the majority of preschools are committed to taking part in a nutrition incentive scheme, the present study demonstrates that in many services parents are providing quite a substantial amount of the food being eaten by children. It is necessary to ensure that a parental perspective is included in the planning and establishment of a future nutrition incentive scheme.

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Nutritional metabolomics: optimisation for general serum metabolic profiling using ultra-performance liquid chromatography (UPLC) coupled with quadrupole orthogonal acceleration time-of-flight (QToF) MS. By M.C.Y. WONG, H. FELDMANN, G. FROST and J. LODGE, Nutrition Research Group, School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH, Surrey, UK

Metabolomics, or the global analysis of metabolites in biological fluids, provides an alternative set of information to understand the complexity of biological processes and how they are influenced by diet and disease. Historically, ¹H-NMR has been used to provide the complex data-sets for multivariate analysis and liquid chromatography (LC)–MS (LC/MS) is still in its infancy. However, sample pretreatment and preparation is usually required before LC/MS analysis, and therefore optimisation is critical in order to produce reliable data for metabolic profiling. The aim of the present study is to develop the use of LC/MS-based metabolomics by optimising the methodology for the processing of human serum samples and then to test this methodology to distinguish metabolic profiles in two groups of human serum samples.

Thus, overnight-fasting serum samples were obtained from healthy human subjects and various sample-preparation procedures and LC/MS conditions were tested to produce optimal conditions for metabolic profiling. The metabolite profiles were acquired using UPLC coupled with QToF MS (Waters Inc., Manchester, UK). After the acquisition of the data MassLynx and MarkerLynx (version 4.1; Waters Inc.) were used to manage and export the data into SIMCA-P+ (version 11.0; Umetrics, Windsor, Berks., UK) for multivariate data analysis. The total number of ions and the total ion count (TIC; the overall strength of signal) were used to assess the efficiency of different extraction methods. Serum metabolites were extracted by acetonitrile, methanol or a combination of acetonitrile and methanol. The effect of sample lyophilisation and sample storage were also investigated. Both UPLC and MS conditions were also varied to optimise for high sample throughput.

Results demonstrate that sample lyophilisation and reconstitution with 100% (v/v) cold methanol yielded the highest number of ions, as well as a higher TIC, detected by the MS. A linear gradient (0–100%; v/v) of acetonitrile over 10 min was found to give suitable chromatographic separation. In addition, it was also found that storage of the metabolite extracts at low temperature (i.e. <4 °C) was stable for ≤1 d. Good within-day and between-day variation was also found.

Furthermore, in order to put the optimised method into practise the metabolic profiles of seven male subjects and seven female subjects were acquired. These data were then used to examine the effect of gender on the metabolite composition of serum. The results of the study showed that 4276 and 2766 ions were detected in the positive and negative ionisation modes respectively. Two groups of samples were clearly separated through partial least squares discriminant analysis. The data was further analysed and a number of potential species identified that contribute to group (gender) separation.

To conclude, an optimised high-throughput method has been developed for LC/MS-based metabolite-profiling studies of human serum, and this technique has been shown to successfully discriminate metabolite profiles for males and females. This highlights the potential use of LC/MS-based metabolomics for nutritional studies.

Is dietary glycaemic index associated with increased risk of metabolic syndrome in young adults? Evidence from the Young Hearts Project, Northern Ireland. By P.M. LUNNY¹, P.J. ROBSON¹, C.E. NEVILLE², L.J. MURRAY², C.A.G. BOREHAM³ and A.M. GALLAGHER¹, ¹Northern Ireland Centre for Food and Health, University of Ulster, Coleraine BT52 1SA, UK, ²Centre for Clinical and Population Sciences, Queen's University of Belfast, Belfast BT12 6BJ, UK and ³Institute for Sport and Health, University College Dublin, Dublin, Republic of Ireland

Metabolic syndrome (MetS) is thought to increase risk of CVD and type 2 diabetes¹. While the aetiology of MetS is not fully understood, it is thought to arise as a consequence of deleterious interactions between genetic, metabolic, environmental and lifestyle factors². One factor that may be of relevance is diet, and specifically dietary glycaemic index (DGI), which provides a quantitative measure of the physiological blood glucose response to different levels of carbohydrate (CHO)-containing foods³. Given the link between CHO type and glycaemic control, it is not surprising that high-GI diets have been implicated as a possible risk factor for MetS. The aim of the present study was to investigate whether DGI was associated with components of MetS in 489 young adults (20–25 years) from Northern Ireland⁴.

Mean DGI for each subject was calculated using published GI tables^{5,6}, with GI values assigned to 81% of dietary CHO. Mean DGI was higher in males than in females (50.2 (SD 6.4) v. 49.1 (SD 6.0), $P=0.05$). Overall, 31.8% of the cohort had at least one component of MetS. When expressed as quintiles of DGI, positive associations between DGI and systolic blood pressure ($P=0.038$) and fasting blood glucose ($P=0.056$) were observed (Table).

	Quintile 1		Quintile 2		Quintile 3		Quintile 4		Quintile 5		P†
	Mean	SD									
DGI	40.6	4.1	46.9	1.1	50.3	0.8	53	0.8	57.6	2.8	
BMI (kg/m ²)	23.8	3.5	23.7	3.4	23.7	3.1	23.9	4.3	24.1	4.3	0.942
Waist (cm)	79.1	12.0	77.7	9.2	76.8	9.5	78.7	10.9	79.0	10.4	0.569
HDL-C (mmol/l)	1.44	0.5	1.36	0.6	1.34	0.6	1.3	0.5	1.31	0.5	0.081
TAG (mmol/l)	0.78	0.4	0.84	0.5	0.75	0.4	0.79	0.5	0.83	0.5	0.588
Fasting glucose (mmol/l)	4.3**	1.8	4.4*	2.0	4.4	1.7	4.4*	2.0	4.5	1.9	0.056
Systolic BP (mm/Hg)	112.2*	16.0	112.4*	10.6	112.4*	14.2	112.1**	12.7	116.8	13.8	0.038
Diastolic BP (mm/Hg)	74.0	11.6	74.4	8.8	72.3	9.9	73.0	8.7	75.6	11.0	0.129

C. cholesterol. Mean values were significantly different from those for quintile 5: * $P<0.05$, ** $P<0.01$. † One-way ANOVA.

These data support the need for further exploration of the relationship between DGI and risk of MetS, and highlight the need for public health strategies aimed at improving the future health of this group.

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Induction of phase II enzymes by sulforaphane leads to cardioprotection. By C. ANGELONI, E. LEONCINI, N. CALONGHI, E. PAGNOTTA, P.L. BIAGI and S. HRELIA, *Department of Biochemistry 'G. Moruzzi', University of Bologna, Bologna, Italy*

There is substantial evidence to support the theory that oxidative stress plays an important role in the pathophysiology of CVD¹. Accordingly, several naturally-occurring antioxidant compounds have been utilized to counteract oxidative cardiac injury^{2,3}. Another promising strategy for protecting cardiac cells against oxidative stress may be through the induction of endogenous antioxidants and phase II enzymes. Sulforaphane (SF) is a naturally-occurring isothiocyanate that is highly concentrated in Cruciferous vegetables. Many studies have shown a strong chemopreventive effect of SF through its ability to induce phase II detoxifying enzymes by activating antioxidant-response element (ARE) through the induction of Nrf2⁴, but no data are available to support a similar role for SF in cardioprotection. Using cultured rat cardiomyocytes, the time-dependent induction of cellular antioxidants and phase II enzymes by SF and its ability to protect cardiac cells against oxidative stress have been characterised, and the translocation of nrf2 to the nucleus after SF supplementation has been investigated.

Cultured rat cardiomyocytes were prepared and grown as described previously⁵. Cells were supplemented with 5 µM-SF for different time periods (6, 12, 24 and 48 h). The activities of glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and NAD(P)H-quinone reductase 1 (NQO1) were determined spectrophotometrically, and the content of intracellular reduced glutathione (GSH) was estimated using the fluorescent indicator monochlorobimane. Western-blot analyses of GR, GST, GPx and NQO1 were performed using specific antibodies and following the manufacturer's recommended protocols. ROS formation was determined by a spectrofluorimetric method using 2',7'-dichlorofluorescein diacetate. Cell viability was evaluated by MTT assay and Nrf2 translocation to the nucleus by laser confocal microscopy using specific antibodies.

Incubation of cardiomyocytes with SF resulted in a significant elevation of cellular GSH content for all exposure times. SF supplementation also led to a time-dependent increase in GR, GST and NQO1 activities. Accordingly, a significant increase in GR, GST and NQO1 expression was observed. In contrast, incubation of cardiomyocytes with SF for 6–48 h did not result in any significant increase in cellular GPx activity and expression, in agreement with published data⁶. Laser confocal microscopy revealed the translocation to the nucleus of Nrf2 after SF supplementation. SF pretreatment led to a decreased intracellular accumulation of ROS and marked cytoprotection after exposure to 100 µM-H₂O₂. The results demonstrate, for the first time, that a number of endogenous antioxidants and phase II enzymes can be induced in cultured cardiomyocytes by low micromolar concentrations of SF, and that this nutritionally-mediated up-regulation of cellular defences is accompanied by a markedly increased resistance to cardiac cell injury elicited by peroxide.

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Biochemical and proteomic changes in the colon and liver of rats in response to long-term folate deficiency. By S.J. DUTHIE¹, G. GRANT², G.J. RUCKLIDGE³, M.D. REID³, G.J. DUNCAN³, L.P. PIRIE¹ and G. HORGAN⁴, ¹Nutrition and Epigenetics Group, Division of Vascular Health, ²Gut Immunology Group, Division of Gut Health, ³Proteomics Unit and ⁴Biomathematics and Statistics Scotland, Rowett Research Institute, Aberdeen AB21 9SB, UK

Folate deficiency has been implicated in the development of colon cancer. Critically, folate deficiency is widespread, affecting a substantial percentage of the population. Here, proteomics and biochemical approaches have been combined to determine metabolic pathways affected by folate deficiency in rats.

Blood, liver and colon were harvested from Rowett strain male Hooded Lister rats fed a control (*n* 10) or folate-free diet (*n* 10) for 24 weeks. Blood and tissue folate were measured by radioimmunoassay. Plasma homocysteine, tissue S-adenosylmethionine (SAM) and tissue S-adenosyl-homocysteine (SAH) were quantified by HPLC. Soluble and insoluble proteins isolated from the distal colon and liver were differentially separated by two-dimensional gel electrophoresis and analysed using PDQuest (BioRad, Hemel Hempstead, Herts., UK). Proteins that differed significantly between treatments (*P*<0.05; Student's *t* test) were identified by searching the Matrix Science Mascot database following nano liquid chromatography–MS–MS analysis.

Blood and tissue folate was significantly decreased in rats fed a folate-depleted diet for 24 weeks. Conversely, plasma homocysteine was significantly increased. The liver SAM:SAH was significantly lower in folate-deficient rats (Table), while the ratio in the colon remained unchanged.

Biomarker	Control		Folate deficient		% change
	Mean	SE	Mean	SE	
Plasma folate (ng/ml)	94.5	2.2	13.2***	1.2	86, decrease
Whole-blood folate (ng/ml)	136.4	4.1	72.9***	4.1	47, decrease
Colon folate (ng/mg tissue)	24.8	1.5	11.9***	2.2	52, decrease
Liver folate (ng/mg tissue)	136.2	6.9	93.9*	16.3	31, decrease
Plasma homocysteine (µmol/l)	5.91	0.28	6.91*	0.32	17, increase
Liver SAM:SAH	5.63	0.12	5.04*	0.13	11, decrease

Mean values were significantly different from those for the controls (Student's *t* test): **P*<0.05, ****P*<0.001.

In excess of eighty liver and fifty colon spots, which differed significantly between treatments, were identified. The majority of proteins were up regulated in response to folate deficiency (81% and 94% for liver and colon proteins respectively). Principal component analysis, performed on spot-density data derived from all spots, revealed that the proteome of replicate gels separated according to folate treatment. Proteins that differed between treatments were categorised broadly according to biochemical function. Treatment effects in both colon and liver were seen on major metabolic pathways related to amino acid and protein biosynthesis (e.g. fumarylacetoacetase, elongation factor 2), lipid metabolism (e.g. propionyl-CoA carboxylase) and glycolysis (e.g. pyruvate kinase, phosphoglucosmutase-1) and on proteins involved in the cell cycle (e.g. profilin, prohibitin), and detoxification (e.g. sulfotransferase 1A1, catalase). Analysis of correlations between biochemical measurements and all colon and liver proteins was carried out using Pearson correlation coefficients. Construction of protein networks from these multiple correlations revealed clusters of highly-correlated proteins that were differentially influenced by blood and tissue folate status, and by plasma homocysteine or SAM and SAH concentrations.

In conclusion, biochemical and proteomic analyses show that both the liver and colon of rats is highly sensitive to changes in both folate status and intermediates in the methionine cycle.

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Phylloquinone intakes and food sources in Irish children aged 5–12 years. By E.M. HANNON, K.D. CASHMAN, M. KIELY and A. FLYNN, *Department of Food and Nutritional Science, University College Cork, Cork, Republic of Ireland*

Poor vitamin K status may have an adverse effect on childhood bone health¹. The objective of the present study was to estimate phylloquinone intakes and food sources in Irish children aged 5–12 years, using data from the National Children's Food Survey (NCFS). The NCFS was carried out between April 2003 and April 2004 to establish a database of habitual food and drink consumption in a representative sample of Irish children aged 5–12 years. A 7 d weighed-food record was used to collect food intake data from 594 children (293 boys, 301 girls). Analysis of dietary intake data was carried out using WISP© (Tinuviel Software, Llanfechell, Anglesey, UK), which contains *McCance and Widdowson's The Composition of Foods, 6th Edition*². Phylloquinone levels were applied to foods consumed in the NCFS from an established database of phylloquinone contents of foods². Mean daily intakes (MDI; µg) increased significantly ($P < 0.05$) with age in boys and nutrient density of intakes (µg/10 MJ) was significantly ($P < 0.05$) higher in 5–8- and 9–12-year-old girls compared with boys.

	Boys				Girls			
	5–8 years (n 145)		9–12 years (n 148)		5–8 years (n 151)		9–12 years (n 150)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Phylloquinone:								
µg/d	34.2	20.4	41.0*	26.0	36.7	22.8	39.7	23.2
µg/10 MJ per d	50.0	28.4	52.1	33.7	57.9†	33.3	57.2†	32.0

* Significantly ($P < 0.05$) higher than that for 5–8-year-old boys, † Significantly ($P < 0.05$) higher than that for boys.

	Total		Boys		Girls	
	5–12 years (n 594)		All ages (n 293)		All ages (n 301)	
	µg	%	µg	%	µg	%
Vegetable & vegetable dishes	15.2	29.2	14.8	28.0	15.6	30.3
Potatoes & potato products	4.3	14.0	4.5	14.9	4.0	13.2
Meat & meat products	3.5	10.1	3.6	10.1	3.4	10.1
Sugars, confectionery, preserves & savoury snacks	2.8	9.1	2.8	9.2	2.8	8.9
Fruit & fruit juices	2.5	6.9	2.3	6.5	2.6	7.2
Milk & yoghurt	1.6	5.8	1.8	6.4	1.4	5.1
Butter, spreading fats & oils	1.6	5.4	1.6	5.4	1.6	5.4
Biscuits, cakes & pastries	1.2	3.9	1.3	4.2	1.2	3.6
Other food groups	5.3	15.8	5.0	15.2	5.6	16.3
Total	37.9	100.0	37.6	100.0	38.2	100.0

Vegetable and vegetable dishes contribute approximately 30% to MDI, with potatoes and potato products, meat and meat products and sugars, confectionery, preserves and savoury snacks also making important contributions. In boys and girls 51% and 48% respectively had MDI < 1 µg/kg body weight⁴.

The project was funded by the Irish Government under the National Development Plan 2000–2006.

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The bioaccessibility of α-tocopherol and retinol from a range of vitamin-enriched foods. By Y.C. O'CALLAGHAN, O. KENNY and N.M. O'BRIEN, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Fortification of frequently-consumed foods, such as milk and other dairy products, with vitamins and minerals is an effective and low-cost means of minimising the risk of nutrient deficiencies in the population¹. The increase in demand for low-fat dairy products has also necessitated the addition of fat-soluble nutrients in order to replace those lost during processing. Data on the bioavailability of fat-soluble vitamins is limited and may be affected by a number of factors including the food matrix, fat content and the level of fibre.

The objective of the present study was to determine the bioaccessibility of retinol and α-tocopherol from a range of vitamin-enriched foods. Bioaccessibility describes the portion of a nutrient that is available for absorption. The foods selected were a low-fat milk, a dairy-free soya milk, a ready-to-use infant formula, a multivitamin juice blend, a rice-based baby cereal, a reduced-fat processed cheese and a full-fat and low-fat variety of vegetable oil spread. Fat-soluble nutrients must be packaged into micelles to facilitate intestinal absorption. The foods were subjected to an *in-vitro* digestion procedure adapted from a method described previously². Following digestion the food samples were centrifuged in order to isolate the aqueous or micellarised fraction. α-Tocopherol and retinol were extracted from both foods and micelles using hexane and were quantified by HPLC.

	α-Tocopherol						Retinol			
	Pack*	Measured in food*		Micellarised (%)		Pack*	Measured in food*		Micellarised (%)	
		Mean	SE	Mean	SE		Mean	SE	Mean	SE
Milk	1.50	3.79	0.85	33.31	6.00	0.12	0.62	0.06	19.38	0.16
Soya milk	1.50	1.45	0.11	50.71	18.02	0.00	nd	nd	nd	nd
Infant formula	0.74	1.56	0.12	52.81	19.66	0.08	0.09	0.01	84.71	3.60
Juice	5.00	7.56	0.51	50.82	4.62	0.40	nd	nd	nd	nd
Rice cereal	2.90	4.23	0.78	52.41	16.07	0.71	0.77	0.03	36.15	3.72
Cheese	2.50	1.35	0.67	63.10	9.49	0.20	0.11	0.05	64.41	35.27
Spread	20.00	16.44	1.00	7.32	2.30	0.80	0.86	0.09	4.17	0.06
Low-fat spread	20.00	20.15	0.99	8.37	3.94	0.80	1.06	0.12	3.92	1.09

Values are means for three independent experiments. nd, not detected. * mg/100 g or mg/100 ml.

Higher levels of both α-tocopherol and retinol were measured in the low-fat milk than were stated on the label, while low-fat cheese was found to contain approximately 50% less α-tocopherol than was reported. Retinol was not detected in the juice as it was added in the form of provitamin A; also, retinol was not detected in the micelles obtained from the juice. The bioaccessibility of α-tocopherol ranged from 7.32% in the full-fat vegetable oil spread to 63.10% in the cheese. The bioaccessibility of α-tocopherol from milk was reported as 21.95% in a previous study¹, which is similar to the level of 33.31% in the present study. The amount of fat present in the food does not appear to influence the bioaccessibility of α-tocopherol, as a similar percentage was micellarised from orange juice (0.1 g fat/100 ml), the rice cereal (6.3 g fat/100 g) and the soya milk (3.3 g fat/100 ml). The bioaccessibility of retinol ranged from 3.92% in the low-fat spread to 84.71% in the infant formula. Bioaccessibility of both vitamins was low in the vegetable oil spreads, which may be related to the food matrix; however, the bioaccessibility may be enhanced when consumed in conjunction with other foods. Fruit juice may provide a suitable alternative to milk for ensuring an adequate supply of fat-soluble vitamins to the general population.

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Effect of fibre on the bioaccessibility of carotenoids from a combination of vegetables. By O.F. O'CONNELL, L. RYAN and N.M. O'BRIEN, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Carotenoids are fat-soluble pigments found in many fruits and vegetables and are of considerable interest because of their antioxidant potential and provitamin A activity. The absorption of carotenoids by the intestine is dependent on these fat-soluble pigments being packaged into micelles. Dietary fibre has been reported to have a negative effect on the bioavailability of carotenoids in human subjects¹. Documenting qualitatively similar results to human studies can help validate an *in vitro* digestion procedure as a rapid model system to screen for the bioaccessibility of phytochemicals. The objective of the present study was to investigate, using an *in vitro* digestion procedure, whether the addition of oat bran, wheat bran or pectin to a vegetable mixture (spinach (*Spinacia oleracea*), tomato and red pepper (*Capiscum annuum* L.) would affect the transfer of lutein, lycopene and β -carotene into micelles. The vegetables were chosen as rich sources of the carotenoids of interest. Equal quantities (2 g) of the three vegetables were mixed with 0.3 g fibre sample (5% (w/w) fibre), homogenised and subjected to an *in vitro* digestion procedure as described previously². Digesta were ultracentrifuged to isolate the aqueous micellar fraction. The carotenoids from digesta and micelles were extracted twice using a solvent mixture of hexane–acetone–ethanol (50:25:25, by vol.). The carotenoid content was quantified by HPLC³. The bioaccessibility of each carotenoid was determined by calculating the transfer from the digestate to the micelles (data not shown).

Table 1. Carotenoid content ($\mu\text{g}/100\text{ g}$)

	Lutein		Lycopene				α -Carotene					
	Digestate		Micelles		Digestate		Micelles		Digestate		Micelles	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
No fibre	2252.0	89.3	1407.6	68.4	1471.2	405.7	455.2	80.3	2826.2	297.9	1066.5	139.11
Oat bran	2941.9	976.5	727.9	329.2	1379.7	502.2	253.3	120.4	3699.7	1685.3	567.0	307.1
Wheat bran	2282.3	380.1	712.2	94.5	1648.3	226.9	326.0	51.5	2800.2	534.5	544.3	96.6
Pectin	2941.4	867.9	1148.4	72.8	1250.9	408.2	170.1	42.1	2090.5	739.0	629.0	85.0

Values are means for three independent experiments. Results were analysed by ANOVA followed by Tukey's multiple rank test.

Table 2. Carotenoid bioaccessibility (%)

	Lutein		Lycopene		β -Carotene	
	Mean	SE	Mean	SE	Mean	SE
No fibre	62.5		30.9		37.7	
Oat bran	24.7		18.4		15.3	
Wheat bran	31.2		19.8		19.4	
Pectin	39.0		13.6		30.1	

Values are means for three independent experiments.

In the absence of fibre the transfer of lutein from the digestate to the micelles was higher compared with that of lycopene and β -carotene. The addition of oat bran, wheat bran or pectin to the combination of vegetables did not decrease the carotenoid content of the digestate. However, these fibres did result in a downward trend in the amount of carotenoids detected in the micelles. Oat bran and wheat bran appeared to have a greater effect on the bioaccessibility of lutein and β -carotene than pectin since there was a ≥ 2 -fold decrease observed in the micelles. The micellar content of lycopene was lowest after the addition of pectin, suggesting that the type of fibre may affect carotenoid transfer to micelles differently. The results from the study, while not statistically significant, indicate that fibre tends to have a negative impact on the bioaccessibility of carotenoids.

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Analysis of the effect of different cooking processes on the percentage bioaccessibility of carotenoids from tomato and red pepper (*Capiscum annuum* L). By L. RYAN, O.F. O'CONNELL and N.M. O'BRIEN, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Numerous studies have documented the carotenoid content of various vegetables in their natural raw state. However, many foods are subjected to a simple cooking procedure in the home before consumption. There are limited data on the availability of carotenoids for absorption by the human body and, in particular, the effect of domestic processing on carotenoid availability. Transfer of carotenoids from digested foodstuffs into bile-salt micelles (% bioaccessibility) is essential for carotenoid absorption. The objective of the present study was to analyse the effect of different cooking processes commonly used in the home (microwaving, grilling, boiling, steaming) on the % bioaccessibility of carotenoids present in tomatoes and red peppers. Raw and cooked tomatoes and red peppers were homogenised and subjected to an *in vitro* digestion procedure as previously described¹. Micellar fractions were isolated from the digesta via ultracentrifugation. Samples were extracted twice under amber light with 1 ml hexane–ethanol–acetone (50:25:25, by vol.). The carotenoid content of the samples was quantified by HPLC². The % bioaccessibility was calculated by measuring the transfer of carotenoids from the *in vitro* digestate into the micellar fraction.

	% Bioaccessibility									
	Raw		Microwaved		Grilled		Boiled		Steamed	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Tomato										
Lutein	82.7	0.9	37.9*	1.7	143.3	62.5	79.9	16.7	56.5	3.7
Lycopene	4.7	0.8	4.8	0.8	5.2	3.7	3.8	2.0	1.5	1.2
α -Carotene	5.7	0.9	39.3*	2.2	40.5*	12.6	48.9*	5.8	28.3*	4.1
Red pepper										
Lutein	81.4	4.1	0.0*	0.0	0.0*	0.0	0.0*	0.0	0.0*	0.0
Lycopene	18.5	0.3	12.5*	1.5	12.6*	0.9	15.5	0.2	8.1*	1.4
α -Carotene	14.2	1.4	38.7	5.3	47.3	9.3	67.7*	15.4	58.1*	5.2

Values are means for three independent experiments. Mean values were significantly different from those for the raw sample:

* $P < 0.01$.

The carotenoids analysed included lutein, lycopene and β -carotene. The % bioaccessibility of lutein was similar from raw tomatoes and red peppers. However, all cooking processes had a negative impact on the % bioaccessibility of lutein from red peppers, while only microwaving significantly decreased the bioaccessibility of this carotenoid from tomatoes. The transfer of lycopene from the digestate to the micelles of tomatoes was quite low and remained unchanged after cooking. All the cooking methods employed significantly ($P < 0.01$) increased the % bioaccessibility of β -carotene from tomatoes and both boiling and steaming significantly ($P < 0.01$) increased the % bioaccessibility of β -carotene from red peppers. The present study demonstrates that the behaviour of carotenoids differs in different vegetables even when the same cooking process is applied. From the results it can be seen that the effect of cooking on the % bioaccessibility of individual carotenoids is dependent on the type of vegetables and the method used.

This research was funded by Science Foundation Ireland.

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Plasma uptake of methyl donors from wheat fractions by human subjects. By R.K. PRICE¹, E.M. KEAVENEY¹, L.L. HAMILL¹, J.M. WALLACE¹, H. McNULTY¹, M. WARD¹, J.J. STRAIN¹, P.M. UELAND², J.M. SCOTT³, A.M. MOLLOY³ and R.W. WELCH¹, ¹Northern Ireland Centre for Food and Health (NICHE), University of Ulster, School of Biomedical Science, Coleraine BT52 1SA, UK, ²Bevital, University of Bergen, Bergen, Norway and ³Department of Biochemistry, Trinity College Dublin, Republic of Ireland

Dietary methyl groups derive from foods that contain methionine, and other carriers of one-carbon groups, including choline and the choline metabolite betaine. Methyl groups play an important role both in decreasing homocysteine, a risk factor for CHD, and in the methylation of DNA, which can prevent the expression of some cancer genes. It is becoming clear that wheat is a good source of methyl donors, in particular choline¹, betaine¹ and folate², which are concentrated in the bran and aleurone fractions of the grain. Although a study has shown significant increases in plasma folate after the consumption of a wheat aleurone cereal², little other work has been carried out to establish if other methyl donors present in wheat are available to the body. The aim of the present study was therefore to evaluate the uptake of the methyl donors from bran and aleurone wheat fractions.

The study was a cross-over design, randomized within subjects, in which fourteen healthy subjects (seven male; seven female; age 27.8 (sd 6.5) years; BMI 22.7 (sd 2.6) kg/m²) participated on three occasions at least 1 week apart. Following an overnight fast the subjects consumed 50 g wheat bran, wheat aleurone fraction or a control product, balanced for energy, macronutrients and fibre. Blood samples were collected at baseline and at 30, 60, 120 and 180 min after the meal. Plasma samples were analysed for folate (microbiological assay), choline, betaine and methionine (liquid chromatography–MS/MS). Results were analysed using repeated measures ANOVA.

Compared with the control meal there were non-significant increases in plasma folate and choline after the consumption of both bran and aleurone fractions (results not shown). Increases in plasma betaine were significantly greater ($P>0.01$) than the control after consumption of both fractions (Figure), reaching a maximum 2 h after the meal. The rise from baseline was significantly greater ($P<0.05$) after consumption of the aleurone fraction (50 µmol/l) than the bran fraction (31 µmol/l). No changes were observed in plasma methionine (results not shown).

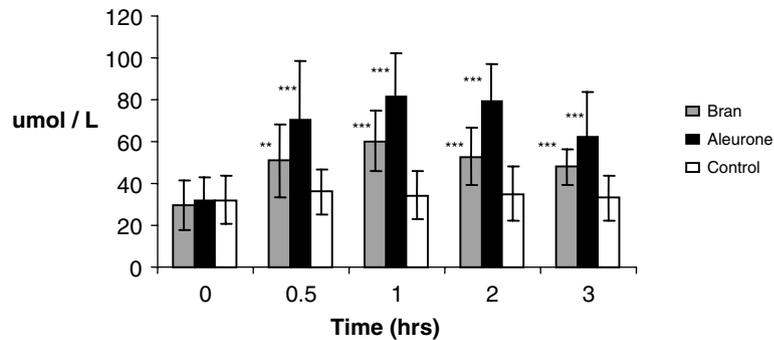


Fig. Plasma betaine levels after the consumption of wheat fractions. Values are means and standard deviations represented by vertical bars for fourteen subjects. Significantly different from control: ** $P<0.01$; *** $P<0.001$.

The study shows that wheat bran and aleurone fractions can provide methyl donors to the body, particularly through their high betaine content, and that this may be one of mechanisms whereby wholegrain wheat cereals protect against CVD and certain cancers.

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