Plasma homocysteine response to folic acid intervention in ischaemic heart disease patients: implications for food fortification policy. By P. TIGHE[1], M. WARD[1], H. McNULTY[1], O. FINNEGAN[2], J.J. STRAIN[1], A. DUNNE[3], A.M. MOLLOY[4] and J.M. SCOTT[4], [1]*Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine, UK, BT52 1SA,* [2]*Coronary Care Unit, Causeway Hospital, Coleraine, UK, BT52 1HS,* [3]*Department of Statistics, University College Dublin, Belfield, Dublin 4, Republic of Ireland and* [4]*Department of Biochemistry, Trinity College, Dublin, Republic of Ireland*

Food fortification with folic acid is primarily aimed at reducing neural tube defects, but is also expected to have some benefit in terms of CVD via a homocysteine (Hcy)-lowering effect. One recent meta-analysis has estimated that a 3 µmol/l decrease in total Hcy (tHcy) concentrations could reduce the risk of IHD by 16%, deep-vein thrombosis by 25% and stroke by 24% (Wald *et al.* 2002). Controversy currently exists, however, regarding the dose of folic acid required to achieve optimal Hcy lowering in IHD patients, with some suggesting that the dose necessary may be as high as 0.8 mg folic acid/d, which is unlikely to be achievable through food fortification (Wald *et al.* 2001). The aim of the present study, therefore, was to determine the minimum effective dose of folic acid required to achieve a significant reduction in tHcy concentration in IHD patients. In an attempt to mimic food fortification, which by its nature involves chronic exposure, the duration of the intervention period was 26 weeks.

IHD patients (*n* 101) were recruited from the coronary care unit, Causeway Hospital on the basis of having previously had a myocardial infarction (at least 3 months before commencing intervention) or having angina diagnosed by electrocardiogram changes. The patients were then stratified into one of four groups on the basis of their initial screening tHcy, to receive either placebo, 0.2, 0.4 or 0.8 mg folic acid daily for 26 weeks. Fasting blood samples (20 ml) were collected at baseline and post-intervention and analysed for tHcy (ImX) and serum folate (microbiological assay). The status of other B vitamins relevant to Hcy metabolism were assessed at baseline and all patients were found to be within the normal range (results not shown).

	Placebo (*n* 22)		0.2 mg (*n* 22)		0.4 mg (*n* 26)		0.8 mg (*n* 31)	
Intervention group …	Median	IQR	Median	IQR	Median	IQR	Median	IQR
tHcy (µmol/l)								
Pre-intervention	12.4	10.1, 17.0	12.1	9.4, 18.4	13.2	10.2, 16.2	13.1	10.7, 16.1
Post-intervention	11.6	10.4, 16.6	9.9	8.1, 15.1	10.7	9.0, 13.2	10.6	8.3, 12.3
Absolute change	−0.4[a]	−1.0, 0.6	−2.7[b]	−4.0, −1.9	−2.3[b]	−3.7, −0.4	−2.7[b]	−4.5, −1.3
Percentage change	−2.7[a]	−7.3, 5.6	−22.0[b]	−27.7, −8.5	−15.8[b]	−24.1, −3.6	−19.5[b]	−31.2, −10.8
Serum folate (µg/l)								
Pre-intervention	7.6	4.7, 11.1	6.4	4.4, 9.4	7.8	5.1, 9.5	7.3	5.2, 10.7
Post-intervention	6.7	4.2, 11.5	13.3	9.0, 16.3	25.0	16.1, 31.0	32.3	26.3, 38.6
Absolute change	−1.1[a]	−1.7, 1.4	4.8[b]	1.8, 10.2	13.3[c]	9.9, 21.4	25.8[d]	19.0, 31.4
Percentage change	−8.8[a]	−26.8, 37.8	87.6[b]	45.1, 187.7	209.8[c]	92.7, 348.7	346.8[d]	167.1, 508.5

IQR, interquartile range.
[a,b,c,d] Median values with unlike superscript letters are significantly different (ANOVA) ($P<0.05$; LSD).

Contrary to previous studies, the results of the present intervention indicate that a dose as low as 0.2 mg folic acid/d will bring about a significant reduction in tHcy and higher doses of folic acid may be neither appropriate nor necessary. The demonstration that higher doses are unnecessary is particularly relevant given that fortification is untargeted; therefore, ensuring that the required dose is reached in a population inevitably means that some people will be exposed to very high levels. Previous studies may have underestimated the effect of lower doses, either because of too short an intervention period or poor compliance with the intervention regimen.

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The use of stochastic modelling to estimate the potential benefit of fortifying white bread with folic acid for the prevention of neural tube defects. By T. JOYCE, E.M. HANNON, M. KIELY and A. FLYNN, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Low compliance by women of reproductive years with advice to use folic acid supplements for the prevention of neural tube defects (NTD) has led to the implementation of mandatory fortification of staple foods with folic acid in some countries. However, this may not be adopted in other countries. An alternative could be to enhance folic acid uptake from foods voluntarily fortified with folic acid. In Ireland, one possible food vehicle for such fortification could be wrapped white bread. In the present study, stochastic modelling (@ Risk, version 4.5; Palisade, Newfield, NY, USA) using dietary intake data from the North/South Ireland Food Consumption Survey (NSIFCS; Irish Universities Nutrition Alliance, 2001) was carried out to assess the impact of folic acid fortification of white bread on folate intakes and on the reduction in risk of NTD (Daly *et al.* 1997) in women aged 18–50 years. The impact of four levels of folic acid fortification (100, 200, 300 and 400 µg/100 g) at four levels of consumption (mean of 10, 20, 50 and 100% of the white bread fortified) was assessed.

	Mean percentage of white bread fortified			
	10	20	50	100
Fortification level of 100 µg/100 g				
Mean increase in folic acid intakes (µg/d)	4	8	19	37
Percentage decrease in NTD risk	1	2	4	8
Fortification level of 200 µg/100 g				
Mean increase in folic acid intakes (µg/d)	9	17	43	86
Percentage decrease in NTD risk	2	4	9	19
Fortification level of 300 µg/100 g				
Mean increase in folic acid intakes (µg/d)	14	27	66	133
Percentage decrease in NTD risk	3	6	14	28
Fortification level of 40 0 µg/100 g				
Mean increase in folic acid intakes (µg/d)	19	36	89	178
Percentage decrease in NTD risk	4	8	19	37

In the NSIFCS, 1.6% of foods (forty-seven) were voluntarily fortified with folic acid. Of these, over 76% were ready-to-eat breakfast cereals (EM Hannon, M Kiely and A Flynn, unpublished results). Food voluntarily fortified with folic acid contributed 31 µg of the mean daily total folate intake (257 µg) of women aged 18–50 years which corresponds to a reduction in NTD risk of almost 7% (Daly *et al.* 1997). Modelling of folic acid fortification of white bread, which was consumed by 90% of women aged 18–50 years (mean daily intake in consumers of 50 g), showed that significant increases in the intakes of folic acid could be achieved by this means. For example, with a mean of 50% of the white bread fortified, up to 19% of a reduction in NTD risk could be achieved at a fortification level of 400 µg/100 g. Modelling studies will also need to be carried out to assess the probability of the occurrence of excess intakes of folic acid in older age groups at risk of vitamin B_{12} deficiency.

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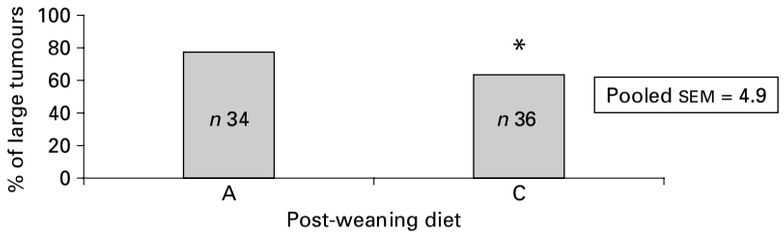
Folate depletion post-weaning suppresses large tumours in the *Apc*$^{+/min}$ mouse intestine. By J.A. McKAY[1], E.A. WILLIAMS[2] and J.C. MATHERS[1], [1]*Human Nutrition Research Centre, School of Clinical Medical Sciences, University of Newcastle upon Tyne, UK, NE1 7RU and* [2]*Human Nutrition Unit, University of Sheffield, Northern General Hospital, Sheffield, UK, S5 7AU*

Colorectal cancer (CRC) is the second most common cause of cancer mortality in the Western world (Houlston & Tomlinson, 2001). Although genetic defects are fundamental to all cancers, there is strong evidence that much variation in CRC incidence is attributable to diet. Several micronutrients appear to modify CRC risk with accumulating evidence of reduced risk in individuals with higher folate intakes (Choi & Mason, 2000).

Given that folate plays a fundamental role in DNA synthesis and methylation and that inadequate folate status may impair genomic stability, we hypothesised that folate insufficiency *in utero* may increase the risk of tumorigenesis, and that continual depletion may exacerbate the effects. To test this hypothesis, we used the *Apc*$^{+/min}$ mouse model which has a point mutation at codon 850 of the *Apc* gene resulting in multiple intestinal tumours (Moser *et al.* 1998). Mutations in the human *APC* gene are causal for familial adenomatous polyposis and are acquired in the majority of sporadic colonic tumours (Su *et al.* 1992). The present study describes the development and testing of a model to distinguish between the effects of altering folate supply *in utero* and during suckling *v.* post-weaning life.

From mating (male *Apc*$^{+/min}$×female C57Bl6/J), mice were randomised to one of two diets based on AIN93A (Reeves *et al.* 1993), namely diet A (control) or diet B (folate depleted) (2 and 0.4 mg folate/kg respectively) *ad libitum*. At weaning, offspring were assigned at random to either diet A or a more folate-depleted diet (diet C; 0.26 mg folate/kg). This resulted in four diet groups from weaning, namely A–A, A–C, B–A, and B–C. At 10 weeks post-weaning, mice were killed for assessment of intestinal tumour number, location and size. Before analysis by ANOVA, data as percentages were arc sine-transformed whilst for presentation in the figure below, conventional means are shown.



In these mice most (96%) of the tumours occurred in the small intestine and the present study showed that feeding the folate-depleted diet from weaning resulted in a significantly reduced percentage of large (>1 mm diameter) tumours (*P=0.039). In human patients, larger polyps are more likely to progress to carcinomas. There was no detectable effect of maternal diet. These results suggest that folate supply has a stronger influence on the progression of tumour development (which occurs after weaning) (Shoemaker *et al.* 1997) than on initiation in this model.

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The relationship between body mass index and B vitamin status. By M.A. KERR[1], M.B.E. LIVINGSTONE[1], I. BRADBURY[1], J.M. SCOTT[2], M. WARD[1], C. J. BATES[3] and H. McNULTY[1], [1]*Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine, UK, BT52 1SA,* [2]*Department of Biochemistry, Trinity College, Dublin, Republic of Ireland and* [3]*MRC Human Nutrition Research, Cambridge, UK*

Obesity at the start of pregnancy is associated with increased risk of many obstetric complications. Less well documented is the positive association between maternal BMI and risk of congenital malformations, including neural tube defects (NTD). It has been shown that compared with non-obese women, obese women are at a greater risk of an NTD affected pregnancy, an effect not explained by lower dietary folate intake or non-use of folic acid supplementation (Shaw *et al.* 1996; Werler *et al.* 1996). It may be possible therefore that impaired status of folate and/or other related B vitamins (B_{12}, B_6 and riboflavin) may be involved in the excess of NTD risk among obese women, or that a factor unrelated to folate may be involved. Our aim was to examine the relationship between BMI, folate and related B vitamins and plasma homocysteine (tHcy, a functional indicator of B vitamin status), using blood and anthropometric data collected as part of the Office for National Statistics Social Survey Division (1997) National Dietary and Nutrition Survey of Young People aged 4–18 years.

	Age- and sex-adjusted percentiles*								
	<9th (*n* 61)		9–91st (*n* 977)		91st–98th (*n* 151)		>98th (*n* 91)		
Variable	Median	IQR	Median	IQR	Median	IQR	Median	IQR	*P*
BMI (wt/ht^2)	15.0^a	13.7, 16.0	17.9^b	16.0, 19.8	22.3^c	19.4, 24.8	27.4^d	22.9, 29.8	0.000
Age (years)	13.2	8.8, 15.9	11.6	8.3, 15.1	11.4	8.7, 15.8	12.1	9.7, 15.9	NS
S.B_{12} (pmol/l)	326^{ab}	251, 448	370^a	269, 508	342^a	261, 452	286^b	211, 366	0.000
RCF (nmol/l)	528	443, 680	569	451, 714	561	451, 688	524	422, 656	NS
SF (nmol/l)	20.4^{ab}	13.7, 26.8	21.1^a	15.2, 27.4	18.1^b	12.9, 24.3	19.0^{ab}	12.9, 25.0	0.039
tHcy (μmol/l)	7.1	5.9, 8.9	6.1	5.0, 7.9	6.7	5.3, 7.9	6.5	5.1, 8.3	NS
PLP (nmol/l)	50.1^a	35.6, 65.4	57.4^b	42.9, 76.3	53.8^{ab}	41.0, 70.9	59.2^{ab}	46.1, 71.3	0.042
EGRac	1.45	1.33, 1.53	1.42	1.32, 1.54	1.46	1.34, 1.58	1.43	1.34, 1.54	NS

IQR, interquartile range.
RCF, erythrocyte folate; SF, serum folate; PLP, plasma pyridoxal phosphate; EGRac, erythrocyte glutathione activation coefficient.
[a,b,c,d]Median values with unlike subscript letters are significantly different (ANOVA) (P<0.05; Tukey).
* Standardised using UK growth reference charts (Freeman *et al.* 1995). <9th Percentile, underweight; 9–91st percentile normal; 91st–98th percentile, overweight; >98th percentile, obese. All data were log-transformed before statistical analysis.

Serum B_{12} (S.B_{12}) was found to be significantly lower among obese compared with normal and overweight subjects (see Table) after adjustment for age, sex and dietary B_{12} intake (multiple regression; P=0.001). We observed a significant, negative correlation between serum B_{12} and BMI (r−0.135; P=0.001). Consistent with its metabolic dependency on vitamin B_{12}, we observed a significant negative correlation between BMI and serum folate (r−0.0728; P=0.001). Like B_{12}, this relationship was not explained by dietary intake. In conclusion, obese individuals may be at an increased risk of lower vitamin B_{12} status, which has in turn been reported to be a risk factor for NTD, independent of folate status (Suarez *et al.* 2003). Further research is warranted to examine the underlying mechanism.

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Diet variety and diet diversity of pregnant and lactating women. By I. BLOSSFELD, C.A. MARTIN and C.M. DELAHUNTY, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

There is evidence that the mother's diet during pregnancy and the first few months after birth when breast-feeding can have long-term effects on the infant's dietary habits and eating behaviour, which can affect the future health and wellbeing (Sullivan & Birch, 1994; Mennella *et al.* 2001). Human infants experience a variety of flavours before weaning because some of the flavours consumed by the mother are transmitted to the amniotic fluid and later to the mother's milk. To date, most studies on diet variety and diet quality of pregnant and lactating women have focused on dietary adequacy and the relationship of the maternal diet and birth outcome.

The purpose of the present study was to characterise the mother's diet in terms of diet variety and diet diversity to form a basis to study the link between the mother's diet and the child's preferences for foods and flavours in later studies. The present study is part of a longitudinal project investigating the development of sensory preferences and eating behaviour in infants up to the age of 18 months. Ninety-three women and their infants participate in this project, but only data from eighty-nine mothers is reported here, as 4 mothers did not complete the second diary. The expectant mothers were recruited from antenatal classes in the three maternity hospitals in Cork. Most of the mothers expected their first child (55.4%). The majority of the women were between 30 and 34 years at the time of recruitment, ranging from 18 to 44 years. Dietary information from the mothers was collected during their last trimester of pregnancy (mean 35.04 (range 24–40) weeks of pregnancy) and again at 2 months postnatally using 7 d dietary records. A diet variety score (DVS) and a diet diversity score (DDS) were calculated for each woman for the two time points. The DVS is defined as the total number of food items consumed over 7 d whereas the DDS counts the number of food groups consumed by each mother. Furthermore, mothers' food neophobia scores and variety-seeking behaviour were calculated.

Diet variety was the same for pregnancy and lactation, (mean score=43.9; ranging from 18 to 61) whereas DDS during pregnancy were significantly higher than during lactation (mean score=0.76 and 0.69 respectively, $p<.05$). Grain and fruits were the most frequent omitted food groups. Furthermore the number of different foods consumed over 7 days (DVS) increases with increasing DDS. Higher DVS were also associated with higher vegetable and fruit group scores indicating an increased consumption. Both DVS and DDS were positively correlated with the subjects' variety seeking behaviour and negatively correlated with their food neophobia scores.

The present study is a first step to relate the mother's diet during pregnancy and lactation to the child's later food preferences and eating behaviour. The results will be used in a later stage of the project to link the mother's diet to the infant's food preferences at 12 and 18 months. Furthermore, we will be able to see if there is a correlation between the mother's diet and flavour variety as well as her food neophobia and variety-seeking behaviour and the infant's food neophobia.

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Vitamin K_1 intake and status in two Irish female population groups. By A. COLLINS, K.D. CASHMAN and M. KIELY, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Attention has been focused recently on the importance of vitamin K for bone health (Weber, 2001) and low vitamin K_1 intakes have been associated with low bone mineral density (Vergnaud *et al.* 1997). Duggan *et al.* (2004) found that habitual intakes of vitamin K_1 in Irish adults (18–64 years) were low relative to data from international studies, primarily due to insufficient intakes of green leafy vegetables. In the present study, vitamin K_1 intakes and status were assessed in ninety-seven apparently healthy free-living Irish women aged 50–75 years from the Cork city region.

Vitamin K_1 intakes were estimated using a detailed dietary history (DH), which measured habitual intakes from a typical 14 d period. The same researcher carried out all the DH and quantified food portion sizes using a photographic food atlas (Ministry of Agriculture, Fisheries and Food, 1997). Recently published food composition data for vitamin K_1 (Bolton-Smith *et al.* 2000) were used to determine vitamin K_1 intakes. An enzyme immunoassay (Takara BIO, Otsu, Shia, Japan) was used to measure undercarboxylated osteocalcin (ucOC) in fasting serum samples.

Dietary intakes of vitamin K_1 were similar between the two age groups. This was also found when vitamin K_1 was expressed as µg per 10 MJ food energy (mean 1.59 µg SD 0.82 µg 50–64 year old women; mean 1.47 µg SD 0.80 µg 65–75 year old women). There were no significant relationships between K_1 intakes and serum ucOC levels. Of the ninety-seven participants in the study, over half of the women aged 65–75 years (52%) and 22% of the 50–64-year-olds had insufficient vitamin K_1 intakes compared with the adequate intake of 90 µg/d recommended by the US Institute of Medicine (2001) for the maintenance of bone health. Serum ucOC values in the current 50–64-year-old women were identical to those reported by Vergnaud *et al.* (1997) in a group of women aged 75 years who had suffered a hip fracture. Surprisingly, the 65–75-year-old women in the present study had slightly lower ucOC values than their younger counterparts, and the values were very similar to those reported by Vergnaud *et al.* (1997) in 75-year-old women who had not experienced a hip fracture.

	Vitamin K_1 intake (µg/d)			ucOC (ng/ml)			Pearson's correlation	
Age groups	Mean	SD	Median	Mean	SD	Median	*r*	*P*
50–64 years (*n* 55)	131.3	54	133.5	6.68	1.2	6.80	0.105	0.509
65–75 years (*n* 42)	117.7	62	100.3	5.54	2	5.90	−0.003	0.813
Total (*n* 97)	123.5	59	108.8	6.0	1.8	6.42	0.042	0.684

The present study is the first to estimate vitamin K_1 intakes and serum levels of ucOC in Irish women. The results show that in a high proportion of these women, intakes appear to be inadequate to maintain bone health, and serum ucOC concentrations are high enough to indicate an increased risk of hip fracture. Research priorities are to carry out similar studies in other age groups, particularly adolescents, and to develop strategies to increase vitamin K_1 intakes in the general population.

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A comparison of vitamin D intake data from a food-frequency questionnaire with a 14 d dietary history and vitamin D status in three Irish female population groups. By A. COLLINS, M.M. O'BRIEN, T.R. HILL, A. FLYNN, K.D. CASHMAN and M. KIELY, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Hill *et al.* (2004) showed that dietary intakes of vitamin D are low in Irish adults (aged 18 to 64 years) and suggested that in the absence of skin biosynthesis of vitamin D in wintertime, a significant proportion of the Irish adult population may benefit from additional intakes. As vitamin D is present in few foods, it is essential to have a reliable tool to measure dietary intakes and food sources, so that potentially effective strategies to increase the supply of vitamin D in the diet can be identified.

In the present study, a sixty-nine-item food-frequency questionnaire (FFQ) was developed using the foods that supplied 95% vitamin D intakes measured in the North/South Ireland Food Consumption Survey (Irish Universities Nutrition Alliance, 2001). Vitamin D intake data from the FFQ were compared with data from a detailed dietary history (DH), which estimated habitual vitamin D intakes during a typical 14 d period.

One hundred and twenty apparently healthy free-living Irish females were recruited from the Cork region. Subjects were from three age groups (11–13 years, 50–64 years, 65–75 years). Each subject completed the FFQ and DH, which were administered by the same researcher, on two separate occasions within 1 month of each other. Food portion sizes were estimated using a photographic food atlas (Ministry of Agriculture, Fisheries and Food, 1997). The vitamin D food composition data were obtained from *McCance and Widdowson's the Composition of Foods* (Holland *et al.* 1991) and recently published vitamin D values (Hill *et al.* 2004). Fasting serum levels of 25-hydroxyvitamin D (25(OH)D) were determined using an enzyme immunoassay (IDS Ltd, Boldon, Tyne and Wear, UK) in the women aged between 50 and 70 years and by HPLC in the girls and the women >70 years.

Estimates of vitamin D intake should always specify nutritional supplement use and the clear differences in the intake values that included (WS) and excluded (NS) supplements are shown in the Table. Intakes were significantly different ($P<0.05$) between the FFQ and DH in the 50–64- and 65–75-year-olds. Significant correlations ($P<0.0001$) were found between estimates of vitamin D from the DH and FFQ in the three age groups separately, 11–13 years (r 0.76), 50–64 years (r 0.48), 65–75 years (r 0.48) and in the entire sample (r 0.81). The FFQ and DH classified 84% of subjects into the same (39%) or an adjacent quartile of the distribution of vitamin D intakes. The FFQ and DH identified similar foods as primary sources of vitamin D. Repeatability was assessed in a subgroup of fifty-six subjects and there were no significant differences in the data from two administrations of the FFQ carried out 6 months apart. There were no significant correlations between vitamin D intake data assessed using either the FFQ or the DH and serum 25(OH)D status measurements.

Age group …	11–13 years (n 22)			50–64 years (n 43)			65–75 years (n 55)		
Vitamin D (μg/d)	Mean	SD	95% CI	Mean	SD	95% CI	Mean	SD	95% CI
FFQ NS	2.47[a]	1.5	1.8, 3.1	4.79[b]*	3	3.9, 5.7	4.38[b]*	2.5	3.7, 5.1
FFQ WS	2.72[a]	1.7	2, 3.5	12.13[b]*	16.1	7.2, 17.1	10.27[b]*	11.9	7, 13.5
DH NS	2.03[a]	0.9	1.6, 2.4	3.28[b]*	2.1	2.6, 3.9	3.47[ab]*	4.6	2.2, 4.7
DH WS	2.6[a]	1.9	1.7, 3.5	12.01[b]*	17.9	6.5, 17.5	9.79[b]*	16	5.6, 14.1

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0.01$).
* Mean values within a column for including (WS) and excluding (NS) nutritional supplements were significantly different ($P<0.0001$).

In conclusion, this newly developed sixty-nine-item FFQ may be a useful tool in identifying potential strategies to increase vitamin D intakes in the Irish population and could replace more onerous and costly methods of assessing intakes of this nutrient in population subgroups.

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Estimation of dietary acidity or alkalinity in British individuals aged 65 years and over from National Diet and Nutrition Survey (NDNS) datasets using estimates of net rate of endogenous non-carbonic acid production (NEAP) and potential renal acid load (PRAL). By R.H.T. GANNON[1], D.J. MILLWARD[1], H.M. MACDONALD[2], L.A. FRASSETTO[3], T. REMER[4] and S.A. NEW[1], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH,* [2]*Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, UK, AB25 2ZD,* [3]*Department of Medicine and General Clinical Research Center, University of California, San Francisco, USA and* [4]*Department of Nutrition and Health, Research Institute of Child Nutrition, Dortmund, Germany*

Imbalances in acid–base homeostasis have implications for a number of diseases states, with increasing interest concerning a relevant impact on skeletal health (New *et al.* 2004). Dietary acidity is particularly influenced by the protein:K ratio, which determines NEAP and contributes to PRAL, each value a predictor of the dietary component of net acid excretion (Frassetto *et al.* 1998; Remer *et al.* 2003). To date there has been little analysis of the actual effects of food groups on acid load predicted from NEAP and PRAL and how this varies in population groups. The aim of the present study was to compare and characterise NEAP and PRAL in relation to food groups likely to influence potential dietary acid load in a representative sample of British individuals. Datasets from the NDNS of the Elderly, (n 1687, of which 412 were institutionalised; aged ≥65), which included a 4 d weighed dietary record and a health and lifestyle questionnaire were examined (Finch *et al.* 1998). PRAL and NEAP values were calculated using Σ ((protein (SO_4)+P)−(Mg+K+Ca)) and protein:K ratio respectively.

				Men (n 836)								Women (n 851)							
Age group (years) …				65–74		75–84		85–94		95–104		65–74		75–84		85–94		95–104	
	Mean	SE	Range	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PRAL	6.6	0.21	−25.9–48.4	7.5	0.5	8.4	0.5	8.2	0.6	8.8	1.0	4.0	0.5	5.8	0.4	5.9	0.4	0.9	1.1
NEAP	47.1	0.3	12.4–106.2	47.8	0.7	49.0	0.7	48.2	0.9	49.4	1.6	44.3	0.7	46.7	0.7	47.0	0.7	37.8	1.7

Units of PRAL (mEq/d) and NEAP (g/mEq per day).

	Quartiles of PRAL								Quartiles of NEAP							
	1		2		3		4		1		2		3		4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total fruits (g)†‡	909	37	574	26	490	24	428	24	921	36	644	29	507	23	334	21
Total vegetables (g)†‡	839	27	624	19	638	20	628	20	820	27	700	20	669	20	543	18
Total meats (g)†‡	632	18	624	18	744	18	948	22	590	18	669	16	789	20	895	22
Total fish (g)†*	184	10	192	9	207	9	258	12	185	10	207	10	236	11	212	10
Protein (g/d)†‡	55.5	0.8	55.9	0.8	61.8	0.7	73.9	0.8	55.9	0.8	60.4	0.8	64.9	0.8	65.7	0.8
K (mg/d)†‡	2621	40	2217	34	2213	30	2343	30	2675	37	2418	31	2319	30	1986	26

† $P<0.001$; ‡ $P<0.001$ for PRAL, * $P<0.01$ for NEAP. ANOVA and Scheffé *post hoc tests* were used for analyses.

PRAL and NEAP values increased significantly with increasing age ($P<0.001$), except in the age group 95–104 years where subject numbers were low (n 32). Regional differences were found for intakes of PRAL, with higher mean intakes in Scotland/North region (8.3 (SE 0.3) mEq/d) compared with Central/South West region (6.1 (SE 0.3) mEq/d) and London/South-East region (4.9 (SE 0.3) mEq/d) Similar results were found for NEAP. With increasing PRAL and NEAP values, fruit and vegetable intakes were lower, and protein, meat and fish intakes were higher. While both protein and K varied with estimates of PRAL and NEAP as expected, the data show that changes in protein intake were more important than changes in K as determinants of PRAL whilst the opposite was true for NEAP. Thus fruit and vegetables impact more on NEAP whilst meat impacts more on PRAL. These novel data provide an insight into the acid-generating potential of the diet in elderly individuals and support other findings that fruit and vegetables reduce the acid load induced by diets rich in acid precursors. Further analyses of additional NDNS datasets and the North/South Ireland Food Consumption Survey are underway.

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Evaluation of a dietary checklist to assess fruit and vegetable consumption. By A.C. HARDCASTLE[2], R. SANDISON[1], S.A. NEW[3], D.M. REID[1,2] and H.M. MACDONALD[1,2], [1]*Osteoporosis Research Unit, University of Aberdeen, Woolmanhill Hospital, Aberdeen, UK, AB25 1LD,* [2]*Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, UK, AB25 2ZD and* [3]*Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford, UK, GU2 7XH*

There are a variety of methodologies available for the assessment of dietary intake. The use of any method should be considered with respect to its validity and reproducibility, especially given that dietary validation studies do not compare a method with the absolute truth, but use a comparison of one technique which has different limitations to that of another (Willett, 1990). The aim of the present study was to evaluate the use of a dietary checklist for assessment of fruit and vegetable consumption which is quick to complete and analyse, in comparison with an estimated portion size food diary which places a greater burden on participants and researchers since they are more time consuming to analyse.

The subjects were women (mean age 59.6±2.1 years) who had been recruited in 1990–3 as part of the Aberdeen Prospective Osteoporosis Screening Study, the majority of whom returned in 1997–2000 for a second visit. A subset (*n* 279) was recruited in 2003 to participate in a 2-year intervention study. Dietary intake was assessed using the EPIC food diary (McKeown *et al.* 2001) and a 3 d fruit and vegetable dietary checklist. The food diary was completed for a period of 4 d (including one weekend day) and was analysed using WinDiets computer program (Robert Gordon University, Scotland). The one-page checklist was analysed using standard food portion sizes. Weighted kappa (K_w) was used as a measure of cross-classification.

Comparisons in fruit and vegetable intakes between the food diary and checklist are shown in the Table. Total fruit included tinned and dried fruits; root vegetables included beetroot and other roots (excluding potatoes); and green and coloured vegetables included salads, tomatoes, onions and peppers.

	Food diary			Checklist			Comparison		
Food group	Median (g/d)	Range (g/d)	% Eating each group	Median (g/d)	Range (g/d)	% Eating each group	Correct classification	Misclassification >one quartile	K_w
Fruit juice	38	0–480	53.4	83	0–1167	58.7	53.0	19.7	0.46
Fresh fruit	185	0–848	95.5	322	0–2670	99.0	39.0	19.0	0.33
Total fruit	251	0–848	97.6	452	0–2670	99.3	38.2	20.4	0.31
Green and coloured vegetables	8	0–309	98.3	144	0–1445	99.0	33.3	21.9	0.25
Root vegetables	8	0–153	54.5	20	0–180	69.0	34.8	30.8	0.20
Total vegetables	130	0–440	98.6	191	17–1502	99.6	34.8	25.4	0.23

One-third of the subjects reported eating the recommended five portions of fruit and vegetables/d in the diaries, and 69.6% reported this in their checklists. Of the participants, 11.8% reported eating ≤two portions of 100 g of fruit per d by the checklist, and 38% reported this in the food diary. More subjects reported eating ≤two portions of vegetables/d (53.4% in the checklist and 74.6% in the diary). Fair agreement was found in the assessment of fruit and vegetable intake between the two techniques (K_w>0.20) (Altman, 1991) with greater misclassifications being found for the vegetable groups compared with the fruit groups, which may be explained in part by the over-estimation of particular items (for example, onions, peppers) in the checklist. Although further evaluation of the dietary checklist is required and its use should be treated with some caution, particularly with respect to portion sizes, it is a useful tool for the rapid assessment of fruit and vegetable intake.

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Factors associated with compliance with the population goals for intakes of fruit and vegetables and dietary fibre in Irish adults. By M. COSGROVE, M.M. O'BRIEN, M.A. GALVIN, A. FLYNN and M. KIELY, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Using data from the North/South Ireland Food Consumption Survey (NSIFCS) (Irish Universities Nutrition Alliance, 2001), Galvin *et al.* (2001) and O'Brien *et al.* (2003) showed that intakes of fruit and vegetables and dietary fibre (DF) are low in Irish adults. The NSIFCS estimated habitual food and beverage intakes using a 7 d food diary in 1379 randomly selected adults, aged 18–64 years. Using the food consumption data constructed by listing each food as consumed including portion weight and nutrients obtained per portion, the present study describes dietary habits that best differentiate compliers and non-compliers with both of the population goals for intakes of fruit and vegetables (≥400 g) and DF (>25 g). Fruit and vegetable intakes are based from discrete portions and composite dishes. Binary logistic regression analysis identified food-related factors that predicted the likelihood of being a member of the compliers group.

Predictors	B	SE	*P*	Increment	OR*	95% CI for OR
Energy (excluding alcohol) (MJ)	1.888	0.457	0.000	0.80	8.12	3.49, 18.41
Vegetables (including carrots) (g/d)	0.091	0.018	0.000	21.65	3.06	2.24, 3.88
Fruit (including apples) (g/d)	0.082	0.020	0.000	19.98	2.72	1.90, 3.53
Citrus fruit (g/d)	0.088	0.017	0.000	14.61	2.37	1.86, 2.89
Salad vegetables (including tomatoes) (g/d)	0.120	0.037	0.001	10.38	2.37	1.54, 3.21
Fruit juice (g/d)	0.060	0.011	0.000	21.28	2.34	1.85, 2.84
Bananas (g/d)	0.076	0.015	0.000	14.80	2.21	1.74, 2.68
Wholemeal bread (g/d)	0.072	0.015	0.000	14.14	2.09	1.65, 2.53
Peas and beans (g/d)	0.140	0.027	0.000	6.60	2.07	1.67, 2.48
Green vegetables (g/d)	0.115	0.027	0.000	5.42	1.75	1.40, 2.10
White bread (g/d)	0.031	0.010	0.002	22.18	1.72	1.26, 2.17
Confectionery (including chocolate) (g/d)	−0.043	0.020	0.036	4.34	0.77	0.56, 0.98
Carbonated beverages (g/d)	−0.008	0.003	0.010	29.23	0.77	0.59, 0.95
Butter and spreading fats (g/d)	−0.064	0.027	0.016	4.86	0.63	0.32, 0.93

* The odds ratio (OR) was based on a 10% increment in the median daily food energy intake and a 33% increment in the median daily food intake (g) in consumers only.

Of the Irish adults, 45% were compliers (of which 53% were men and 47% women) while 23% (of which 28% were men and 72% women) were non-compliers with both of the population goals for fruit and vegetables and DF intakes. Food energy was a significant (P<0.001) predictor of compliance with a 8-fold increase in the odds of being a complier for every 0.80 MJ increase in energy. Frequency of food intake was a more significant factor than portion size in predicting compliance. Compliers consumed wholemeal bread, ready-to-eat breakfast cereals, biscuits, cheeses, yoghurts, butter and spreading fats, fruit, potatoes and vegetables significantly more frequently (P<0.001) than non-compliers. However, compliers also had significantly larger (P<0.001) portion sizes of fruit, potatoes and vegetables. A 33% increase in the median daily intake of fruit and vegetables was associated with a 1.75- to 3.06-fold increase in the odds of being a complier. An increase of 33% in the median daily intakes of confectionery, carbonated beverages and butter was associated with a reduced likelihood in the odds of being a complier. Targeted strategies to increase the percentage of consumers and frequency of consumption of fruit and vegetables will improve compliance with both of the population goals for fruit and vegetables and DF intakes in Irish adults.

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Size does not matter: validation of a micro-method for adipose tissue biopsy for measurement of fatty acid composition. By F.F.M. CHONG, A.S.T. BICKERTON, B.A. FIELDING and K.N. FRAYN, *Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK, OX3 7LJ*

The fatty acid composition of subcutaneous adipose tissue (AT) obtained using the methods of Hirsch *et al.* (1960) and Beynen & Katan (1985) for AT sampling have been shown to reflect the long-term dietary intake of fatty acids in interventional and cross-sectional studies (Garland *et al.* 1998).

The minimum requirement of the biopsy needle is 1.5 mm diameter (19-gauge (19G) for Hirsch's method and 17G for Beynen's method) so that small, but visible, amounts of AT can be collected for fatty acid analysis (Beynen & Katan, 1985). We hypothesised that fatty acid analysis can be carried out with even smaller amounts of AT and we sought to develop a less invasive method of biopsy.

We performed two AT biopsies on ten normal, healthy subjects under local anaesthesia. The first biopsy was taken using an 18G needle and syringe from one side of the abdomen and used directly for lipid extraction. The other biopsy was taken using a 21G butterfly needle that was part of a Vacutainer blood collection system, analogous to Beynen's method. AT was initially not clearly visible in the tubing of the 21G needle. When flushed with saline (3 ml) into a glass tube and centrifuged (1000 rpm, 1 min), glistening fat globules were seen on the surface. These were aspirated and used for lipid extraction. Two further layers (each approximately 0.5 ml) were then aspirated and used for lipid extraction. Fatty acid composition of the lipid extracts was analysed by GC. No bruises were seen using the 21G needle, compared with visible blue-black marks by the 18G needle after 24 h.

Fatty acid (g/100 g) ...		14:0	16:0	16:1*n*-7	18:0	18:1*n*-9	18:2*n*-6	20:1*n*-9	20:4*n*-6	20:5*n*-3	22:6*n*-3
21G (*n* 10)	Mean	2.66	21.71	5.47	3.44	49.84	13.05	0.70	0.29	0.23	0.10
	SEM	0.19	0.39	0.32	0.39	0.61	0.54	0.10	0.06	0.14	0.04
18G (*n* 10)	Mean	2.78	22.02	5.22	3.61	49.99	12.88	0.68	0.31	0.11	0.11
	SEM	0.24	0.53	0.27	0.45	0.48	0.55	0.12	0.05	0.05	0.04

The fatty acid composition of AT taken using the 18G needle and the 21G needle are very similar (P=0.6 using repeated measures analysis), proving that only very tiny amounts of AT are needed to analyse fatty acid composition. The composition of the next layer aspirated was also similar (results not shown), but the third layer showed a fatty acid composition resembling that of phospholipids (for example, 20:2, 1.77 g/100 g; 20:4, 4 g/100 g). We conclude that washing with saline and centrifugation effectively separated AT triacylglycerol from blood contamination.

The implication of the present study is that it is unnecessary for subjects to undergo uncomfortable and invasive methods of sampling to obtain AT for fatty acid analysis. This method of sampling is easy and similar to a routine venepuncture, allowing application on a large scale.

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Deleterious effects of skipping breakfast on lipid and carbohydrate metabolism in lean healthy women. By H.R. FARSHCHI[1], M.A. TAYLOR[1] and I.A. MACDONALD[1], *School of Biomedical Sciences, Queen's Medical Centre, Nottingham, UK, NG7 2UH*

Breakfast consumption appears to have declined in the last few decades (Haines *et al.* 1996) and eating breakfast, especially cereal, is associated with a lower risk of obesity (Cho *et al.* 2003). Serum cholesterol concentration is reported to be lower in adults eating breakfast (EB) and higher among those not (Stanton & Keast, 1989). No study, to our knowledge, has investigated the effect of skipping breakfast (SB) on various aspect of energy metabolism. Thus, the present study evaluated the effect of EB or SB on adult energy, carbohydrate and lipid metabolism.

Ten healthy women (BMI 23.2 (SD 1.4) kg/m^2) were recruited after giving informed consent. Each subject participated in a randomised cross-over trial which encompassed two 14 d intervention periods; EB in one of them and SB in the other with a 2-week washout period between. In EB, subjects were asked to consume a pack of wholegrain cereal (Kellogg's, UK, 45 g) with 200 ml semi-skimmed milk between 07.00 and 08.00 hours and eat a chocolate bar (Nestle, 48 g) at 10.30–11.00 hours. Then, they consumed four further meals of similar content to usual in the rest of the day at predetermined times every day for 2 weeks. In SB, subjects consumed the chocolate at 10.30–11.00 hours, and then had the cereal and semi-skimmed milk at 12.00–12.30 hours. Then, they consumed four further meals of similar content to usual as for EB. Subjects consumed their normal diet for a 2-week washout period between the two intervention periods. Subjects recorded their food intake on 3 d during each intervention, and came to the laboratory after an overnight fast at the start and end of each intervention period and their weight and anthropometric variables were measured. Blood samples were taken for glucose, lipids and insulin before and for 3 h after a test meal (milk shake containing 30 kJ/kg, 50% carbohydrate). RMR was measured by indirect calorimetry before and after the test meal. Repeated-measures ANOVA, and paired t tests were used for the statistical comparisons.

SB was associated with higher fasting total cholesterol (3.4 (SD 0.4) compared with 3.1 (SD 0.4) mmol/l after EB; P<0.02) and LDL-cholesterol (1.8 (SD 0.3) compared with 1.6 (SD 0.3) mmol/l after EB; P<0.04). Fasting glucose and insulin were not affected by breakfast but mean area under the curve of insulin responses to the test meal was higher after SB compared with after EB (82.6 (SD 44.0) and 73.6 (SD 42.0) mIU/l over 3 h respectively; P<0.01). Mean recorded energy intake was lower during EB (0.38 MJ/d lower than the mean energy intake of SB; P<0.002), while fasting RMR and postprandial thermogenesis were not different between the two periods.

In conclusion, skipping breakfast appears to produce higher total and LDL-cholesterol and lower insulin sensitivity, which are known cardiovascular risk factors. SB may also produce positive energy balance if the observed higher energy intake continued in the long term.

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Dietary advice for prevention of type 2 diabetes mellitus in adults. By H.J. MOORE[1], C.D. SUMMERBELL[1], L. HOOPER[2], V.J. ASHTON[1], P. JOHNSTONE[3] and P. KOPELMAN[4], [1]*University of Teesside, Middlesbrough, UK, TS1 3BA,* [2]*University of Manchester, Manchester, UK, M15 6FH,* [3]*Department of Health, Leeds, UK, LS1 4US and* [4]*University of London, London, UK, E1 2AD*

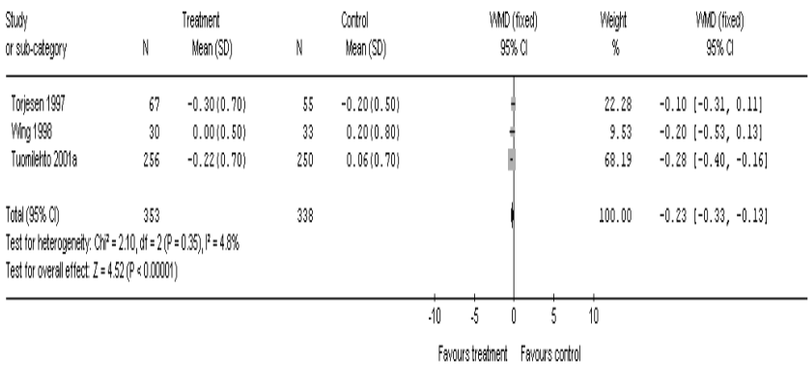
The changing of dietary habits may lessen the chance of developing type 2 diabetes. Given the prevalence and the potentially serious costs of the disease, it is important to establish which type of dietary advice, either alone or in combination with other interventions (the addition of exercise, behavioural approaches and alternative treatments), is most effective in preventing development of the disease.

The present systematic review assesses the effect of type and frequency of different types of dietary advice to all adults on morbidity, weight, quality of life, total mortality and measures of diabetic control.

We searched The Cochrane Library, MEDLINE, EMBASE, CINAHL, AMED, bibliographies and contacted relevant experts. All randomised controlled trials, of 12 months or longer, where dietary advice was the main intervention in adults were included.

Fourteen articles reporting nine trials were included. Various dietary approaches were considered. Two trials compared the usual dietary habits with a low-fat diet. Six studies compared dietary advice with dietary advice plus exercise and one other study looked at dietary advice *v.* dietary advice plus a behavioural approach. The studies all measured weight and glycaemic control although not all studies reported these outcomes. Four studies reported the number of participants who developed diabetes and only two studies reported mortality. Other outcomes measured in these studies included blood pressure, serum cholesterol (including LDL- and HDL-cholesterol), serum triacylglycerols, maximal exercise capacity and compliance.

Using exercise as an adjunct to dietary advice appears to improve fasting plasma glucose at 12 months in normoglycaemic individuals. Dietary advice plus exercise, as compared with dietary advice alone, was associated with a statistically significant mean (pooled weighted mean difference) decrease in fasting plasma glucose of 0.2 (95% CI 0.1, 0.3) mmol/l at the 12-month follow-up.



Three studies (Torjesen *et al.* 1997; Wing *et al.* 1998; Tuomilehto *et al.* 2001) contributed data to this meta-analysis. The adoption of regular exercise is a good way to promote better glycaemic control in adults.

Torjesen PA, Birkeland KI, Anderssen SA, Hjermann I, Holme I & Urdal P (1997) *Diabetes Care* **20**, 26–31.
Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Aunola S, Cepaitis Z, Moltchanov V, Hakumaki M, Mannelin M, Martikkala V, Sundvall J & Uusitupa M (2001) *New England Journal of Medicine* **344**, 1343–1350.
Wing RR, Venditti E, Jakicic JM, Polley BA & Lang W (1998) *Diabetes Care* **21**, 350–359.

Dietary advice for treatment of type 2 diabetes mellitus in adults. By H.J. MOORE[1], C.D. SUMMERBELL[1], L. HOOPER[2], K. CRUICKSHANK[2], A. VYAS[2], P. JOHNSTONE[3], V.J. ASHTON[1] and P. KOPELMAN[1], [1]*University of Teesside, Middlesbrough, UK, TS1 3BA,* [2]*University of Manchester, Manchester, UK, M15 6FH,* [3]*Department of Health, Leeds, UK, LS1 4US and* [4]*University of London, London, UK, E1 2AD*

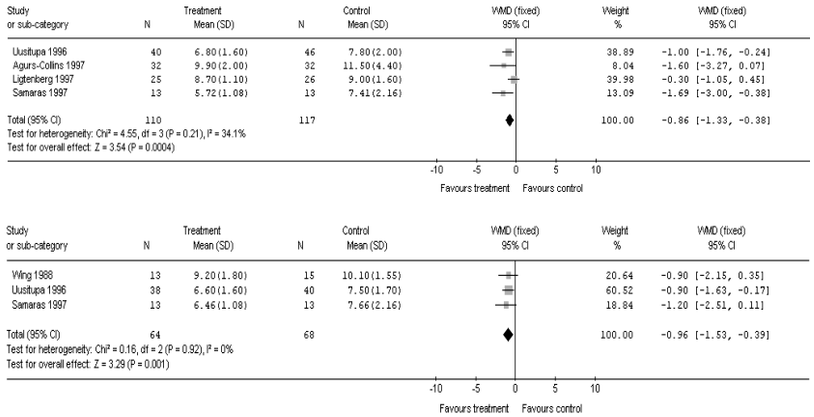
While initial dietary management immediately after formal diagnosis is an 'accepted' cornerstone of treatment of type 2 diabetes mellitus, a formal and systematic overview of its efficacy and method of delivery is not currently available.

The present Cochrane systematic review assesses the effect of type and frequency of different types of dietary advice to all adults with type 2 diabetes on weight, measures of diabetic control, morbidity, total mortality and quality of life.

We searched The Cochrane Library, MEDLINE, EMBASE, CINAHL, AMED, bibliographies and contacted relevant experts. All randomised controlled trials, of 6 months or longer, where dietary advice was the main intervention in adults with type 2 diabetes mellitus were included.

Thirty-six articles reporting eighteen trials were included. Various dietary approaches were considered. Nine studies compared dietary advice with another diet. Six studies compared dietary advice with dietary advice plus exercise and three other studies looked at dietary advice *v.* dietary advice plus behavioural approaches. The studies all measured weight and glycaemic control although not all studies reported these outcomes. Other outcomes measured in these studies included mortality, blood pressure, serum cholesterol (including LDL- and HDL-cholesterol), serum triacylglycerols, maximal exercise capacity and compliance.

Using exercise as an adjunct to dietary advice appears to improve glycated Hb at 6 and 12 months in individuals with type 2 diabetes. There were small, yet significant changes in glycated Hb in the four (Uusitupa *et al.* 1996; Agurs-Collins *et al.* 1997; Ligtenberg *et al.* 1997; Samaras *et al.* 1997) and three (Wing *et al.* 1988; Uusitupa *et al.* 1996; Samaras *et al.* 1997) studies that contributed data to these analyses.



Dietary advice plus exercise was associated with a statistically significant mean (pooled weighted mean difference) decrease in glycated Hb of 0.9 (95% CI 0.4, 1.3)% at 6 months and of 1.0 (95% CI 0.4, 1.5)% at 12 months. The adoption of regular exercise is a good way to promote better glycaemic control in type 2 diabetic patients.

Agurs-Collins TD, Kumanyika SK, Ten Have TR & Adams-Campbell LL (1997) *Diabetes Care* **20**, 1503–1511.
Ligtenberg PC, Hoekstra JB, Bol E, Zonderland ML & Erkelens DW (1997) *Clinical Science* **93**, 127–135.
Samaras K, Ashwell S, Mackintosh AM, Fleury AC, Campbell LV & Chisholm DJ (1997) *Diabetes Research and Clinical Practice* **37**, 121–128.
Uusitupa M, Laitinen J, Siitonen O, Vanninen E & Pyorala K (1996) *Annals of Medicine* **28**, 445–449.
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Investigation into the effects of initiating insulin therapy in type 2 diabetes. By R.M. BARRATT[1], H. TRUBY[1], D.J. MILLWARD[1] and G.S. FROST[2], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, UK, GU2 7XH and* [2]*Nutrition and Dietetic Department, Hammersmith Hospitals NHS Trust, Du Cane Road, London, UK, W12 0HS*

Since the publication of the UK Prospective Diabetes Study (United Kingdom Prospective Diabetes Study (UKPDS) Group, 1995) insulin therapy is being used earlier and more extensively in the treatment of type 2 diabetes mellitus (type 2 DM). Insulin therapy undoubtedly improves glycaemic control and reduces the risk of microvascular complications. However, it has not been shown to significantly impact or reduce macrovascular complications (UKPDS Group 1998). Iatrogenic weight gain after initiating insulin is a common problem, which may impact further on the risk of macrovascular complications (Reaven, 2002). The present study aimed to investigate changes in body weight following the introduction of insulin therapy in adults with type 2 DM and to determine the influence that this change in treatment has on lipid metabolism, body composition and CHD risk. An intensive dietetic weight-management intervention was also tested to determine whether weight gain can be minimised and outcome optimised in this notoriously difficult-to-treat group.

Subjects with type 2 DM have been recruited during the transition period to insulin therapy (±4 weeks of initiating insulin). As subjects are recruited (target number *n* 74) they are randomly assigned to either the control or intervention group. The control group will continue with standard care, while the intervention group will follow a dietitian-led intensive weight-management programme. A sub-cohort from each group will have magnetic resonance imaging scans completed to measure body fat deposition and muscle and liver triacylglycerols, and postprandial studies will be carried out to demonstrate changes in lipid handling. All measurements are completed at baseline and endpoint (6 month); then inter- and intra-group analysis will be undertaken.

Currently half of the total number of patients have been recruited (57% male, 43% female) and 12 patients (16%) have completed the study; these numbers are continually increasing. Initial analysis indicates that there is a wide variability in weight change and other biochemical markers following the initiation of insulin. Of those that have completed, the initial mean BMI was 34.7 (SD4.3, range 29.7–44.6) kg/m^2, meaning that all patients, except one, were clinically obese. The mean HbA1c at baseline was 10.01 (SD 1.77, range 7.8–12.5) % and at endpoint, after 6 months, was 10.10 (SD 1.74, range 8.5–12.4) %, which actually indicates a worsening in glycaemic control at this stage. The weight change of those in the control group have ranged from −1.9 to +8.3 kg (mean+3.52 kg). For those in the intervention group the change in weight has ranged from −1.6 TO +2.5 kg (mean+0.43 kg). Therefore, initially it appears that an intensive dietetic intervention can, on average, prevent some weight gain following the initiation of insulin therapy. It is anticipated that as additional patients complete the study the results will indicate more comprehensively the risks and benefits of insulin therapy in type 2 DM and reveal whether intensive dietetic input at this time is of significant advantage.

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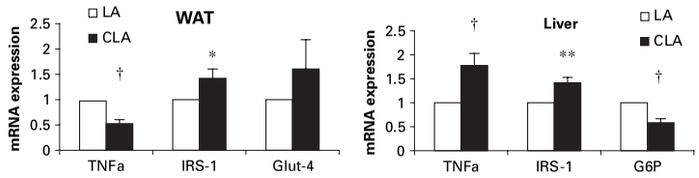
***Cis*-9, *trans*-11 conjugated linoleic acid (CLA) improves metabolic and molecular markers of insulin sensitivity in adipose tissue and liver.** By F. MOLONEY, E. NOONE, M.J. GIBNEY and H.M. ROCHE, *Nutrigenomics Research Group, Department of Clinical Medicine, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Republic of Ireland*

Conjugated dieonic derivatives of linoleic acid are a mixture of geometric and positional isomers of linoleic acid. The effects of CLA on glucose and insulin metabolism have yielded controversial results. Feeding a blend of CLA isomers (*trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA; 50:50) improved insulin sensitivity and glucose tolerance in Zucker diabetic fa/fa rats (Roche *et al.* 2002). In contrast, a CLA-enriched diet induced insulin resistance and hyperlipidaemia in C57BL/6J mice (Roche *et al.* 2002). It has become evident that the health effects of CLA are isomer specific. Both animal and human studies have indicated that the pro-diabetic effects of CLA may be attributable to the *trans*-10, *cis*-12 CLA isomer (Risérus *et al.* 2002; Roche *et al.* 2002). However, there is little information regarding the effects of the *cis*-9, *trans*-11 isomer (*cis*-9, *trans*-11 CLA) on molecular markers of insulin sensitivity in adipose tissue and liver.

Eleven 12-week-old male ob/ob mice were randomly allocated to receive either a high-fat diet supplemented with *cis*-9, *trans*-11 CLA or linoleic acid (control) for 5 weeks. Glucose and insulin concentrations were measured using an enzymatic colorimetric assay and ELISA kit, respectively. RNA was extracted from white adipose tissue (WAT) and liver. Gene expression was investigated using real-time PCR. Statistical analysis was completed using a pooled *t* test.

Feeding a diet enriched with the *cis*-9, *trans*-11 CLA isomer significantly reduced fasting glucose (12.06 (SEM 0.61), 10.76 (SEM 0.71)mmol/l; control, CLA respectively; $P<0.05$) and insulin concentrations (1.16 (SEM 0.32), 0.65 (SEM 0.21) ng/ml; control, CLA respectively,$P<0.05$). In WAT, IRS-1 mRNA expression was significantly increased ($P<0.05$). Glut-4 mRNA expression was also increased, but this did not reach statistical significance. CLA significantly reduced TNFα mRNA expression ($P<0.001$). In liver, both IRS-1 mRNA expression and TNFα expression were significantly increased ($P<0.01$, $P<0.001$ respectively). Glucose 6 phosphatase (G6Pase) mRNA expression was significantly decreased ($P<0.001$).

The Figure shows mRNA expression (ng target gene/ng GAPDH) of molecular markers of insulin sensitivity in WAT and liver following linoleic acid (LA) or CLA diets. Values represent group means and SEM. Mean value was significantly different from LA * $P<0.05$, ** $P<0.01$, † $P<0.001$.



Insulin resistance is a classic feature of type 2 diabetes and CVD. Thus, it is important to identify potential therapeutic nutrients, which may have the ability to reduce metabolic impact. The present results indicate that the insulin-sensitising effects of *cis*-9, *trans*-11 CLA may be mediated through molecular mechanisms in both WAT and liver. Increased expression of IRS-1 (WAT and liver) and Glut-4 (WAT) is likely to promote glucose uptake and storage in cells. G6Pase catalyses the hydrolysis of the terminal step in the gluconeogenic and glycogenolytic pathways, allowing the release of glucose into the bloodstream. Thus the altered expression profile observed following the *cis*-9, *trans*-11 CLA-enriched diet could contribute to lower circulating glucose and insulin concentrations. TNFα is a key molecular link between obesity and insulin resistance, and represents a potential mechanism for the observed improvement in insulin sensitivity.

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Conjugated linoleic acid (CLA) regulates basal and inducible cytokine expression in endothelial cells. By A.A. SNEDDON[1], E. MACLEOD[1], K.W.J. WAHLE[2] and J.R. ARTHUR[1], [1]*Cellular Integrity Division, Rowett Research Institute, Aberdeen, UK, AB21 9SB and* [2]*School of Life Sciences, Robert Gordon University, Aberdeen AB25 1HG, UK*

Dietary fats and cholesterol in excessive amounts have been strongly associated with an increased risk of CVD. Conversely, some types of fats, such as MUFA and PUFA, may be associated with a decreased risk of disease. One such PUFA is CLA. CLA elicit a multiplicity of effects in animal models of human diseases and in animal and human cells that are considered to have positive health benefits in man (MacDonald, 2000). It is thought that the modulation of lipid metabolism by CLAs is believed to underlie their observed anti-cancer, anti-obesity, anti-inflammatory and anti-atherogenic effects observed in animals, and man. Since one of the earliest steps in the development of CVD is thought to involve damage to the endothelial cells that line the arteries, which then invoke an inflammatory response, we have determined the effects of CLA on endothelial cell inflammation. Human umbilical vein endothelial cell cultures were treated for 24 h with the predominant CLA isomers, *cis*-9, *trans*-11 and *trans*-10, *cis*-12. The effect of these treatments on inflammatory signalling and cytokine expression was then monitored by semi-quantitative PCR. We showed that the CLA regulate both the basal and the inducible levels of certain pro-inflammatory cytokines. Cytokine-induced mRNA levels of TNFα are down regulated by up to 40%, whereas levels of IL-1β are reduced by around 20% (see Table).

The Table shows the effect of 24 h fatty acid treatment on TNFα-induced levels of TNFα mRNA as measured by semi-quantitative RT-PCR.

	TNFα		IL-1β	
	Mean	SEM	Mean	SEM
Control (untreated)	3	1	20	3
+TNFα	100	3	100	1
Linoleic acid	76	4	79	5
9-*cis*, 11-*trans* CLA	78	6	82	5
10-*cis*, 12-*trans* CLA	68	4	77	4
CLA mix	76	6	80	5

Basal levels of these cytokines were also affected by the CLA. Down regulation of both macrophage (Yu *et al.* 2002) and endothelial cytokine production may play a role in the reported beneficial effects of this fatty acid in the development of CVD.

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Energy and nutrient intake during the menstrual cycle in females living in the United Arab Emirates. By L.I. SHEIKH-ISMAIL, C.J.K. HENRY and H.J. LIGHTOWLER, *Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus Headington, Oxford, UK, OX3 0BP*

The influence of the menstrual cycle on energy and nutrient intake has been documented in the literature. In menstruating human females, a cyclic pattern of food intake has been observed (Dalvit-McPhillips, 1983). Some researchers have shown a higher energy intake during the luteal phase of the menstrual cycle (Barr *et al.* 1995), while others report no variations in energy intake across the menstrual cycle (Fong and Kretsch, 1993). Remarkably, few studies have been reported from communities outside the Western world. The aim of the present study was to compare energy and nutrient intakes during different phases of the menstrual cycle in females living in the United Arab Emirates.

Forty-three females, aged between 18 and 30 years, were recruited to the present study. All subjects were non-oral-contraceptive users and experienced regular menstrual cycle lengths between 25 and 35 d. Subjects were asked to record everything they ate in a food diary. Portion sizes were determined by retrospective weighing of food by L.I.S-I. Energy and nutrient intakes were analysed using the Diet5 for Windows program.

	Premenstrual phase		Menstrual phase		Postmenstrual phase	
	Mean	SD	Mean	SD	Mean	SD
Energy (kJ)	570	548	4711**	462	5247	526
Energy (kcal)	1363	548	1126**	462	1254	526
Carbohydrate (g)	182.0	74.4	149.8**	66.0	170.5	90.5
Protein (g)	54.8	24.0	43.2**	20.7	49.0	20.3
Fat (g)	51.1	24.4	43.7*	20.3	47.2	19.1

Mean values were significantly lower than in the premenstrual phase: * $P<0.05$, ** $P<0.01$.

The results show that the mean energy intake was significantly lower in the menstrual phase compared with the premenstrual phase ($P=0.002$), but not the postmenstrual phase. Similarly, intakes of carbohydrate, protein and fat were significantly higher in the premenstrual phase than in the menstrual stage ($P=0.008$, $P=0.001$, $P=0.013$, respectively). Increased protein, carbohydrate and fat intake during the premenstrual phase all played a role in affecting the changes in total energy intake observed in the present study and this is consistent with a previous study conducted by Martini *et al.* (1994).

In conclusion, the data indicate that energy and nutrient intakes varied during the different phases of the menstrual cycle. These results confirm the hypothesis that food intake differs between different phases of the menstrual cycle. The study has important implications for food and nutrient intake studies performed on women. Future studies on energy metabolism may need to control for the phase of the menstrual cycle. Moreover, the role of the menstrual cycle on energy regulation remains a key area for further research.

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'Freshman fifteen' in Liverpool, UK? By L.J. BEASLEY and A.F. HACKETT, *Liverpool John Moores University, IM Marsh Campus, Barkhill Road, Liverpool, UK, L17 6BD*

Living away from home for the first time is likely to have a profound effect on young individuals' dietary habits. Students are reported to have sub-optimum nutritional intakes, mainly due to a reliance on takeaway and convenience foods and lack of practical cookery skills (Eves *et al.* 1995). Longitudinal studies of changes in dietary habits of students starting university have reported a decline in intakes of energy, some micronutrients and fruit and vegetables (Moynihan *et al.* 1999; Edwards & Meiselman, 2003).

In the USA, the tendency for students to gain weight is notoriously known as 'freshman fifteen', although recent research suggests weight gain is an average 4 lb, not 15 lb (Tufts, 2002). The purpose of the present study was to investigate whether British students gain weight. One hundred and seventeen students were recruited; fifty-eight completed all aspects of the study (29% male, 71% female). Dietary habits before and after leaving home were assessed by 24 h recall. Weight and height were measured during the first 2 weeks of university and after 6 months.

	Male (*n* 17)				Female (*n* 41)			
	Baseline		6 months		Baseline		6 months	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Height (m)	1.75	0.09	–	–	1.65	0.05	–	–
Weight (kg)	74.5	11.3	75.2	10.7	65.1	9.8	66.2**	10.2
BMI (kg/m^2)	24.2	2.9	24.4	2.6	23.8	3.1	24.2**	3.3
	Home		Semester One		Home		Semester One	
Energy (kJ)	8005	2015	8306	2173	8715	1634	8226	1743
Fat (%)	34.4	5	35.0	5	35.0	5	35.6	6
Saturated fatty acids (%)	13	2	15	3	14	3	13	4
MUFA (%)	11	2	10	2	10	2	10	3
PUFA (%)	5	1	4	2	4	1	5	2
Alcohol (%)	0.1	0.5	7.0*	7	0.5	1	6.9**	6

Mean values were significantly different from those at baseline: * $P<0.05$, ** $P<0.01$.

Females gained an average 1.1 kg (2.4 lb) and males 0.7 kg (1.4 lb) during their first 6 months of independent living. No significant changes to energy and fat intakes were observed. Saturated fat intakes were high, and mono- and polyunsaturated fat intakes low in both sexes. Females' fat intakes improved after leaving home to comply with recommendations to decrease saturated and increase mono- and polyunsaturated fat intakes, but male intakes changed to be contrary to recommendations. It is probable that decreased levels of physical activity at university contributed to weight gain. Alcohol intakes increased significantly in both sexes, which is likely to contribute to weight gain.

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What's in a snack? Analysis of meals, snacks and drinks in the UK Women's Cohort Study. By N. SULLIVAN and V.J. BURLEY, *Nutrition Epidemiology Group, The Nuffield Institute for Health, University of Leeds, 71–75 Clarendon Road, Leeds, UK, LS2 9PL*

Over the last 20 years the prevalence of overweight and obesity has almost trebled (National Audit Office, 2001). It has been suggested by some that one reason for this dramatic increase is the rise in the consumption of snack foods. There is an increasing tendency amongst Western populations to adopt a grazing rather than meal eating pattern. Snacking could potentially influence body weight both through effects on eating frequency, and also by altering the macronutrient balance of the diet. The present study aimed to investigate which foods women in the UK Women's Cohort Study (UKWCS) choose to snack on and to explore what constitutes a snack and how snacks vary in nutritional composition compared with meals and drinks.

The subjects studied formed a sub-sample of the UKWCS. This is a national study which aims to investigate the relationship between diet and cancer incidence in a cohort of women (*n* 34 374). All subjects, initially aged 35–69 years, completed a food-frequency questionnaire. The subjects were contacted a second time, 2–5 years after baseline. At this stage, all subjects were sent a 4 d food diary and a further health and lifestyle questionnaire. From these food diaries, an exploration was undertaken of the number and nature of meals, snacks and drinks consumed in 186 randomly selected participants of the UKWCS. A 'meal' was defined as an eating episode occurring at conventional meal times (breakfast, lunch or evening meal). Snacks were defined as an eating event containing solid or semi-solid food that occurred outside of conventional meal times, but that was separated from the next event by at least 15 min. Drinks included all beverage types including alcoholic beverages and water. Within DANTE (our in-house ACCESS-based diary analysis program), we categorised each food code used as meal, snack or drink in origin.

The Table describes the percentage contribution made by meals, snacks and drinks to energy and macronutrient intakes in this sample.

Percentage derived from …	Meals		Snacks		Drinks	
	Mean	SD	Mean	SD	Mean	SD
Energy	73.2	9.4	14.9	9.1	11.7	6.1
Fat (g)	79.5	11.4	16.5	11.4	3.9	3.5
Protein (g)	82.4	7.9	7.9	5.8	9.6	5.8
Carbohydrate (g)	70.6	11.7	17.1	10.3	12.3	8.4
Englyst fibre (g)	85.7	10.1	13.5	9.6	0.8	2.7
Sugar (g)	55.3	16.2	22.5	14.4	22.5	14.4
Nutrient density						
Energy density (kJ/g)	5.7	1.2	8.6	5.0	0.6	0.3
Fat density (g fat/100 g food)	5.5	2	9.2	7	0.2	0.2

Between-meal eating provided approximately 15% of dietary energy. This is similar to previous reports in the literature for adult UK and US women. Interestingly, drinks provided an additional 12% of dietary energy in these women. It was found that the top ten sources of energy from drinks were reduced-fat milks, wine and various fruit juices. The greatest contributors to snack energy were found to be bananas, chocolate, cakes, biscuits, potato crisps and chocolate-coated biscuits. Contrary to expectations, in this health-conscious cohort, snacks were found to be almost twice as energy dense and fat-rich as meals. These data provide a basis for further exploration of the effects of the distribution of energy between meals, snacks and drinks on energy balance.

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Symptoms of constipation in children: the influence of physical activity and water intakes. By A. JENNINGS[1], G.J. DAVIES[1], M.F. CHAPLIN[1], V. COSTARELLI[1] and P.W. DETTMAR[2], [1]*Academy of Sport, Physical Activity and Wellbeing, London South Bank University, London, UK, SE1 0AA and* [2]*Reckitt Benckiser Healthcare (UK) Ltd, Dansom Lane, Hull, UK, HU8 7DS*

Holt *et al.* (1992) suggested the possibility that exercise may play an important role in determining bowel habit in children. However, a search of the current literature showed a distinctive lack of studies investigating the possible relationship between bowel habit parameters and physical activity levels in children. The proposed link between constipation and fluid intakes would also need to be considered when investigating these parameters. Therefore the aim of the present study was to concurrently assess and evaluate specific parameters of bowel function, physical activity levels and water intakes in pre-adolescent children (aged 7–10 years) in the UK.

Eighty-four subjects, twenty male and sixty-four female (mean age 9.2 (SD 0.9) years), were recruited from twelve primary schools in the London area. The children's guardians completed consent forms before the subjects' entry into the study. Bowel habit parameters and physical activity levels were assessed by the use of two specifically designed 7 d diaries completed by the children. Dietary intake was also measured, using the 7 d weighed inventory method.

Parameters	Mean	SD	Range	Pearson correlation
Time spent in vigorous activity (h/d)	0.15	0.24	0–1.12	–
Water (g/d)	894	375	300–2197	0.031
Frequency of defecation (per d)	1.3	1	0.1–4.7	0.179
Straining to start (% defecations)	29.8	31.8	0–100	0.032
Straining to finish (% defecations)	16	24.6	0–100	0.148
Incomplete evacuation (% defecations)	18	23.9	0–100	0.162

Findings from the present study indicate that parameters of bowel habit and water intakes are not significantly correlated with children's physical activity levels. This is in contrast to studies conducted in adults, which have indicated that subjects with lower levels of physical activity have a lower prevalence of constipation (Dukas *et al.* 2003). One possible explanation for the above finding could be that children's gastrointestinal function responds differently to physical activity compared with adults. More studies are needed to better understand possible factors that determine bowel habit parameters in children.

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Presentation patterns in childhood obesity. By R. DYKES[1], E. DICKSON[2], D. HARVEY[2] and A. DALE[2], [1]*The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne, UK, NE2 4HH and* [2]*The Children's Unit and the Department of Nutrition and Dietetics, Queen Elizabeth Hospital, Gateshead, UK, NE9 6SX*

In 1998, the WHO designated childhood obesity a global epidemic (World Health Organization, 1998). One national study of children aged 4–12 years found that the prevalence of obesity doubled between 1984 and 1994 (Chinn & Rona, 2001), and the most recent statistics suggest that around 8.5% of 6-year-olds, and 15% of 15-year-olds are now obese (Department of Health, 2003). The consequences of obesity in adulthood are well recognised and it has been shown that over 80% of obese adolescents will remain obese as adults (Whitaker *et al.* 1997). However, it now established that obesity in childhood also has immediate clinical effects, probably the most important being adverse effects on the cardiovascular system similar to those seen in adults (Reilly *et al.* 2003). Childhood obesity is also associated with considerable psychological morbidity and therefore tackling the problem is a public health priority.

To date there is little research on patterns of presentation in childhood obesity. Such information would help in the planning of appropriate services and focusing of health education. All paediatric referrals due to obesity, to dietetic services at the Queen Elizabeth Hospital, Gateshead, over the last 3 years were audited to compare actual referral practice with the standards outlined in the Scottish Intercollegiate Guidelines Network (SIGN) guideline (Scottish Intercollegiate Guidelines Network, 2003). Demographic details were obtained to provide baseline data on presentation patterns.

A total of 115 children were referred to the dietetic services over the 3-year period. Of these, 52% were female and 48% male. The number of referrals increased over the 3 years from twenty in 2001 to fifty in 2003. Mean age at presentation was 10.51 years and mean BMI 28.5 kg/m^2 with no difference between the sexes for either value. Of the children, 97% had a BMI greater than the 98th centile and their referral was therefore appropriate according to the SIGN guidelines. Of the children, 52% were referred to dietitians by hospital paediatricians, and 31% by their GP. The remainder were referred by school health advisors, social workers, health visitors, etc. Of the children, 74.5% had a BMI greater than the 99.6th centile. The SIGN guidelines recommend that all these children should be referred to a paediatrician before treatment is considered. In practice, this happened in less than half of these cases. Co-morbidity was documented in 30%. There was a significant rate (44%) of discharge due to failure to attend clinic (35% of whom did not attend their initial appointment). Less than 15% completed their treatment and 40% children are still under review. Of those who completed their treatment and for whom complete data are available, 75% improved their BMI.

The demographic data show that the problem of obesity arises in primary-school-aged children, and suggest that health education should be targeted at this age group. The vast majority of referrals to dietitians were appropriate. However, a significant proportion of children with a BMI >99.6th centile, and who were therefore at increased risk of obesity-related morbidity, were not referred to a paediatrician. It is hoped that implementation of the new 'pathway of care' will improve referral practice. The results indicate that dietetic intervention is successful in part. However, success is clearly dependent on motivation and there was a substantial rate of non-attendance. This may suggest the need for a more diverse approach, involving agencies such as the food, sports and leisure industries, and a role for a wider range of health professionals to provide an integrated service to address the needs of these children in an acceptable and sustainable way.

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Association between body mass index, health-related quality of life and use of commercial weight-loss services in a sample of obese individuals. By A. McCONNON, S.F.L. KIRK, D.C. GREENWOOD and J.K. RANSLEY, *Nutrition Epidemiology Group, University of Leeds, 50 Clarendon Road, Leeds, UK, LS2 9PL*

Despite the existence of evidence-based guidelines for the treatment of obesity (Scottish Intercollegiate Guidelines Network, 1996) and numerous studies investigating different methods for treating obesity and maintaining weight loss, the prevalence of obesity is still on the increase. Literature on interventions examining the effect of diet, physical activity and behavioural therapy on obesity is plentiful. However, to date, very little research has been undertaken to examine the effect of more common, commercial services on weight loss in an obese population.

The aim of the present study was to investigate the association between both BMI and self-reported health-related quality of life (HRQOL) and a range of widely available weight-loss services. The data presented have been collected in the UK Weight Control Trial (UKWCT). The UKWCT is a Leeds-based randomised controlled trial investigating the role of a web-based weight-support programme in a sample of obese participants (Kirk *et al.* 2003). For this trial, 214 obese individuals with a mean BMI of 36 kg/m^2 were recruited. This sample is predominately white (95%), married (76%) and middle aged (mean age 46 years), with a male:female ratio of 24:76.

		BMI			HRQOL		
		Median	Interquartile range	*P* value	Median	Interquartile range	*P* value
Member of a commercial slimming club	Yes	34.2	31.8–37.1	0.267	75	63–83	0.004*
	No	35.3	32.1–39.2		60	40–75	
Gym member	Yes	34.3	31.5–38.0	0.900	65	40–80	0.522
	No	35.4	32.6–39.2		65	40–80	
Internet weight-loss advice	Yes	33.0	31.4–37.7	0.129	74	53–80	0.059
	No	35.4	32.4–39.2		61	40–76	
Diet books and magazines	Yes	34.4	31.8–39.4	0.646	65	40–80	0.984
	No	35.3	32.4–39.0		65	41–80	

* Statistical significance.

A Spearman correlation highlighted a significant inverse correlation between BMI and HRQOL (P=0.001). The P values presented in the Table were generated from Mann–Whitney tests. The only statistically significant result was the association between HRQOL and membership of a slimming club. A borderline non-significant result (P=0.059), with a thirteen-point difference in HRQOL for participants who use the Internet for weight-loss advice compared with those who do not, is a noteworthy finding. Research has shown that currently available weight-loss methods, which have been evaluated, are notoriously unsuccessful. The need to explore the use of novel treatment methods in obesity research has been recognised. These data are presented from The UK Weight Control Trial. This trial marks the first attempt in the UK to investigate and evaluate the use of the Internet in obesity treatment. The results from the present preliminary analysis support the investigation of this potential method of obesity treatment.

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The role of meat protein in influencing satiety. By F.A. PUGLIA and C.J.K. HENRY, *Nutrition and Food Science Group, School of Biological and Molecular Sciences, Gipsy Lane Campus, Oxford Brookes University, Oxford, UK, OX3 0BP*

Obesity is a worldwide epidemic that touches both developed and developing countries. In the UK, 32% men and 27% women are currently classified as overweight and 17% men and 21% women as obese (National Audit Office, 2001). Obesity results from a slow process that converts the energy derived from food into fat. This phenomenon takes place in the case of a positive energy balance, i.e. when energy intake exceeds energy expenditure. A complex network of signals regulates energy intake. This project focuses on the role of signals arising from food consumption; appetite and satiety (Blundell, 1999). Recent studies have shown that among the different dietary components, proteins can increase satiety more than carbohydrates or fats (Stubbs, 1995). In light of this, the present study aims to investigate the role of different protein sources on satiety.

The experimental protocol was tested in a pilot study and subsequently improved to match our goals. In the present study, twenty volunteers were recruited among Oxford Brookes University staff and students. Approval from the Oxford Brookes University ethics committee was obtained for the present study. Subjects were fed on four separate occasions with a high-protein mixed meal prepared with beef, lamb, pork or soya protein. Subjects rated their appetite feelings before and at various intervals during the following 3 h after the meal using visual analogue scales (VAS) (Flint *et al.* 2000). Energy expenditure was measured on a sub-sample (n 10) to calculate the diet-induced thermogenesis (DIT) of each meal. Incremental areas under the curve (AUC) were calculated for VAS and DIT using the trapezoid method.

At this time the study is ongoing, therefore the results presented are preliminary. The Table shows the AUC for DIT, hunger and prospective consumption of food (PCF) for the four protein sources used. Differences in delaying the return of hunger (hunger and PCF rating) and in DIT response can be observed between the different protein sources especially between the meats (beef, lamb and pork) and non-meat protein sources (soya protein) but no significance can be yet established.

AUC		Beef (n 7)	Lamb (n 6)	Pork (n 8)	Soya protein (n 4)
DIT	Mean	6025	8561	4866	2199
	SD	8123	10549	6717	807
Hunger	Mean	10248	7184	9894	10627
	SD	3307	2205	4330	3515
PCF	Mean	9226	7778	9597	12838
	SD	2849	2620	2967	2484

Uhe *et al.* (1992) reported that fish was more satiating than beef and chicken. The next stage of the study will include fish (cod). Our further work will investigate the link between satiety, physiological parameters and the biochemical composition of the different sources.

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Implementation of dietary and general lifestyle advice in women with polycystic ovarian syndrome. By L. HUMPHREYS[1], V. COSTARELLI[1] and G. CONWAY[2], [1]*Academy of Sport, Physical Activity and Wellbeing, London South Bank University, London, UK, SE1 0AA and* [2]*Department of Endocrinology and Diabetes, Middlesex Hospital, Mortimer Street, London, UK, W1T 3AA*

Polycystic ovarian syndrome (PCOS) is a common endocrine condition affecting about 5–10% of women of reproductive age (Lakham *et al.* 2000). PCOS has serious reproductive and metabolic consequences, including anovulation, infertility and diabetes mellitus. Central obesity and insulin resistance are strongly implicated in its aetiology and the reduction of these risk factors is a central treatment focus. In addition, weight loss has been consistently successful in reducing insulin resistance and restoring ovulation and fertility in women with PCOS (Norman *et al.* 2002). The purpose of the present study is to investigate the general attitudes towards dieting and exercise in women with PCOS together with the extent of implementation of the dietary and lifestyle advice that these women are given as part of their treatment. Fifty-two premenopausal women, over the age of 18 years, with a confirmed diagnosis of PCOS, were recruited from the Endocrinology Department, Middlesex Hospital, London. The subjects were interviewed individually using an interviewer-guided questionnaire. Each interview lasted about 15–20 min and took place in a separate room before the woman's scheduled appointment to the endocrinology clinic. The baseline characteristics of the subjects are summarised in the Table.

	Age (years)		Weight (kg)		Height (m)		BMI (kg/m^2)		Age at diagnosis of PCOS (years)	
n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
53	32.8	6.7	76.5	19.6	1.64	0.07	28.7	7.2	22.5	7.1

Of the subjects, 64% were overweight (BMI>25 kg/m^2) and 18% of those were obese (BMI>30 kg/m^2), which was anticipated given the high incidence of weight problems in women with PCOS. Out of the thirty-five women who were overweight and obese, twenty-two reported constant dieting from their late teens in order to reduce their weight and twelve out of thirty-five were currently on a weight-reducing diet. Thirty-six out of a total of fifty-three patients reported an average weight fluctuation of about 6 (SD 4.8) kg over the past 12 months. Only the overweight women were given dietary advice from their consultant endocrinologist, which, according to the subjects, was very general and inadequate. All subjects identified the importance of weight control in the management of their condition; however, only nine out of the thirty-five overweight women had actually been referred to a dietitian and only twenty-two reported taking moderate exercise once per week in order to improve their health. Most of the subjects had a good understanding of healthy-eating principles; however, the main source of information was the Internet and their consultant endocrinologist who they normally see only twice annually. It is important to note that a general lack of motivation to improve dietary and exercise habits was evident particularly in the obese subjects. Currently the emphasis in the management of PCOS focuses on the administration of different drug treatments and general advice on lifestyle issues such as weight loss, exercise and smoking. In spite of the fact that weight loss and weight maintenance are absolutely vital in the treatment of PCOS, the present study has shown that the support given to PCOS patients to help reduce and control their weight could be inadequate and needs to be improved.

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Perceived dietary change following advice to simultaneously reduce both dietary fat and sucrose or dietary fat. By C. WHITE[1], A. DELOOY[2] and S. DRUMMOND[1], [1]*Centre for Nutrition and Food Research, Queen Margaret University College, Edinburgh, UK, EH12 8TS and* [2]*University of Plymouth, Drake Circus, UK, PL5 3UB*

The escalating obesity levels in the UK indicate an urgent need for effective weight-loss programmes. In addition to promoting weight loss, all weight-loss programmes should also include a weight-maintenance component (Scottish Intercollegiate Guidelines Network, 1996). To ensure long-term maintenance of a healthy weight, some degree of behaviour modification is required by the individual.

The present study aimed to measure subject's perception of the dietary changes made by them following one of two types of dietary advice to elicit a weight loss for 3 months (White *et al.* 2004) with a further 9 months of weight maintenance.

A parallel-design intervention study was employed and subjects were randomly assigned to group 1 (advice to reduce energy, fat and sucrose), group 2 (advice to reduce energy and fat, maintaining sucrose at 10% energy) or a control group 3 (no advice). Baseline dietary intakes were assessed using a 7 d unweighed diet diary and compliance with the dietary advice was assessed by two 4 d food diaries after 3 and 9 months. Perceived dietary change was assessed by a Lickert scale questionnaire. For example, subjects were asked to estimate their current fat and sugar intake measured on a seven-point scale from 'extremely low' to 'extremely high' and current fat and sugar intake compared with before the study began from 'much lower than before' to 'much higher than before'.

	Baseline				Month 3				Month 9			
	Group 1		Group 2		Group 1		Group 2		Group 1		Group 2	
Dietary analysis	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
n	44		43		44		43		44		43	
Energy (MJ/d)	8.04	0.3	8.61	0.3	5.34*	0.2	5.94*	0.2	5.41*	0.2	6.15*	0.2
Fat (% energy)	35.6	0.7	36.0	0.8	25.4*	0.9	26.8*	1.1	27.1*	0.8	28.8*	0.8
Carbohydrate (% energy)	43.6	0.7	44.7	0.8	49.7*	0.9	50.8*	1.4	48.6*	0.9	48.9*	1.0
Starch (% energy)	27.1	0.6	26.7	0.6	31.7*	0.9	30.1*	1.0	31.6*	0.9	29.0	0.9
Sucrose (% energy)	6.4	0.4	7.4	0.5	4.8*	0.3	6.8	0.4	5.6	0.4	7.7	0.5
Protein (% energy)	15.1	0.4	14.6	0.4	19.3*	0.5	18.7*	0.7	18.8*	0.6	17.8*	0.5

* Mean value was significantly different from baseline (P<0.001).

Over the 9-month study period group 3 reported no significant changes to their diet, except that between 3 and 9 months they significantly decreased their energy intake by 0.16 MJ. After 3 months, and maintained at 9 months, both groups 1 and 2 reported a significant decrease in energy intake and percentage energy from fat, and reported a significant increase in percentage energy from total carbohydrate and protein. Group 1 reported a significant reduction in percentage energy from sucrose after 3 months, but intakes at 9 months were no different from baseline intake.

Both intervention groups enjoyed complying with the dietary advice; however group 1 showed a significant decrease between 3 and 9 months in their enjoyment. Both intervention groups correctly perceived their fat and sugar intake to have fallen and be lower than the average individual of their sex and age in the UK by the end of 3 and 9 months. Group 1 perceived their sugar intake to increase between 3 and 9 months. All these perceived dietary changes are in agreement with the diet diaries.

By recognising beneficial dietary changes and being able to comply with them long term is essential for successful weight loss and maintenance. The present study has demonstrated that advice to reduce the energy content of the diet by either reducing fat and sugar or fat alone can be effective in changing dietary behaviour which is recognised by the individual and sustainable over 9 months. Also, compliance with dietary advice to reduce both fat and sugar was seen to be not as enjoyable as prioritizing a reduction in dietary fat only, in the longer term.

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A comparison of the effect of advice to simultaneously reduce both dietary fat and sucrose or dietary fat on perceived quality of life. By C. WHITE[1], A. DELOOY[2] and S. DRUMMOND[1], [1]*Centre for Nutrition and Food Research, Queen Margaret University College, Edinburgh, UK, EH12 8TS and* [2]*University of Plymouth, Drake Circus, UK, PL5 3UB*

Obesity is now acknowledged as one of the most important public health problems in developed countries contributing to an ever-increasing non-communicable disease burden. Excess weight can cause cardiovascular diseases, respiratory disorders, osteoarthritis, sleep apnoea, low self-esteem and other psychological disorders. Moderate weight loss can improve these health risks, leading to a better quality of life for obese individuals.

The present study aimed to compare the effect of two types of dietary advice on perceived quality of life throughout a 3-month weight-reduction and 9-month weight-maintenance intervention.

One hundred and sixty nine overweight or obese women were randomly assigned to group 1 (advice to reduce energy, for 3 months only, fat and sucrose), group 2 (advice to reduce energy, for 3 months only and fat, maintaining sucrose at 10% energy) or a control group 3 (no advice). Baseline dietary intakes were assessed using a 7 d unweighed diet diary and compliance with the dietary advice was assessed by four 4 d food diaries after 1.5, 3, 6 and 9 months. At baseline and after 3, 9, and 12 months change in perceived quality of life was assessed by subjects responding to a series of statements on a Lickert seven-point scale questionnaire ranging from 1 ('strongly disagree') to 7 ('strongly agree') (only baseline and 3-month results presented).

	Baseline				Month 3			
	Group 1 (*n* 44)		Group 2 (*n* 43)		Group 1 (*n* 44)		Group 2 (*n* 43)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Feel less worried about their health	3.07	0.20	3.63	0.20	4.80*†	0.19	4.42*†	0.16
Feel less worried about their weight	3.05	0.23	2.91	0.19	4.50*†	0.23	4.21*†	0.20
Feel more energetic	3.61	0.16	4.07	0.19	5.27*†	0.17	5.09*†	0.16
Feel more satisfied with their self in general	3.59	0.19	3.60	0.16	5.16*†	0.17	4.91*†	0.16
Feel happier in general	3.74	0.19	3.88	0.17	5.12*†	0.16	5.02*†	0.17
Feel more satisfied with personal appearance	3.36	0.19	3.51	0.17	4.93*†	0.18	4.70*†	0.16
Enjoy social situations more	3.88	0.16	3.88	0.14	4.56*†	0.15	4.47*†	0.15
Food eat now makes it easier to control their weight	3.80	0.15	3.98	0.10	5.23*†	0.14	5.02*†	0.16
Food eat now reduces risk of heart disease	3.82	0.17	4.19	0.14	5.50*†	0.14	5.40*†	0.15
Food eat now is more interesting	3.91	0.13	3.98	0.06	4.20	0.17	4.56*†	0.19
Overall quality of life	4.11	0.07	4.19	0.08	4.94*†	0.13	4.72*†	0.12

* Mean values were significantly different from baseline ($P<0.01$).
† Mean values were significantly different from control ($P<0.05$).

Both groups 1 and 2 reported a significant decrease in percentage energy from fat ($P<0.001$) and group 1 reported a significant reduction in percentage energy from sucrose after 3 months ($P<0.001$), but could not maintain that reduction at 9 months despite advice to do so.

After 3, 9 and 12 months both intervention groups perceived their overall quality of life to have increased, as well as feeling less worried about their health and weight, more energetic, more satisfied with themselves in general, more satisfied with their personal appearance, feeling happier in general and enjoying social situations more. They also perceived that the diet they were now following made it easier for them to control their weight and reduce their risk of heart disease. In addition group 2 found the food that they were now eating to be more interesting than at baseline. There was no significant change in perceived quality of life in the control group over the 9 months.

The two sets of dietary advice given in the present study were perceived as having a positive effect on subjects' quality of life. However, advice given to group 2, maintaining sucrose at 10% energy, may have resulted in a more palatable low-fat diet as indicated by their significant agreement that the food they were now eating was more interesting. Long-term dietary change may be difficult to sustain if individuals have to make too many significant dietary changes but may be promoted by the inclusion of sucrose in a low-fat diet.

Can urinary sugars and thiamine be used as biomarkers to assess measurement error in dietary intake data? By N. TASEVSKA, S. RUNSWICK and S. BINGHAM, *MRC Dunn Human Nutrition Unit, Hills Road, Cambridge, UK, CB2 2XY*

Measurement errors associated with different methods for assessing dietary intake are generally assessed by comparison with another assumed more accurate reference dietary method. However, if errors between the reference and the test method are correlated, correction for regression dilution arising from measurement error will be substantially underestimated. Using existing biomarkers of intake, it has recently been shown that errors from methods to measure the diet used in epidemiological studies are far greater than previously thought (Day *et al.* 2001; Kipnis *et al.* 2002). Nevertheless, only limited biomarkers to assess accuracy of dietary intake are available and there are many foods and nutrients for which no suitable biomarker has been identified (Bingham, 2002).

To assess whether 24 h urinary sucrose and thiamine might serve as biomarkers of dietary intake, two studies have been conducted with volunteers living in the MRC Dunn Human Nutrition Unit volunteer suite, where diet can be carefully controlled and all specimens collected. Study 1 was designed to assess whether there is a dose response of sugars and thiamine in urine to increased levels in food, giving a predictive response to varying intakes, independent of individual variations. Study 2 (in progress) is investigating the ability of these markers to estimate the intake of thiamine and sugars while the subjects consume their normal varying diet.

In the first study, twelve subjects were fed three different diets of constant and known composition during 30 d of study: (a) low thiamin (0.7 mg), high sugars (250 g); (b) medium thiamin (1.9 mg), medium sugars (150 g); (c) high thiamin (3.5 mg), low sugars (60 g). In study 2, thirteen subjects were given their normal varying diet (replicated from food menus kept beforehand) for 30 d and amounts consumed assessed by weighing dishes before and after consumption. The subjects collected 24 h urine on a daily basis throughout both of the studies. Sucrose and fructose in urine were determined using a UV method for determination of sucrose, D-glucose and D-fructose in foodstuffs (Bergmeyer, 1974). Thiamine in urine was determined using an HPLC method for rapid determination of thiamine in foods, body fluids, urine and faeces (Bötticher & Bötticher, 1986). Intakes were calculated using Diet 5 and DINER software for studies 1 and 2 respectively (Forestfield Software, 1991; Welch *et al.* 2002).

Results from study 1 indicated that the excretion value of both sucrose and fructose depended primarily on the intake level, with a dose-related response between dietary sugars and the excretion level. In all of the subjects the urinary excretion level of thiamine was distinguishable on an individual basis between different dietary periods. Individual mean excretion levels of both sucrose (r 0.782; $P<0.001$) and fructose (r 0.786; $P<0.001$) were significantly correlated with the level of sugars intake. In the linear regression model urinary fructose (R^2 0.58; $F_{1,119}=167.41$; $P<0.001$) was found to be a better predictor of sugars intake than urinary sucrose (R^2 0.48; $F_{1,125}=119.1$; $P<0.001$). A significant correlation was found between individual mean thiamine excretion level and thiamine dietary intake (r 0.652; $P<0.001$). The multiple regression model showed that thiamine intake is the main significant predictor of thiamine output (R^2 0.309; $F_{1,260}=117.83$; $P<0.001$) explaining 31% of the variability of the urinary level, whereas urine volume and age each contribute about 10%.

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Using the Internet as a tool for finding reports on childhood obesity-prevention and -treatment programmes, with a focus on immigrants new to industrialised countries. By C.A. FLYNN[1] and D. McNEIL[2], [1]*University of Ulster, Cromore Road, Coleraine, UK, BT52 1SA and* [2]*Calgary Health Region, 29th Street NW, Calgary, Alberta, Canada, T3B 3Y2*

Searching published literature is the search strategy primarily employed in systematic review approaches; yet to be truly comprehensive, multiple literature types and sources should be utilised (Conn *et al.* 2003). Our aim was to develop an Internet search strategy as part of a systematic approach to finding reports on interventions and strategies for promoting healthy weights in children. There was a particular focus on immigrants new to industrialised countries.

There were three elements to the search strategy developed. Firstly, a list of twenty-two relevant organisation websites was compiled. Then, due to its high ratings and comprehensiveness (Sullivan, 2003) the Google search engine was chosen to carry out the search for sites containing both the words **obesity** and **children**, and either the term **program**, **prevention** or **treatment**. Due to the Internet's constant changes, a brief search of the hits generated (*n* 137 000) was carried out to create a list of potentially relevant sites. These were subsequently searched in greater detail to select relevant reports. Criteria adopted to eliminate irrelevant sites excluded those linked to library databases, which were already being searched, those of a commercial nature, and those in our opinion from unreliable sources. Following examination of the first 550 hits, this initial brief search was concluded as the number of relevant sites per page of hits occurred less frequently. This search identified 152 sites, including twelve from the first list of twenty-two sites.

The final element of the search strategy involved creating a list of government sites for the forty-eight non-industrialised countries, which currently contribute most significantly to Canada's immigrant population. A Google search was not useful because most of the official sites for these countries were in foreign languages or were poorly managed. Instead, four reliable (in our opinion) sites identified 288 government sites:

http://www.gksoft.com/govt/en/
http://www.psr.keele.ac.uk/official.htm
http://www.voww.net/countries/countries.htm
http://www3.who.int/whosis/national_sites/index.cfm?path=whosis,links,links_national&language=english

Of these, fifty-one in English were added to the list of sites to be searched. A detailed search of these 213 sites resulted in the selection of 249 reports for appraisal (247 from the Google–Investigator list and two from the country list). Ten of these were included in the synthesis phase of the review, including five published reports, which did not emerge in any of the published literature database searches (i.e. Medline, Cinahl etc.).

Having an Internet component to this synthesis research facilitated consideration of relevant reports from both grey and published literature. Limitations are to be expected, as the Internet is an enormous uncontrolled database of information. There was potential to miss relevant sites due to searcher judgement error. However, carrying out a duplicate search by another searcher to minimise this error was impractical due to the constant evolution and variability of the Internet. Also, reliance on the Google page-ranking system risks potentially missing smaller, yet relevant, sites that are ranked lower. However, using the Internet as a search tool in systematic research lowers publication bias and creates a more comprehensive review by not systematically excluding grey literature and providing an additional source for harder-to-find published literature.

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A Cochrane systematic review of low-glycaemic-index diets for coronary heart disease. By S.A.M. KELLY[1], G. FROST[2], V.J. WHITTAKER[1] and C.D. SUMMERBELL[1], [1]*Univesity of Teesside, Middlesbrough, UK, TS1 3BA and* [2]*Department of Nutrition and Dietetics, Hammersmith Hospital, London, UK, W12 0HS*

Glycaemic index (GI) is a physiological measure of a carbohydrate's ability to affect blood glucose. Originally introduced as a method of enhancing nutritional advice to individuals with diabetes, it has been linked to other possible health benefits (Jenkins *et al.* 2002). There is mounting epidemiological evidence that low-GI carbohydrates have a positive effect on cardiovascular risk factors (Leeds, 2002). There is a growing interest in this concept in the clinical management of individuals at risk or with established CHD and debate whether this concept should be moved into the public health arena.

The present systematic review assesses the relationship between the consumption of low-GI diets and CVD and risk factors.

We searched The Cochrane Library, CCTR, MEDLINE, EMBASE (from start date to present) and CINAHL (1982 to present) bibliographies. We also contacted experts in the field. All randomised controlled trials and controlled clinical trials with concurrent controls that had assessed the effect of low-GI diets, compared with control diets, over a minimum of 4 weeks, on CVD and risk factors, in adults with at least one major risk factor for CVD were included.

Fifteen studies (from seventeen papers) were identified. No studies were found which reported the effect of low-GI diets on CHD mortality or CHD events and morbidity. All fifteen included studies reporting the effect of low-GI diets on major risk factors for CHD. Nine of the studies were of randomised cross-over design and six studies were parallel randomised controlled trials.

Systematic review and meta-analysis of the parallel-design (but not cross-over-design) studies showed a weak but significant relationship between low-GI diets and lower total cholesterol, Hba1c and insulin compared with the control diets. Also, most of the other risk factors, except HDL-cholesterol, were improved in groups of individuals who consumed a low-GI diet, although this was not statistically significant.

The evidence from controlled trials showing that low-GI diets reduce CVD and risk factors is weak. Of the trials that met the review criteria, there is a large variation in methodological quality, with many poor-quality studies. Many of the trials identified were short term (between 3 and 4 weeks in duration), with small sample sizes, and different comparison methodology. The current evidence suggests that low-GI diets may offer some small advantage in the management of CVD. However, there is a need for a well-designed, adequately powered, randomised controlled study, using standard methodology and of greater than 4 weeks duration to assess the usefulness of low-GI diets for the reduction in CVD.

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An evaluation of dual-energy X-ray absorptiometry for assessing body composition. By J.E. WILLIAMS, M.S. FEWTRELL, N.J. FULLER, C.M. WILSON and J.C.K. WELLS, *MRC Childhood Nutrition Research Centre, Institute of Child Health, London, UK, WC1N 1EH*

Dual-energy X-ray absorptiometry (DXA) is increasingly used as a rapid, precise technique for assessing bone mineral content (BMC), fat mass (FM) and lean mass in both adults and children. Its use is likely to increase with the rising incidence of obesity. However, few studies have compared DXA with a reference method such as the four-component model (4CM; Fuller *et al.* 1992). We evaluated DXA against the 4CM in normal-weight and obese adults and children.

A total of 157 subjects (children, normal weight *n* 52, obese *n* 19; adults, normal weight *n* 70, obese *n* 16) were recruited for an ongoing study of body composition in healthy subjects at Great Ormond Street Children's Hospital. Obese was defined as greater than the 95th percentile, and mean age for the children was 10.62 (SD 3.42) years and for the adults 20.35 (SD 0.43) years. BMC, weight, FM and lean mass were determined by DXA (GE Lunar Prodigy; Waukesha, WI, USA). Fat-free mass (FFM) was calculated as lean mass plus BMC and percentage FM as FM/weight×100. The 4CM derives values for mineral, water, fat and protein and hence provides values for FFM and percentage fat. A Bland & Altman (1986) analysis was used to assess systematic difference between methods (bias) and random variability (95% limits of agreement).

			Bias (DXA–4CM)	95% Limits of agreement	Correlation
Body weight (kg)	Child	Non-obese	−0.11*	−0.82, 0.60	0.25
		Obese	−0.07	−2.00, 1.85	−0.02
	Adult	Non-obese	0.38***	−0.78, 1.54	0.44††
		Obese	−0.29*	−1.19, 0.61	0.42
FFM (kg)	Child	Non-obese	0.18	−1.51, 1.88	0.06
		Obese	−1.33**	−4.58, 1.91	0.67††
	Adult	Non-obese	−0.88***	−3.49, 1.73	0.15
		Obese	−1.90***	−5.08, 1.28	0.06
Percentage fat	Child	Non-obese	−1.02***	−4.90, 2.86	0.43††
		Obese	1.93***	−1.72, 5.59	−0.20
	Adult	Non-obese	1.87***	−2.00, 5.75	0.29†
		Obese	2.21***	−2.20, 6.62	−0.07

Mean values for the two methods were significantly different: * $P<0.05$, ** $P<0.01$, *** $P<0.001$ (paired t test).
Correlation significance between the difference and the mean: † $P<0.05$, †† $P<0.01$, ††† $P<0.001$.

DXA measured body weight, FFM and fatness with significant error in almost all cases. DXA underestimated fatness in normal children but overestimated it with increasing body size and fatness such that the largest bias occurred in the obese adults. Within normal-weight children and adults the magnitude of the bias significantly increased as percentage fat increased.

The present results suggest that DXA–FM values differ significantly from those derived from the 4CM, and that the bias varies with size and fatness. Consequently, it may not be possible with this instrumentation to apply a single 'correction' figure for the bias as recently proposed (Wong *et al.* 2002). The findings also suggest caution is required when using DXA to monitor longitudinal changes in body composition (for example, during weight-loss programmes for the obese) since the measurement bias will alter with weight loss. A further complication is that that the biases are likely to be make- and model-specific.

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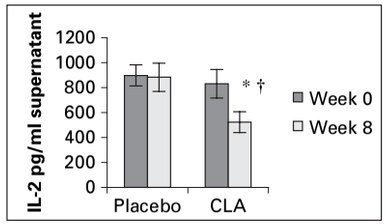
Conjugated linoleic acid supplementation decreases *ex vivo* human peripheral blood mononuclear cell interleukin 2 expression and secretion. By A. MULLEN, F. MOLONEY, A. NUGENT, M.J. GIBNEY and H.M. ROCHE, *Nutrigenomics, Institute of Molecular Medicine, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Republic of Ireland*

Conjugated linoleic acid (CLA) refers collectively to the positional and geometric isomers of linoleic acid. The biologically active *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA dienes have been shown *in vitro* and in animal models to modulate immune function. In animal studies CLA has induced lymphocyte proliferation, increased concanavalin A (Con A)-stimulated IL-2 and decreased TNF-α and IL-6 expression (Roche *et al.* 2001). However, there is relatively little information in relation to the effects of CLA supplementation on the human immune response.

The present double-blind, placebo-controlled intervention trial investigated the effects of CLA supplementation (*cis*-9, *trans*-11 CLA–*trans*-10, *cis*-12 CLA isomeric blend, 50:50) on the expression of inflammatory mediators associated with atherosclerosis in middle-aged men. Thirty healthy male volunteers (mean age 49.4 years, mean BMI 26.1 kg/m^2) were randomly assigned to receive 3 g CLA or placebo in softgel capsules daily for 8 weeks. Fasting blood samples were drawn pre- and post-supplementation and serum, plasma and cultures of unstimulated and Con A-stimulated peripheral blood mononuclear cells (PBMC) were prepared. ELISA determined PBMC supernatant IL-2, TNF-α and IL-10 concentrations. Serum IL-6 and plasma C reactive protein (CRP) were measured by ELISA. Plasma fibrinogen activity was measured using an automated clotting assay. PBMC IL-2 mRNA expression was analysed by TaqMan® real-time PCR with GAPDH as an endogenous control. Statistical significance was determined by two-way ANOVA and pooled t test, after data normalisation as required.

CLA supplementation significantly decreased Con A-induced PBMC IL-2 secretion ($P\leq0.05$). Con A-stimulated PBMC IL-2 mRNA expression was reduced by 14.4% post-CLA supplementation and was 14.8% lower than the placebo group, but this change was not statistically significant. CLA had no significant effects on PBMC TNF-α or IL-10 secretion. The serum and plasma concentrations of other inflammatory markers involved in CVD, including IL-6, CRP and fibrinogen, were not affected by CLA supplementation. The Table shows peptide expression of inflammatory mediators from Con A-stimulated PBMC, serum and plasma. The Figure shows Con A-induced IL-2 expression from PBMC in placebo and CLA groups.

	CLA				Placebo			
	Week 0		Week 8		Week 0		Week 8	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
IL-10 (pg/ml)	587.4	177.9	384.8	123.5	517.9	115.5	487.7	116.3
TNF-α (pg/ml)	2041.5	309.5	2200.7	401.3	2015.7	212.9	1920.4	243.4
IL-6 (pg/ml)	2.75	2.12	2.1	1.5	2.5	2.3	3.9	2.5
CRP (mg/l)	1.1	0.3	1.2	0.3	1.8	0.5	1.0	0.3
Fibrinogen (mg/l)	2799	79	2784	53	2820	87	2842	69



* Significant decrease in CLA group ($P\leq0.05$).
† Significant difference from placebo group ($P\leq0.05$).

The present study showed that CLA supplementation down regulated PBMC IL-2 secretion, a Th1 signature cytokine, which may indicate some anti-inflammatory potential. However CLA supplementation had no effect on the other markers of the human inflammatory response associated with CVD. The present study determined the effect of a heterogeneous blend of CLA. The isomer-specific effects of CLA on inflammatory mediators and immune pathways associated with human disease requires further investigation.

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Conjugated linoleic acid supplementation in type 2 diabetes. By F. MOLONEY[1], A. MULLEN[1], T.P. YEOW[2], J.J. NOLAN[2] and H.M. ROCHE[1], [1]*Nutrigenomics Research Group, Department of Clinical Medicine, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Republic of Ireland and* [2]*Metabolic Research Unit, St James's Hospital, Dublin 8, Republic of Ireland*

Type 2 diabetes mellitus (T2DM) is associated with significant health and socio-economic burdens. Therefore there is a need to identify effective dietary therapies to attenuate modifiable risk factors. Conjugated linoleic acid (CLA) refers to a collection of positional and geometric isomers of linoleic acid with a conjugated double-bond system. It is a natural food component found in the lipid fraction of ruminant meat and dairy products. Most research has investigated the effects of two isomers: *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, which are thought to have the most biological activity. Animal feeding studies have shown beneficial effects on lipid and glucose metabolism (Roche *et al.* 2002). In healthy subjects it has been shown to improve lipid metabolism (Noone *et al.* 2002). The aim of the present study was to examine the impact of CLA supplementation on lipid, insulin and glucose metabolism in a group of patients with diet-controlled T2DM.

In a randomised, double-blind, placebo-controlled trial, thirty-two subjects with stable diet-controlled T2DM received 3.0 g CLA isomer blend/d (50:50 blend of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA) or placebo (blend of palm oil and soyabean oil) for 8 weeks. A 3 h 75 g oral glucose tolerance test (OGTT), fasting plasma lipid concentrations, and inflammatory markers were assessed pre- and post-intervention. Insulin sensitivity was assessed by homeostasis model assessment (HOMA) and oral glucose insulin sensitivity (OGIS). Values represent means with their standard errors. Three-way ANOVA was used to identify significant changes in biochemical parameters as a result of CLA intervention.

Fasting glucose concentrations were significantly increased following CLA supplementation ($P<0.001$). Plasma glucose, insulin and c-peptide concentrations were not significantly altered at other time points during the OGTT. HOMA was significantly increased following CLA supplementation ($P=0.05$). Insulin sensitivity measured by OGIS was unchanged. Supplementation with CLA had no effect on total cholesterol, LDL-cholesterol, plasma triacylglycerol, triacylglycerol rich lipoprotein (TRL) cholesterol or TRL triacylglycerol concentrations. Total HDL-cholesterol concentration was significantly increased following CLA supplementation ($P<0.05$). This 7.9% increase in total HDL-cholesterol concentration was due to a significant increase in HDL_2-cholesterol concentrations ($P<0.05$). LDL:HDL-cholesterol was significantly reduced after supplementation ($P<0.01$). CLA supplementation significantly reduced fibrinogen concentrations ($P<0.001$), but had little effect on either C-reactive protein or IL-6. Compliance was assured as plasma CLA increased by 89% following CLA supplementation ($P<0.001$). Mean daily intake, assessed by two 4 d food diaries, was unchanged during the supplementation period.

In conclusion, CLA had a negative effect with respect to fasting glucose metabolism in the fasting state. In contrast, OGIS, which reflects both fasting and postprandial glucose metabolism, was not adversely affected by CLA supplementation. The potentially negative effect of CLA on fasting glucose metabolism may be due to the recently reported pro-diabetic effect associated with *trans*-10, *cis*-12 CLA. CLA supplementation had positive effects on lipoprotein metabolism, including HDL-cholesterol and LDL:HDL-cholesterol, and fibrinogen concentrations. Further studies are required to investigate the effect of pure sources of the *cis*-9, *trans*-11 CLA isomer.

Noone E, Roche HM, Nugent AP & Gibney MJ (2002) *British Journal of Nutrition* **88**, 243–251.
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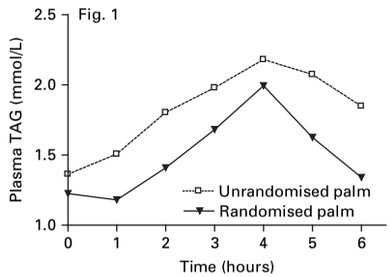
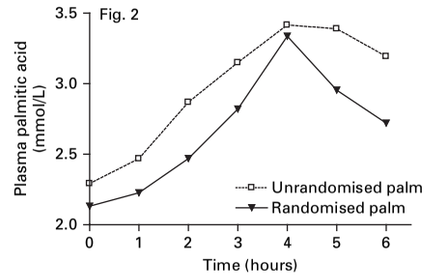
Influence of triacylglycerol structure on the postprandial lipaemic response to a symmetrical palmitic acid-rich fat and an asymmetrical palmitic acid-rich fat. By S.E.E. BERRY and T.A.B. SANDERS, *Nutrition, Food and Health Research Centre, King's College London, 150 Stamford Street, London, UK, SE1 9NN*

Palm oil consists mainly of the symmetrical triacylglycerol (TAG) 1-palmityl, 2-oleyl, 3-palmityl glycerol (POP). The digestibility of saturated fatty acids in position *sn*-2 of TAG is believed to be greater than when in *sn*-1 and *sn*-3. However, we have shown that cocoa butter, a fat naturally rich in symmetrical TAG; 1-stearyl, 2-oleyl, 3-palmityl glycerol (SOP) or POP, decreases postprandial lipaemia following randomisation (31 mol% palmitic acid (16:0) in the *sn*-2 position) (Sanders *et al.* 2003). Yli-Jokipii *et al.* (2001) reported that randomised palm oil produced a reduced postprandial lipaemia compared with unrandomised palm oil.

The present study was designed to confirm this observation using our test meal model and to ascertain whether any such differences could be attributed to the physical characteristics of the TAG. The effects on postprandial lipaemia of test meals containing 50 g fat (either randomised or unrandomised palm oil) were compared in twenty healthy male subjects (age 20–50 years) using a randomised cross-over design. Plasma TAG, chylomicron TAG and plasma fatty acid concentrations were determined over a 6 h period. Data were log-normalised before statistical analysis by repeated measures ANOVA. The results are shown in Figs. 1 and 2.





The pattern of response of plasma TAG (Fig. 1) following the two meals differed (meal×time interaction $P=0.002$). The incremental area under the TAG curve was 43% greater following the unrandomised *v.* the randomised palm oil. A similar postprandial pattern for plasma 16:0 concentration was observed (meal×time interaction $P<0.001$) (Fig. 2). Chylomicron TAG following the randomised palm oil was enriched with 16:0 in the *sn*-2 position (44 *v.* 24 mol% for unrandomised palm oil; $P<0.001$); the proportions of 16:0 in the *sn*-2 position of the dietary fats were 38 *v.* 7 mol% respectively.

The lower postprandial increase in TAG following the randomised palm oil may be due to differences in the proportion of solid fats (the solid fat content measured by NMR at 37 °C was 15.2 *v.* 3.6% for unrandomised oil). The present study suggests that the pattern of absorption differs between symmetric and asymmetric palmitic acid-rich TAG.

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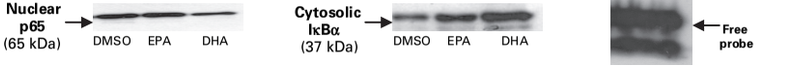
Eicosapentaenoic acid and docosahexaenoic acid decrease the production of pro-inflammatory cytokines by human THP-1 macrophages. By S.M. WELDON, L. HURLEY, C. LOSCHER, M.J. GIBNEY and H.M. ROCHE, *Nutrigenomics, Institute of Molecular Medicine, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Republic of Ireland*

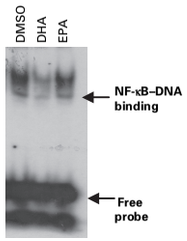
A number of studies have investigated the effects of *n*-3 PUFA on the production of pro-inflammatory cytokines (Calder, 2002). The majority of human studies have employed *ex vivo* peripheral blood mononuclear cell models and have used various concentrations of fish oils as a source of long-chain (LC) *n*-3 PUFA. Fish oils contain a heterogeneous blend of the LC *n*-3 PUFA EPA and docosahexaenoic acid (DHA). Consequently, findings to date do not permit examination of the differential effects of EPA and DHA.

To investigate the relative potencies of EPA and DHA on macrophage pro-inflammatory cytokine production, we treated THP-1 monocyte-derived macrophages with 100 μM-EPA or 100 μM-DHA for 48 h. Cells were stimulated with lipopolysaccharide (LPS; 1 μg/ml). TNF-α, IL-1β and IL-6 production by THP-1 macrophages were quantified by ELISA. To determine if the effects of EPA and DHA on cytokine production were mediated transcriptionally, the effects of EPA and DHA on cytokine mRNA expression were analysed by TaqMan® real-time PCR. To examine whether the NF-κB pathway was implicated in the anti-inflammatory effects of EPA and DHA in this cell model, the binding of NF-κB to κB enhancer elements on DNA was measured by the electrophoretic mobility shift assay. In addition, the effects of EPA and DHA on components of the NF-κB signalling cascade (p65 and IκBα) were assessed by Western blotting. The Table shows the data for five to six independent experiments.

	Concentration in medium (pg/ml)						mRNA					
	TNF-α		IL-1β		IL-6		TNF-α		IL-1β		IL-6	
Treatment	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
DMSO	8000[a]	527	273[a]	20	318[a]	59	1[a]	0	1[a]	0	[a]	0
EPA	6161[b]	243	193[b]	16	181[b]	28	0.6[b]	0.05	0.9[a]	0.2	0.3[b]	0.1
DHA	5485[b]	405	106[c]	9	54[c]	5	0.3[c]	0.02	0.4[b]	0.09	0.05[c]	0.02

[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P \leq 0.05$; ANOVA).





Compared with vehicle control-treated cells, both EPA and DHA significantly decreased the production of TNF-α, IL-1β and IL-6 by THP-1 macrophages stimulated with LPS. In all cases, the effect of DHA was significantly more potent than that of EPA. EPA and DHA reduced the mRNA levels of TNF-α, IL-1β and IL-6. Again, for all cytokines the effects of DHA were significantly greater than those observed with EPA. Both EPA and DHA down regulated NF-κB–DNA binding in LPS-stimulated THP-1 macrophages by approximately 13% ($P \leq 0.03$). This reduction in NF-κB–DNA binding by DHA was associated with a significant decrease in macrophage nuclear p65 expression ($P \leq 0.05$) and a significant increase in cytoplasmic IκBα expression ($P \leq 0.05$). Similar trends were observed with EPA; however, these effects did not reach statistical significance. Further work is required to elucidate the divergent mechanisms of action of EPA and DHA in this cell model.

Calder PC (2002) *Proceedings of the Nutrition Society* **61**, 345–358.

The role of the mitochondria in apoptosis induced by cholesterol 5β,6β-epoxide and 7β-hydroxycholesterol. By L. RYAN, Y.C. O'CALLAGHAN and N.M. O'BRIEN, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Oxysterols are oxygenated derivatives of cholesterol that may be produced endogenously or absorbed from the diet and are commonly found in processed foods of animal origin. Some oxysterols have previously been shown to induce apoptosis in certain cell lines. To date, most studies have focused on 7β-hydroxycholesterol (7β-OH), a potent inducer of cell death. The proposed mechanism of 7β-OH-induced apoptosis involves the generation of an oxidative stress, perturbation of the mitochondria, release of cytochrome *c* and activation of the executioner of apoptosis, caspase-3. Little is known about the mechanism of cell death induced by other oxysterols such as cholesterol 5β,6β-epoxide (β-epoxide). The objective of the present study was to examine the role of the mitochondria in 7β-OH- and β-epoxide-induced apoptosis in U937 cells, a human monocytic blood cell line. We examined the effects of a range of inhibitors of the mitochondrial permeability transition pore on 7β-OH- and β-epoxide-induced apoptosis.

U937 cells were adjusted to a density of 2×10^5 cells/ml in RPMI medium supplemented with fetal bovine serum (25 ml/l). Cells were treated with 30 μM-7β-OH or 30 μM-β-epoxide in the presence or absence of bongkrekic acid (BA), a potent inhibitor of the mitochondrial megachannel, cyclosporin A (CsA), a specific inhibitor of the mitochondrial permeability transition, carboxyatractyloside (CATR), a highly selective inhibitor of the mitochondrial ADP–ATP carrier, or ADP, an inhibitor of the opening of the permeability transition pore and cytochrome *c* release from the mitochondria. Viability was assessed by the fluorescein diacetate–ethidium bromide assay and apoptotic nuclei were quantified following staining with Hoechst 33342. The Table shows the data for three independent experiments.

	7β-Hydroxycholesterol									
	Positive control		BA		CsA		CATR		ADP	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viable cells (% control)	63	6	93*	2	93*	1	68	3	90*	1
Apoptotic cells (% increase)	27	4	9*	1	7*	1	23	2	8*	2

	β-Epoxide									
	Positive control		BA		CsA		CATR		ADP	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viable cells (% control)	56	4	58	5	59	3	57	1	67	1
Apoptotic cells (% increase)	28	1	30	3	29	1	23	2	22	2

* Mean value was significantly different from control ($P<0.05$).

BA, ADP and CsA protected against the decrease in cell viability and the increase in apoptotic nuclei induced by 7β-OH but had no observed effect on the cells exposed to β-epoxide. CATR did not significantly protect against either of the oxysterols employed for the present study. Results suggest that the pathway of 7β-OH-induced apoptosis involves opening of the mitochondrial megachannel and the permeability transition pore. The mitochondria do not appear to have a role in the pathway of β-epoxide-induced apoptosis.

The effect of antioxidants and inhibitors of apoptosis on cholesterol 5β,6β-epoxide-induced cell death in U937 cells. By L. RYAN, Y.C. O'CALLAGHAN and N.M. O'BRIEN, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Cholesterol oxidation products, known as oxysterols, are commonly found in processed foods of animal origin. Oxysterols have been shown to induce apoptosis in a range of cell lines and have been implicated in the process of plaque development in atherosclerosis. To date, much work has concentrated on oxysterols oxidised at C7, in particular, 7β-hydroxycholesterol and 7-ketocholesterol, both potent inducers of cell death. Little is known about the mechanism of cell death induced by other oxysterols such as cholesterol 5β,6β-epoxide (β-epoxide). The objective of the present study was to investigate the apoptotic pathway induced by β-epoxide in U937 cells, a human monocytic blood cell line. We examined the effects of a range of inhibitors of apoptosis and of different antioxidants on cells incubated with β-epoxide.

U937 cells were adjusted to a density of 2×10^5 cells/ml in RPMI 1640 medium supplemented with fetal bovine serum (25 ml/l). Cells were treated with 30 μM-β-epoxide in the presence or absence of a range of inhibitors of apoptosis; broad-spectrum caspase inhibitor (Broad-spec; 25 μM), specific caspase-3 inhibitor (Cas-3; 25 μM), inhibitor of cytochrome *c* release (Cyt *c*; 100 nM) or a variety of antioxidants; trolox (10 μM), ebselen (2 μM) or resveratrol (10 μM). Viability was assessed by the fluorescein diacetate–ethidium bromide assay and apoptotic nuclei were quantified following staining with Hoechst 33342. The Table shows the data for three independent experiments.

	Positive control		Broad-spec		Cas-3		Cyt *c*		Trolox		Ebselen		Resveratrol	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viable cells (% control)	68	5	88*	2	79	4	68	1	73	2	69	11	68	4
Apoptotic cells (% increase)	13	2	4*	1	8	3	13	1	20	6	14	2	17	2

*Mean value was significantly different from control ($P<0.05$).

The broad-spectrum caspase inhibitor significantly ($P<0.05$) protected U937 cells against β-epoxide-induced apoptosis. Caspase-3 inhibition slightly protected against the decrease in viability and the increase in apoptotic nuclei induced by β-epoxide. The inhibitor of cytochrome *c* release did not prevent apoptosis in cells treated with β-epoxide. U937 cells were incubated with a variety of antioxidants: trolox, ebselen and resveratrol. None of the antioxidants had a protective effect in β-epoxide-treated cells. Oxysterols have previously been shown to induce apoptosis through generation of an oxidative stress, release of cytochrome *c* from the mitochondria into the cytosol, activation of caspase-3 and subsequent degradation of the poly ADP-ribose polymerase. We present evidence that β-epoxide induces apoptosis in U937 cells through a mechanism that is independent of cytochrome *c* release and that may not be prevented by a range of antioxidants.

Associations between cardiovascular risk factors, anthropometric characteristics and gene polymorphism distribution in postmenopausal women: data from a pan-European study (ISOHEART). By K. VAFEIADOU, W.L. HALL, A.M. MINIHANE, C.M. WILLIAMS and the ISOHEART consortium, *Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading, Reading, UK, RG6 6AP*

Many studies in men have demonstrated associations between anthropometric characteristics (BMI, waist:hip ratio (WHR) and circulating markers of cardiovascular risk (total and LDL-cholesterol, triacylglycerol (TAG) and HDL). However there are limited data for postmenopausal women who represent a high cardiovascular risk group. Furthermore, although genetic background can influence an individual's susceptibility to CVD, it is not yet clear whether polymorphisms in the oestrogen receptor (ERα and ERβ) genes influence cardiovascular risk. The investigation of cardiovascular risk factors in relation to ERα and ERβ polymorphism in women would appear to be of particular importance, given the increased risk of CVD that accompanies the menopause. The aim of the present study was to determine relationships between cardiovascular risk markers and anthropometric characteristics, as well as relevant gene polymorphisms, in postmenopausal women.

The data were derived from the baseline measurements of postmenopausal women (*n* 117) who were recruited in four European countries (Germany, Denmark, UK and Italy) to participate in an intervention study which aimed to investigate the effects of isoflavone supplementation on circulating biomarkers of CVD risk (ISOHEART Study). These results will be reported elsewhere (Hall *et al.* 2004). CVD risk measurements taken at baseline included total cholesterol, LDL-cholesterol, HDL-cholesterol, TAG, %LDL3, lipoprotein(a), glucose, blood pressure, BMI, waist and hip circumferences and markers of endothelial function including von Willebrand Factor (vWF), cell adhesion molecules VCAM-1 and ICAM-1 and e-selectin. Polymorphisms in ApoE, cholesteryl ester transfer protein (CETP) and ERα (XbaI and PvuII) were determined and allelic distributions evaluated by country and for the group as a whole.

No significant differences were found between the country groups for age, diastolic blood pressure and BMI. However, the Italian women showed significantly lower systolic blood pressure, total cholesterol, HDL-cholesterol, LDL-cholesterol and TAG ($P<0.05$) whilst the Danish women showed significantly lower fasting glucose ($P<0.05$) but significantly greater WHR ($P<0.05$) compared with the other countries. Differences in the frequencies of the ApoE genotype were observed between northern and southern countries with allelic distributions of the ApoE4 allele of 0.089, 0.130, 0.139 and 0.250 observed in Italy, UK, Germany and Denmark respectively. Genotype distribution of CETP and ERα genes were in accordance with what has been previously reported. ERα polymorphisms were associated with TC, TAG and vWF levels ($P<0.05$). CETP polymorphisms were associated with HDL levels ($P<0.05$). No association was found between ApoE polymorphisms and markers of CVD. Correlation analysis demonstrated positive associations between measures of adiposity, such as BMI and WHR, and lipid levels ($P<0.05$). Strong positive correlations were found between %LDL3 and inflammatory markers ($P<0.05$).

Between-country differences in cardiovascular risk markers, as well as allelic distributions of apo E variants, were generally consistent with the observed differences in risks of CVD between northern and southern Europe. Associations between anthropometric measures and CVD risk markers were similar to those reported in men and younger women. ERα and CETP polymorphisms were associated with CVD risk markers. Future analyses will include investigation of relationships with polymorphisms in ERβ.

This work was funded by the European Union (Framework 5) Isoheart QLK1-CT-2001-00221.

Hall WL, Vafeiadou K, Williams CM and the ISOHEART consortium (2004) *Proceedings of the Nutrition Society* **63**, (In the Press).

Increased wholegrain food consumption and indicators of adiposity: preliminary results from the CHEW-IT study. By A.R. JONES[1], M.L. HEPPLES[1], J. COULSON[1], S. KUZNESOF[1], D.P. RICHARDSON[2] and C.J. SEAL[1], [1]*Human Nutrition Research Centre, School of Agriculture, Food and Rural Development, University of Newcastle, Newcastle upon Tyne, UK, NE1 7RU and* [2]*DPR Nutrition Limited, 34 Grimwade Avenue, Croydon, Surrey, UK, CR0 5DG*

Wholegrain foods (WGF) offer an abundance of nutrients and phytoprotective substances which are in short supply in the Western diet (Richardson, 2000). An increasing amount of evidence indicates that a diet rich in WGF is associated with a reduced risk of several chronic diseases including heart disease, certain cancers and type 2 diabetes (Smith *et al.* 2003). Currently, US dietary guidelines advise the consumption of at least three servings WGF/d. Recent research has also raised awareness of the role whole grains may play in the treatment and prevention of obesity due to the effects whole grain components may have on hormonal factors, satiety and satiation (Koh-Banerjee & Rimm, 2003). The present study examines the effect of increased WGF consumption on body weight and indicators of adiposity.

Ten females (mean age 35.5 years) habitually consuming less than three servings WGF/d were recruited into a 16-week intervention study. Subjects were asked to consume three servings WGF/d for the first 8 weeks and six servings WGF/d for the final 8 weeks. Subjects were provided with prescribed quantities of WGF in order to aid compliance (quantities derived from analysis by Jones *et al.* 2003). Food intake was assessed at baseline, at 8 weeks and at 16 weeks using a 4 d food diary. Body weight, BMI, waist and hip circumference and percentage body fat (by bioelectrical impedance) were measured at 4-weekly intervals.

Results are reported here for the first 8 weeks of the intervention and show a significant increase in total WGF consumption, total whole grain intake and number of servings of WGF consumed. For all anthropometric measures including body weight and indicators of adiposity, there were no changes observed between baseline and 8 weeks of the intervention.

	Baseline (*n* 10)		4 Weeks (*n* 10)		8 Weeks (*n* 10)		
	Mean	SEM	Mean	SEM	Mean	SEM	*P*
Total WGF intake (g/4 d)	82.1	29.58			484.1	41.56	<0.001*
Total whole grain intake (g/4 d)	52.3	18.81			351.6	30.59	<0.001*
Total servings of WGF consumed (servings/4 d)	2.2	0.82			15.3	1.33	<0.001*
Weight (kg)	61.5	3.26	61.1	3.34	61.1	3.39	NS†
BMI (kg/m^2)	23.0	1.41	22.9	1.43	22.9	1.45	NS†
Waist circumference (mm)	788	34.2	755	33.0	745	35.3	NS†
Hip circumference (mm)	1016	29.2	997	26.3	996	29.8	NS†
Waist:hip ratio	0.8	0.12	0.8	0.16	0.8	0.17	NS†
Percentage body fat	28.6	2.69	29.1	2.82	30.4‡	3.01	NS†

* *P* values determined using paired Student's *t* test.
† *P* values determined using one-way ANOVA.
‡ *n* 9.

Preliminary findings from the present study indicate that the US recommendation to consume three servings WGF/d can be readily achieved. Consumers should be able to respond to positive health promotion relating to WGF and we are advocating a 'Three are Key' health message. Whether this can be exceeded in the second part of the study is currently being evaluated. At this stage in the present study, increased WGF consumption did not affect body weight or indicators of adiposity, but further information from a larger population will offer a greater insight into the relationship between WGF consumption and indices of obesity.

A.R.J. is in receipt of a BBSRC CASE studentship with Cereal Partners, UK.

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A within-laboratory-based evaluation of the self-testing BOD POD. By L. DOYLE[1], J. GANDY[1] and H.D. McCARTHY[2], [1]*Research Centre for Health Studies, Buckinghamshire Chilterns University College, Gorelands Lane, Chalfont St Giles, Buckinghamshire, UK, HP8 4AD and* [2]*Institute of Health Research & Policy, London Metropolitan University, Holloway Road, London, UK, N7 8DB*

Air displacement plethysmography has recently been developed into a viable commercially available technology for measuring body composition, known as the BOD POD measurement system (Dempster & Aitkens, 1995). Within the last year, the self-testing (S/T) version of the BOD POD, designed specifically for the health and fitness club environment, has become available. The S/T system has the added features of being easier, quicker, cheaper, self-calibrating, and capable of self-testing, and therefore does not require an operator. To date its performance under laboratory conditions has not been evaluated. The aim of the present study was to determine variability in volume measurements of inanimate objects and to make a within-laboratory evaluation of its performance to measure percentage body fat (%BF).

Volume measurements were performed by repeat testing of known volume water canisters (30 litres and 60 litres). Five consecutive tests were conducted each day for 10 d. %BF was determined in five adult subjects (mean age 37.6 (SD 17.0) years and mean BMI 28.2 (SD 5.8) kg/m^2). Subjects followed the recommended procedure and refrained from eating, drinking and exercise during the 1 h before testing. Subjects also voided the bladder before measurement. Each subject underwent three test procedures on nine separate days.

The maximum deviation from the measured volume across the 10 d was 26 ml for the 30 litre canister and 37 ml for the 60 litre canister. Repeated measurements of the 30 litre and 60 litre canisters over the 10 d resulted in a mean volume of 29.670 (range 29.610–29.746) litres and 59.393 (range 59.298–59.581) litres and corresponding CV of 0.177% and 0.158%, respectively. Mean %BF measurements ranged from 13.0 to 40.6%, resulting in an overall mean of 31.9%. Mean within-subject CV for %BF ranged from 1.7% to 8.6% corresponding to a mean CV of 3.4%.

The results of the canister study indicate good reproducibility by the BOD POD S/T with a CV less than 1%. The CV for %BF are comparable with previously reported values using the research BOD POD (Collins *et al.* 2003). However this evaluation was undertaken by trained, knowledgeable operators utilising strict testing protocols in a laboratory setting, a setting for which this model is not primarily intended. It is not known what level of variability might be observed in a non-laboratory setting. The BOD POD S/T offers a method for quick, multiple within- and between-day testing, which could determine usual values in large numbers of subjects. The BOD POD S/T is a reliable technique that, like the original BOD POD, can safely evaluate body composition. Further inter-laboratory comparative studies are needed using both the original research model and the S/T model.

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Programming of appetite and adiposity in male rats is dependent on timing of prenatal nutritional insult. By L. BELLINGER and S.C. LANGLEY-EVANS, *Centre for Reproduction and Early Life, School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, UK, LE12 5RD*

Studies of animal models have shown that maternal undernutrition has a significant influence on the long-term health of the developing fetus. Restricted or excessive intakes of a range of macro- and micronutrients in pregnancy programme the risk of major diseases of adult life (Langley-Evans, 2001). However, the majority of this research has tended to maintain the nutritional insult throughout the entire pregnancy. Considering the fact that there are critical time points for fetal development throughout pregnancy, it would seem more likely that the effects of prenatal exposure to nutritional insults depend on timing. We have previously shown that rats exposed to a low-protein (LP) diet *in utero* throughout the entire pregnancy exhibited an increased preference for high-fat (HF) food when allocated to a self-selection diet (Bellinger *et al.* 2004). The aim of the present trial was to determine the critical time points for the programming of appetite and food preferences.

Pregnant, Wistar rats were fed either a control diet (180 g casein/kg diet; *n* 5) or a LP diet (90 g casein/kg diet) during either early pregnancy (LPE, days 0–7; *n* 5), mid-pregnancy (LPM, days 7–14; *n* 5), or late pregnancy (LPL, day 14–term; *n* 5). Half of the resulting male offspring from all litters were allocated to a self-selection diet protocol to assess appetite and food preferences at 12 weeks of age. All rats were individually caged and had free access to three different food types: HF (40 g fat/100 g diet), high carbohydrate (HC; 62 g carbohydrate/100 g diet); high protein (HP; 48.5 g protein/100 g diet).

At 12 weeks there were significant differences in feeding behaviour between the groups. Unlike animals exposed to a LP diet throughout gestation (Bellinger *et al.* 2004), single-week exposures appeared to promote reduced intakes of HF food (see Table). At cull gonadal, perirenal and intrascapular adipose tissue depots were dissected and weighed. Variation in fat deposition at these sites was noted with control animals exhibiting greatest adiposity and LPM animals the least (NS). Variation in adiposity was associated with marked differences in feed efficiencies between the groups.

		Food intake (g/kg body weight per d)					
		HF		HC		HP	
Maternal diet	*n*	Mean	SE	Mean	SE	Mean	SE
Control	5	48.28	4.58	19.31	2.53	4.47	2.98
LPE	5	41.51	2.08	19.72	2.48	6.15	1.58
LPM	5	51.86	5.78	20.52	5.68	2.34	0.97
LPL	5	40.42	4.95	23.53	3.40	6.12	3.72

Intra-uterine influences upon feeding and the deposition of adipose tissue may well be mediated at the level of the hypothalamus. DNA microarray studies comparing expression of 10 000 genes in hypothalamus samples from control and LPE animals indicated an altered expression of genes encoding important olfactory and taste receptors. These may, in part, determine the differences in food preferences between these animals. The present study has shown that the timing of nutritional insults in fetal life is important in determining the nature of the interaction between the genome and the environment and hence the risk of obesity.

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Prenatal exposure to a low-protein diet and programming of fat metabolism. By L. BELLINGER and S.C. LANGLEY-EVANS, *Centre for Reproduction and Early Life, School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, UK, LE12 5RD*

A wide variety of animal studies of nutritional programming has been used to determine the relationship between nutritional insults to the fetus and disease in adult life. Studies of animal models of nutritional programming have demonstrated that prenatal dietary restrictions lead to an increase in adiposity and adult conditions that are associated with obesity, such as hypertension, glucose intolerance and insulin resistance (Anguita *et al.* 1993; Vickers *et al.* 2000). We have previously shown that rats exposed to a low-protein (LP) diet *in utero* throughout the entire pregnancy exhibit an increased preference for high-fat food when allocated to a self-selection diet (Bellinger *et al.* 2004). The present study aimed to assess feeding behaviour and body composition of male and female rats subjected to protein restriction *in utero* and then fed a high-fat diet in adult life. Pregnant, Wistar rats were fed either a control diet (180 g casein/kg diet; *n* 5), or an LP diet (90 g casein/kg diet; *n* 5) throughout the entire pregnancy, as previously described (Langley-Evans *et al.* 1994). All rats were individually caged and had free access to high-fat food (40 g fat (provided as lard)/100 g diet), continuously, for 4 weeks.

				Intrascapular fat (% body weight)				Total cholesterol (mg/l)	
				BAT		WAT			
Sex	Maternal diet	Postnatal diet	*n*	Mean	SE	Mean	SE	Mean	SE
Male	Control	Chow	7	0.17	0.02	0.19	0.02	726	71.1
Male	Control	High-fat	7	0.21	0.02	0.25	0.04	1019†	69.8
Male	LP	Chow	7	0.13	0.01	0.21	0.02	822	45.9
Male	LP	High-fat	7	0.18	0.02	0.35†*	0.03	1898†*	189.3
Female	Control	Chow	6	0.20	0.02	0.20	0.03	951	79.0
Female	Control	High-fat	7	0.28†	0.03	0.35†	0.03	928	149.3
Female	LP	Chow	9	0.18	0.01	0.20	0.02	1171	149.3
Female	LP	High-fat	9	0.21	0.02	0.44†	0.04	1013	152.2

* Mean value was significantly different for LP *v.* control of the same sex and postnatal diet group ($P<0.05$).
† Mean value was significantly different for chow *v.* high-fat of the same sex and maternal-diet group ($P<0.05$).

Studies of 12-week-old offspring indicated that overall weight gain was only significantly influenced by postnatal diet and sex ($P<0.001$). No significant difference between maternal dietary groups was noted for the total amount of food consumed over the 4 weeks, in contrast to previous trials where LP-exposed offspring had shown a preference for high-fat food (Bellinger *et al.* 2004). Feeding a high-fat diet to animals from all groups increased fat deposition at the intrascapular depot. In control rats the increase reflected the deposition of brown adipose tissue (BAT) and white adipose tissue (WAT) in similar proportions. LP-exposed rats deposited more WAT and less BAT than control animals. Total plasma cholesterol was significantly different ($P<0.0025$), with male LP-exposed animals responding dramatically to the high-fat postnatal diet compared with the chow-fed animals. Blood glucose did not differ significantly between groups.

Prenatal undernutrition appears to determine the intrascapular white:brown fat ratio. BAT plays a role in thermogenesis and so this could have some implications for the programming of relative resistance or susceptibility to obesity.

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Associations between body mass index and nutrient intakes in early pregnancy. By E.J. DERBYSHIRE[1], G.J. DAVIES[1], V. COSTARELLI[1] and P.W. DETTMAR[2], [1]*Academy of Sports, Physical Activity and Wellbeing, London South Bank University, London, UK, SE1 0AA and* [2]*Reckitt Benckiser Healthcare Ltd, Dansom Lane, Hull, UK, HU8 7DS*

Adequate maternal nutrition is of paramount importance in pregnancy, particularly in the first trimester where fetal development is crucial (Anderson, 2003). Research conducted by Fairburn & Welch (1990) has suggested that 40% of UK primigravidae fear weight gain in pregnancy and 72% fear they will be unable to return to their pre-pregnancy weight.

The aim of the present study was to investigate possible correlations between pre-pregnancy BMI and nutrient intakes in the first trimester. Sixty-nine Caucasian, primiparous non-smokers of mean age 33.4 (SD 4.6) years completed a background information questionnaire and a 4 to 7 d weighed inventory food diary during the first trimester of pregnancy. The mean gestation period was 12.7 weeks and pre-pregnancy BMI was 23.7 (range 17.4–32.0) kg/m^2. Subjects were recruited from three London teaching hospitals and all gave ethical approval. The main findings are summarised in the Table.

	Mean	SD	Range	Pearson correlation (*r*)	Significance(*P*)
BMI (kg/m^2)	23.7	3.74	17.5–32.0	–	–
Energy (kJ)	7895	1912	2828–14104	−0.245	
Energy (kcal)	1887	457	676–3371	−0.245	0.042
Southgate fibre (g)	20.8	6.83	5.90–47.9	−0.284	0.018
Englyst fibre (g)	14.5	5.01	4.10–27.2	−0.289	0.016
Folate (μg)	267	89.7	70.0–590	−0.339	0.004
Vitamin E (mg)	6.45	3.10	1.07–24.0	−0.253	0.036
Fe (mg)	12.8	3.71	2.92–33.2	−0.258	0.032

The data suggest that pre-pregnancy BMI is negatively correlated with the intake of energy, fibre, vitamin E, Fe and folate in the first trimester of pregnancy. The correlation is particularly strong in the case of folate ($P<0.01$). Low intakes of folic acid in early pregnancy have been associated with an increased risk of neural tube defects (Finglas *et al.* 2003). The most plausible explanation could be that women with a higher pre-pregnancy BMI may be restraining their food intake to prevent additional weight gain during pregnancy. Alternatively, it is possible that women with a higher pre-pregnancy BMI were more likely to under-report their nutrient intakes (Macdiarmid & Blundell, 1998). More studies are needed to confirm the original hypothesis.

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Why don't women breast-feed for longer? By K. BERRIDGE and J.C. ABAYOMI, *Liverpool John Moores University, School of the Outdoors, Leisure, and Food, IM Marsh Campus, Barkhill Road, Liverpool, UK, L17 6BD*

The benefits of breast-feeding are well documented, and encompass improved health outcomes for both baby and mother, including less risk of the infant becoming obese in later life (von Kries *et al.* 1999). Despite the numerous health benefits, the incidence and duration of breast-feeding in the UK is low; 69% of mothers initiated breast-feeding, but this dropped to 42% by 6 weeks postpartum, and only 21% by 6 months (Hamlyn *et al.* 2002). The lowest rates are seen amongst mothers who fall into the lowest socio-economic groups, and the impact of this on future health is probably substantial.

Many studies have tried to explain why breast-feeding rates are low; typical reasons given by women include perceived insufficient milk, pain, lack of support and embarrassment (Hamlyn *et al.* 2002). The present paper presents some findings from a study carried out in Liverpool, UK which aimed to look at reasons for cessation of breast-feeding.

A total of 149 women were recruited from Liverpool Women's Hospital. A structured informal interview was carried out when the infant was between 12 and 16 weeks of age, and the mothers completed a questionnaire about infant feeding practices. For the purpose of the present study, exclusively breast-fed (EBF) was defined as never having been given any formula, partially breast-fed (PBF) as being given some breast-feeds and some formula feeds, and formula-fed (FF) as never having been breast-fed.

The mean age of mothers was 31 years, and the mean age of babies was 13 weeks. The mothers were predominantly white (95%), and well educated; 38% were educated to degree level, whilst only 8% had left school with no qualifications. Thirty-seven mothers (25%) EBF, forty-eight (32%) FF and sixty-four (43%) PBF. EBF women were significantly more likely to be a non-smoker ($P=0.00$), educated to degree level ($P=0.00$), married or living with a partner ($P=0.010$), to have been breast-fed as a baby ($P=0.012$), belong to a higher social class ($P=0.00$), older ($P=0.011$), and to have only one child ($P=0.038$).

Reasons given by the formula-feeding mothers for not breast-feeding included having a big baby, could involve father or others in feeding routine, embarrassing, lack of privacy in home, previous difficulties with breast-feeding, formula is as good as breast milk, just didn't want to breast-feed, difficult to breast feed with demands of other children, in pain after the birth, not enough milk, not having to feed continuously, not as tiring, baby wouldn't latch on, working mother and more freedom. Of the FF women, 69% gave more than one reason for not wanting to breastfeed. Of the FF women, 15% had decided before the baby was born to breast-feed, but following the birth found that they were either too exhausted or in pain following the delivery or their baby wouldn't latch on or they had no milk.

Of the PBF mothers, forty-three (67%) had stopped breast-feeding altogether, half of which were first-time mothers. Reasons given by PBF mothers for stopping breast-feeding included returning to work, not convenient with demands of other children, baby very demanding, slow weight gain, too time consuming, too painful and difficult, too tired or baby wouldn't latch on properly.

Many of the reasons given for either not breast-feeding or ceasing prematurely could have been overcome with the right support and encouragement. Women need to be given consistent accurate advice in order to regain confidence in their body's ability to breast-feed successfully.

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Supporting women to breast-feed for longer at an infant feeding clinic in Liverpool. By K. BERRIDGE[1], K. McFADDEN[2], J.C. ABAYOMI[1] and J. TOPPING[2], [1]*Liverpool John Moores University, School of the Outdoors, Leisure, and Food, IM Marsh Campus, Barkhill Road, Liverpool, UK, L17 6BD and* [2]*Liverpool Women's Hospital, Crown Street, Liverpool, UK, L8 7SS*

It is now widely recognised that breast-feeding confers many health benefits to both mother and baby and recommendations in most European countries (Kaftos & Coddrington, 2001) advise exclusive breast-feeding for the first 4 to 6 months after birth. However, breast-feeding rates in the UK are amongst the lowest in Europe, and improving breast-feeding rates in the UK remains a challenge. With only a modest improvement in the initial incidence of breast-feeding, 66% in 1990 compared with 69% in 2000 (Hamlyn *et al.* 2002), supporting and encouraging breast-feeding is a priority. Women cite numerous reasons for stopping breast-feeding, with insufficient milk, painful nipples and breasts, and refusal of the baby to suck or latch on to the breast being the most common (Hamlyn *et al.* 2002). Support from others is also a factor affecting breast-feeding success. The aim of this study was to investigate reasons for attendance at an infant feeding drop-in clinic at Liverpool Women's Hospital. A clinic is provided once a week to support women who are having problems or need advice with infant feeding (breast-feeding, bottle-feeding and weaning). A telephone help line is also provided.

A short questionnaire was devised to identify reasons for attendance at the clinic, and women were approached to complete this once they had been seen by one of the infant-feeding team. Many of the women took this opportunity to talk about their experiences with feeding, or life with a new baby in general.

During the study period (21 weeks), 198 women attended the drop-in clinic (mean of nine women/week; range of two to sixteen women/week). Of the women, 108 visited the clinic only once, while ninety (46%) returned more than once; women were only asked to complete a questionnaire the first time they attended. In total, eighty women completed questionnaires. The average age of the babies was 34 d (ranging from 4 d to 5 months). Of the women, 98% were breast-feeding (either partially or fully), and 2% were formula-feeding.

Of the eighty women, fifteen (19%) cited reasons for attendance relating just to themselves as mother. These reasons included cracked or sore nipples, mastitis or breast abscess, not producing enough milk, advice about expressing milk, advice about own diet, or other. Fourteen (18%) cited reasons just relating to their baby (i.e. not latching on properly, not taking enough milk, advice about making up bottles, advice about what formula to use, advice about weaning or other), and fifty-one (64%) cited reasons relating to both themselves and the baby. The most common reasons for attendance were baby not latching on properly (46% of all mothers attending), advice about expressing milk (33% of all mothers) and cracked or sore nipples (33% of all mothers).

A common theme that many of the women verbalised was the benefit of being able to meet other mothers who were either having similar problems or had overcome problems with feeding. This seemed to counter the feelings of isolation that many of the breast-feeding women felt. Being supported and able to talk to somebody who would understand what they were going through was also important to this group of women. Inconsistent advice, or lack of knowledge by health professionals, was highlighted by several women as contributing to their breast-feeding difficulties.

As demonstrated by the findings of the present study, important factors for the duration of breast-feeding will be found in the support systems that exist and their accessibility to women. Although breast-feeding support has progressed in recent years, there is still much to be achieved.

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Ethnic differences in parents' attitudes towards feeding their infants in Singapore. By W.M. HAN[1], Y.H. CHAN[2], C.B. TAN[3] and J.B. MORGAN[1], [1]*School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, UK, GU2 7XH,* [2]*Clinical Trials and Epidemiology Unit, 226 Outram Road, Block A, #04–02, Singapore 169039 and* [3]*Singhealth Polyclinics, 3 Second Hospital Avenue, #06–03, Singapore 168937*

There is growing recognition that diet during infancy has an important effect on later health (Barker, 1999). Available data also suggest possible ethnic differences in diet-related attributable risks though these are limited (Kumanyika, 1993). A recent national health survey in Singapore found adult obesity rates were highest among Malays and Indians compared with the Chinese, and that Indians have the highest rates of diabetes, followed by Malays and Chinese (Lee, 2000). A previous study in England in the 1990s (Morgan *et al.* 1995) showed that parents were misinterpreting adult healthy eating guidelines for their infants. We were interested to establish if this is the case in Singapore, as well as evaluate for any possible ethnic differences in parents' attitudes towards feeding their infants.

Parents were interviewed in Singapore Polyclinics between July 2003 and December 2003 on their attitudes to certain dietary guidelines for their infants who were aged between 4 and 15 months of age at the time when the questionnaire was administered. There was considerable misunderstanding of the healthy-eating guidelines for the infants, regardless of race and social background. Nearly one-quarter of the parents incorrectly reported that 'plenty of energy' was not important, while more were concerned with added sugar, salt and additives. Besides that, a large proportion of the three races reported, inappropriately, that a high fibre, low fat intake was important or very important (see Table).

Percentage of parents who responded 'important' or 'very important'	Total (*n* 245)	Chinese (*n* 148)	Malay (*n* 76)	Indian (*n* 21)	*P* value
Wide variety of foods	92.0	96.0	88.0	81.0	0.019*
Plenty to drink	99.0	99.0	99.0	100.0	NS
Low sugar intake	85.0	90.0	79.0	71.0	0.026*
Additive-free foods	81.0	86.0	74.0	71.0	NS
Low salt intake	93.0	95.0	92.0	81.0	0.026*
High fibre intake	95.5	95.0	97.0	95.0	NS
Low fat intake	72.0	72.5	71.0	75.0	NS
Plenty of energy	73.0	71.5	75.0	75.0	NS

Variables entered: income level, mother's education, mother's employment status and race groups.
* Significant differences were found between Chinese and Indian groups only.

There were notable differences between the Indian parents' attitudes towards feeding their children compared with the Chinese. The Chinese were 5.4 times more likely than the Indians to think that a wide variety of foods was important for their children (95% CI 1.33, 2.22). The Chinese also had 3.6 and 4.8 times more tendency than the Indians to report that they restricted their children to a low sugar intake and to control the salt content of their children's diet respectively (95% CI 1.17, 11.24; 95% CI 1.21, 19.23). The Malays had similar attitudes to the Chinese, with the only exception being that of sugar intake, where the Malays were more likely to be less restrictive with sugar intake compared with the Chinese (OR 0.4; 95% CI 0.18, 1.00).

These results suggest that parents are inappropriately placing a greater emphasis on the natural state of foods rather than their nutritional quality, reflecting the same situation as that in England in the 1990s (Morgan *et al.* 1995). This raises a concern about the provision of optimal nutrition for growth and for later health. Furthermore, the parents' attitudes towards healthy eating may be a reflection of their own diets. If this is so, it could help to explain the higher prevalence of weight-related diseases amongst the Indians and Malays relative to the Chinese.

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Effect of maternal cold exposure and nutrient restriction on the abundance of mitochondrial cytochrome c and voltage-dependent anion channel (VDAC) in the ovine kidney. By D.P. YAKUBU, S. PEARCE, A. MOSTYN, T. STEPHENSON and M.E. SYMONDS, *Centre for Reproduction and Early Life, Institute of Clinical Research, University Hospital, Nottingham, UK, NG7 2UH*

Kidney development can be programmed by reduced maternal nutrition *in utero* (for example, Whorwood *et al.* 2001), an effect that may be mediated in part by changes in mitochondrial protein abundance, which are involved in both cellular energy metabolism and solute exchange. Mitochondria achieve this function acting through specific proteins present in the mitochondria including cytochrome c and VDAC (for example, Mostyn *et al.* 2004). However, the combined effects of maternal cold exposure and nutrient restriction on mitochondrial protein in the developing kidney are yet to be established and the aim of the present study was to address that.

Thirty-three twin-bearing, multiparous Bluefaced Leicester cross Swaledale ewes of similar body weight and parity were entered into the study. All ewes were housed indoors from 68 d gestation and randomly allocated to four groups, with fifteen ewes being shorn at 70 d gestation. From 110 d gestation ewes were offered straw *ad libitum* and a fixed amount of concentrate (sufficient to fully meet their total metabolisable energy requirements) or were fed 50% of this amount. The groups were: shorn control (SC; *n* 7); shorn nutrient restricted (SNR; *n* 8); unshorn control (UC; *n* 9); unshorn nutrient restricted (UNR; *n* 9). After lambing, one randomly selected lamb was humanely euthanased and tissues were dissected and weighed. The remaining lamb remained with its mother until 30 d of postnatal life when it was similarly sampled. Mitochondrial fractions were prepared from kidney tissue and abundance of each protein was determined by immunoblotting. Results in arbitrary units (a.u.) are presented as means with their standard errors. Statistically significant effects ($P<0.05$) were assessed by Mann-Whitney U Test.

	VDAC (au)								Cytochrome c (au)							
	SNR		SC		UNR		UC		SNR		SC		UNR		UC	
Age	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S
1d	77	3	98*	2	91	2	78	2	43**	0.2	24	1	29*	0.4	24	0.5
30d	53	4	58	1	70	3	78	1	27	0.1	25	1	32*	2	39	0.2

M, mean; S, SEM. Mean values were significantly different from controls: * $p<0.05$, ** $p<0.01$.

Maternal cold exposure resulted in enhanced kidney weight (data not shown) and abundance of VDAC and Cytochrome c at 1 but not 30 day of postnatal life. Nutrient restriction had opposite effects on the abundance of cytochrome c in kidney mitochondria that was dependent on maternal cold exposure. Consequently nutrient restriction resulted in the up regulation of cytochrome c at 1 d of age but a reduction at 30 d in offspring of unshorn but not shorn ewes. In the latter group a reduction in cytochrome c was apparent at 1 but not 30 d of age. In conclusion, maternal cold exposure has a marked affect of mitochondrial protein abundance in the kidney after birth. This may reflect differences in kidney function at birth with respect to the large increase in metabolic energy requirements.

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The development of a caffeine assessment tool (CAT) for use in pregnancy. By S.M. BOYLAN[1], J.E. CADE[1], S.F.L. KIRK[1], D.C. GREENWOOD[2], K.L.M. WHITE[3], S. SHIRES[3], A.W.M. HAY[3], N.A.B. SIMPSON[4] and C.P. WILD[3], [1]*The Nutrition Epidemiology Group, The University of Leeds, 50 Clarendon Road, Leeds, UK, LS2 9PL,* [2]*Biostatistics Unit, University of Leeds, 24 Hyde Terrace, Leeds, UK, LS2 9LN,* [3]*The Molecular Epidemiology Unit, School of Medicine, University of Leeds, Leeds, UK, LS2 9JT and* [4]*Obstetrics and Gynaecology, D Floor, Clarendon Wing, Leeds General Infirmary, Leeds, UK, LS2 9NS*

There is no consensus on whether there is any link between caffeine consumption, caffeine metabolism and pregnancy outcome. Previous studies have shown mixed results, mainly due to methodological problems such as imprecise or biased measurement of caffeine intake (Committee on Toxicology of Chemicals in Foods, Consumer Products and the Environment, 2001). We present results of the development of a detailed caffeine assessment tool (CAT) for use prospectively in pregnancy to assess caffeine intake.

The aim was to pilot and validate the CAT in the antenatal clinic at the Leeds General Infirmary. A total of sixty-three pregnant women (aged 18+ years) were recruited; however, only twenty-four pregnant women completed the pilot study. Subjects who gave their written consent were given a background questionnaire, CAT, and 3 d food and drink diary. They were also given nine Salivettes® to take home for the collection of saliva samples over 2 consecutive days. The saliva samples were used to assess variation in salivary caffeine concentrations at different times during the day, along with day-to-day variation.

A computer algorithm was developed so that the daily caffeine intakes (mg) could be calculated from the CAT for each subject. The food and drink diaries were analysed manually for each subject's daily caffeine intake. Times of caffeine intake were recorded in the diaries to allow comparison with the salivary caffeine concentrations. The saliva samples were analysed for concentrations of caffeine and paraxanthine using a HPLC with UV detection. Intra-class correlation coefficients (ICC) measured agreement. A variance components model was used to investigate the variance structure of the data.

The average caffeine intake was approximately 113 mg/d from the diary, and 128 mg/d from the CAT. On average, the daily caffeine intakes from the CAT were 15 mg more than the diary, equivalent to one-fifth of a cup of instant coffee. The caffeine intakes from the CAT and the diary showed adequate agreement (ICC 0.5). The CAT agreed with the saliva concentrations just as well as the food and drink diaries (ICC 0.5). The salivary caffeine and paraxanthine concentrations shadowed each other closely. For both salivary caffeine and paraxanthine, the between-sample variation (50 and 61% of total variation, respectively) was even greater than between-women variation (39 and 38% of total variation, respectively) and between-day variation (11 and 0.1% of total variation, respectively).

The difference in the caffeine intakes between the CAT and diary could be due to the variation of food and drink consumption over 3 d. Women tended to provide brand information in the CAT, but not in the diary, despite being instructed to do so. Therefore, standard caffeine values were used in the analysis for most of the diaries. There was a greater between-sample variation than between-women variation, as saliva concentrations reflect only very recent exposure. However even with the large variability between concentrations, there was adequate agreement between these measures and the CAT. A large prospective study is now being undertaken in Leeds and Leicester using the CAT. Using this tool and a more detailed exploration of the interindividual variations in caffeine metabolism, a conclusive answer to whether there is any link between caffeine and pregnancy outcome may be demonstrated.

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Methyl group deficiency and tissue methionine cycle activity. By F.A. WILSON, W.D. REES and G.E. LOBLEY, *The Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 9SB*

Reduced birth-weight babies have an increased risk of developing CVD, hypertension and diabetes later in life (Godfrey & Barker, 2000). Maternal folate deficiency has been linked to an increased risk of low birth weight (Hoffbrand, 2001). Folate along with choline is essential for the re-methylation of homocysteine to methionine; as part of the methionine cycle for production of S-adenosyl methionine (SAM), the major precursor of methyl group transfers. Homocysteine, synthesised from SAM, is either re-methylated to methionine or converted to cysteine via the trans-sulfuration pathway. The aim of the present study was to identify, in non-pregnant rats, those tissues with the greatest methionine cycle activity and most sensitive to methyl group deficiency.

Groups (each *n* 6) of female Hooded Lister rats from the Rowett Institute colony were maintained on one of four diets (for 5 weeks). These contained either adequate or excess methionine, and with or without folate and choline (FC). Animals were infused for 4 h using the tracer bis-labelled [1-^{13}C, $^{2}H_5$] methionine then killed, with tissues and plasma collected. Enrichments and concentrations of methionine and homocysteine in tissues and plasma were analysed using GC-MS. Cycle activity was measured using the labelled re-methylated methionine (M+1): tracer methionine (M+4) ratio.

Removal of dietary FC significantly decreased methionine cycle activity based on plasma enrichment under adequate methionine conditions ($P<0.001$). With excess methionine, removal of FC from the diet resulted in a non-significant decrease in methionine cycle activity. At both methionine statuses plasma homocysteine concentration increased ($P<0.001$) with FC deficiency. Methionine cycle activity was absent or low in most tissues examined, but was high in the liver and pancreas, with limited activity also noted in the heart and spleen (see Fig. 1).

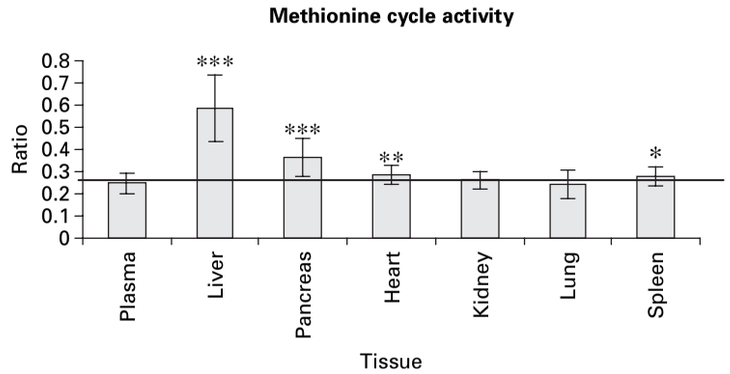

Fig. 1. Methionine cycle activity across tissues. All four diet groups combined. Significance of cycle activity determined by comparison in tissues compared with plasma M+1 : M+4 ratio. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Removal of FC resulted in decreased methionine cycle activity in the liver and pancreas similar to those detected in plasma ($P<0.001$ and $P<0.016$ respectively).

Excess dietary methionine is a precursor for SAM, reducing the requirement for homocysteine re-methylation by FC. With adequate methionine intake, however, increased re-methylation of homocysteine and cycle activity is required. This appears to occur mostly in the liver and pancreas. Future work shall adopt this approach to examine the impact of FC on tissue metabolism in pregnancy.

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Hoffbrand AV (2001) Folate deficiency in humans. In *Encyclopaedia of Life Sciences*, pp. 1–7.

A comparison of the sources of vitamin K_1 in the diets of individuals assessed in childhood and in adult life. By K.J. JACKSON[1], C.J. PRYNNE[1] and M.E.J. WADSWORTH[2], [1]*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL and* [2]*MRC National Survey of Health and Development, University College and Royal Free Medical School, 1–19 Torrington Place, London, UK, WC1 6BT*

Phylloquinone (vitamin K_1) is known primarily for its role in blood coagulation, but it is also essential for conferring functional Ca binding to several bone proteins, which are important for bone calcification and for inhibiting vascular calcification (Shearer *et al.* 1996). A sub-optimal intake of phylloquinone in the elderly may contribute to osteoporosis and increased fracture risk. The major dietary source of vitamin K_1 is leafy green vegetables. Vegetable oils such as olive, rapeseed and soyabean are also important sources, as are the wide range of products that include vegetable oils in their manufacture.

The MRC National Survey of Health and Development provides an opportunity to compare dietary sources of vitamin K_1 at two time points, 49 years apart. Dietary data were collected from members of the cohort aged 4 years in 1950 (24 h recall) and again, from 1669 of the original subjects, in 1999 (5 d diary). These records were coded and analysed using in-house programs and the nutrient intakes of the same subjects at both time points were compared. The vitamin K_1 content of a wide range of foods was obtained from Bolton-Smith *et al.* (2000) and from other data (C Bolton-Smith, unpublished results). The vitamin K_1 derived from fats in the foods of 1950 was estimated according to the types of fats and oils in use at the time.

	1950 (4 years)		1999 (53 years)		Paired sample *t* test		
	Mean	Median	Mean	Median	Mean difference	SD	*P*
Vitamin K_1 from total cereals (%)	14	10	10	8	−3.3	14.2	<0.001
Of which: Bread, breakfast cereals and cereals (%)	10	7	5	3	−5.0	10.7	<0.001
Cakes, puddings and biscuits (%)	4	3	6	4	1.5	7.7	<0.001
Vitamin K_1 from total vegetables (%)	61	69	61	64	0.5	34.4	0.6
Of which: Leafy green vegetables (%)	33	0	36	38	2.8	44.6	0.01
Peas and green beans (%)	17	0	7	5	−9.7	24.6	<0.001
Other vegetables (%)	6	0	10	7	4.2	13.4	<0.001
Potatoes (%)	5	2	7	4	1.6	9.9	<0.001
Vitamin K_1 from meat, fish and dishes (%)	3	1	8	6	4.7	11.6	<0.001
Vitamin K_1 from fat spreads (%)	5	3	4	3	−0.9	7.2	<0.001
Vitamin K_1 from milk and milk products (%)	8	5	2	2	−5.6	8.2	<0.001
Total daily vitamin K_1 intake (μg)	68		91				

Vegetables remain the principal source of vitamin K_1 but the proportion from leafy, green vegetables was increased in the adults. This was due to the greater contribution from raw salad vegetables. The contribution from other vegetables and potatoes was also greater in the adults. However, this reflects the vitamin K_1 in the oils in vegetable dishes and the oils in which potato products are prepared rather than the vitamin intrinsic to the vegetables themselves. Fats used in cooking and manufacture in 1950 were mainly animal fats, low in vitamin K_1, or oils such as coconut and palm which have a very low vitamin K_1 content compared with soya or rapeseed oil which predominate now. This also accounts for the greater contribution from cakes and biscuits and meat and fish dishes in the adults. Bread was an important source of vitamin K_1 in 1950. Not only was it consumed in large quantities; it was also made from high-extraction-rate flour which has a higher vitamin K_1 content than white flour. Similarly, the significantly higher contribution from milk and milk products in 1950 reflects the high milk consumption of the children.

There would appear to be a shift towards more fat-derived vitamin K_1 in the adult diets. However, the fact that leafy green vegetables still provide the greatest percentage of vitamin K_1 indicates that these adults do retain some of the good dietary habits acquired in childhood.

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Shearer MJ, Bach A & Kohlmeier M (1996) *Journal of Nutrition* **126**, S1181–S1186.

Habitual dietary calcium intake and body weight in 7–10-year-old children. By A. JENNINGS[1], V. COSTARELLI[1], G.J. DAVIES[1] and P.W. DETTMAR[2], [1]*Academy of Sport, Physical Activity and Wellbeing, London South Bank University, London, UK, SE1 0AA and* [2]*Reckitt Benckiser Healthcare (UK) Ltd, Dansom Lane, Hull, UK, HU8 7DS*

Dietary Ca plays a pivotal role in the regulation of energy metabolism (Teegarden, 2003). Recently, several observational studies detected inverse associations between dietary Ca intake and body weight (Davies *et al.* 2000). It was demonstrated that low-Ca diets lead to an increase in intracellular Ca concentrations, which in turn act to promote body fat deposition, reduce lipolysis and reduce thermogenesis (Zemel *et al.* 2000). High-Ca diets reverse these trends, and it appears that Ca in the form of dairy products may be more effective than elemental Ca (Shi *et al.* 2002). Most of the studies have been conducted on adults. However, Skinner *et al.* (2003) have recently demonstrated that longitudinal Ca intake is negatively associated with children's body fat levels. The purpose of the present study was to investigate possible associations between habitual Ca intake and body weight in a group of 7–10-year-old children. Eighty-five children, twenty-one boys and sixty-four girls (mean age 9.2 (SD 0.9) years) were recruited from twelve primary schools in the London area. Consent forms were completed by the children's guardians before their entry into the study. Dietary intake was measured using the 7 d weighed inventory method. Body weight and height measurements were also recorded. The Table summarises the main findings.

	All subjects (*n* 85)		Boys (*n* 21)		Girls (*n* 64)	
	Mean	SD	Mean	SD	Mean	SD
Weight (kg)	38	11	36	10	39	12
Height (m)	1.4	0.10	1.37	0.09	1.4	0.1
BMI (kg/m^2)	19.19	3.63	19.1	3.4	19.16	3.7
Ca intake (mg/d)	560	263	648*	252	531	267
Energy intake (kJ/d)	6521	2450	7150	2205	6313	2531

* Mean value was significantly different compared with the Ca intake of the girls ($P<0.05$) (one-way ANOVA).

The data suggest that girls have significantly lower intakes of Ca than boys and that 48% of boys and 38% of girls were overweight (above the 91st centile). However, there are no significant correlations between body weight or BMI and habitual intake of dietary Ca in this age group, which is in contrast with the results of similar studies conducted in adults. One explanation could be that the possible effect of Ca on adiposity and body weight is more pronounced in adulthood than in childhood. It is important for future studies to measure levels of body fat in children together with body weight in conjunction with Ca intake in order to elucidate the original hypothesis.

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Zemel MB, Shi H, Greer B, DiRienzo D & Zemel PC (2000) *FASEB Journal* **14**, 963–970.

The effect of increased red and processed meat consumption on endogenous formation of *N*-nitroso compounds in ileostomists. By J.C. LUNN[1], J.R.A. POLLOCK[2] and S.A. BINGHAM[1], [1]*Diet and Cancer Group, Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Cambridge, UK, CB2 2XY and* [2]*Pollock and Pool Ltd, Reading, UK, RG5 4DX*

Epidemiological studies have suggested an association between high-red-meat diets and an increased risk of colorectal cancer (Norat & Riboli, 2001). The present research is further investigating whether increased endogenous formation of *N*-nitroso compounds (NOC), measured by an increase in faecal apparent total *N*-nitroso compounds (ATNC) content, could account for this association. In man, there is a dose response to increased red meat consumption, and faecal ATNC levels increase when a low-red-meat diet is supplemented with haem, an effect not seen with inorganic Fe supplementation or when white meat or vegetarian diets are consumed (Bingham *et al.* 2002; Cross *et al.* 2003). The mechanism of NOC formation in the large intestine has previously been attributed to microbial fermentation (Calmels *et al.* 1985). However, the specific effect of haem would suggest an additional chemical effect. *In vitro* studies have shown that haem and myoglobin are readily nitrosated and can act as nitrosating agents (Hirst & Goodwin, 2000). The present study was designed to investigate NOC formation in the absence of a colonic flora using an ileostomy model.

Twenty-seven ileostomists were studied in the volunteer suite at the Dunn Human Nutrition Unit. Over 6 d three diets, high (240 g) red meat, high (240 g) processed (bacon, ham, corned beef) meat and a no-meat control diet were consumed in a randomised cross-over design in which each diet was investigated twice over 24 h. Every 2 h participants collected all ileal output which was snap-frozen on dry ice and then stored at –20 °C until required. A portion of the sample was homogenised and NOC content was measured by chemical denitrosation and thermal energy analysis (Pignatelli *et al.* 1987). A further portion of the sample was centrifuged and the resultant water fraction was used for genotoxicity testing.

NOC levels in the ileal output were significantly increased (mean 1469.96 (SEM 189.90) μg ATNC/kg; $P<0.0001$) following a high-red-meat diet and to a greater extent following a processed-meat diet (mean 2132.70 (SEM 263.12) μg ATNC/kg; $P<0.0001$) compared with the control diet (mean 366.93 (SEM 46.52) μg ATNC/kg). This compared with 22, 37 and 9 μg ATNC/kg in the red-meat, processed-meat and control-diet samples respectively.

We have used the Comet assay to assess the genotoxicity, as DNA strand breaks, of the sample. Preliminary results for only four volunteers indicate that the output following a red-meat diet is more genotoxic (mean Tail Extent Moment (TEM) 17.17 (SEM 7.70)) when compared with the control no-meat diet (mean TEM 4.62 (SEM 1.39)) and the processed-meat diet (mean TEM 5.53 (SEM 1.29)).

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Vitamin B_{12} status in elderly subjects with low haemoglobin. By K.M. MOONEY[1], I.S. YOUNG[1], C.C. PATTERSON[2] and G.J. CUSKELLY[1], [1]*Department of Medicine and* [2]*Department of Epidemiology & Public Health, Queen's University Belfast, Grosvenor Road, Belfast, UK, BT12 6BJ*

The prevalence of anaemia in the institutionalised and community-living elderly ranges from 8 to 26% (Dunn *et al.* 2003). Anaemia of chronic disease has been identified as the most common cause of anaemia in this population followed by Fe deficiency, then folic acid or vitamin B_{12} deficiency (Dunn *et al.* 2003). The aim of the present study was to assess the prevalence of haematological abnormalities in vitamin B_{12} and folate status among elderly subjects referred for vitamin B_{12} and folate biochemical assessment.

Data for all patients who had serum B vitamins (vitamin B_{12} and folate) measured by Belfast Link Laboratories (during a 6-month period; *n* 2853) were compiled. Patients' data were included if serum vitamin B_{12}, serum folate, Hb, mean cell volume (MCV) and serum ferritin were measured within ±4 d of each other. Data remaining (*n* 905) were divided into quintiles (Q) of B vitamins and ferritin. Percentages of those with low Hb (<130 g/l (male; M); <115 g/l (female; F) and high MCV (>100 fl) are shown per quintile (Table 1). Patients were also stratified according to vitamin B_{12} status as frankly deficient (def; <200 ng/l), suboptimally deficient (200–249 ng/l), possibly deficient (250–349 ng/l) and normal (>350 ng/l; Table 2). Nutritional status values shown in the tables are means and SEM.

Table 1.

	Q1		Q2		Q3		Q4		Q5	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Serum vitamin B_{12} (ng/l)	154.6	2.4	231.4	1.5	307.9	2.0	425.8	3.3	891.8	27.3
Patients with low Hb (%)	55		53		52		54		63	
Patients with high MCV (%)	10		10		7		7		12	
Serum folate (μg/l)	2.34	0.04	3.75	0.03	5.17	0.04	7.43	0.06	14.74	0.33
Patients with low Hb (%)	61		56		52		52		56	
Patients with high MCV (%)	15		8		9		4		10	
Serum ferritin (μg/l)	13.1	0.5	39.0	0.6	80.7	1.2	161.5	2.6	1180	657
Patients with low Hb (%)	71		46		43		48		70	
Patients with high MCV (%)	2		3		9		11		20	

Table 2.

Category (%) ...	Frankly def (20%)		Suboptimally def (14%)		Possibly def (24%)		Normal (41%)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Serum vitamin B_{12} (ng/l)	156.1	2.4	223.5	1.2	294.0	2.0	648.1	18.0
Patients with low Hb (%)	54		53		54		58	
Patients with high MCV (%)	10		12		7		9	

Vitamin B_{12} deficiency is common in this group. The incidence of elevated MCV was not significantly different in subjects with frank vitamin B_{12} deficiency when compared with the other three categories of vitamin B_{12} status. When vitamin B_{12}, folate and ferritin (by quintiles) were included simultaneously in a logistic regression analysis as predictors of high MCV, the only independent predictor was ferritin ($P<0.0001$). Given the possibility that folic acid supplementation may further mask haematological manifestations of vitamin B_{12} deficiency, work is required to demonstrate the safety of folic acid supplementation in this group.

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A pilot survey to investigate the nutritional status of patients with a fractured neck of femur and level of nutritional support provided during treatment. By M. NEMATY[1], M. HICKSON[1], A.E. BRYNES[1], C. RUXTON[2] and G.S. FROST[1], [1]*Nutrition and Dietetic Research Group, Imperial College London, Hammersmith Hospitals Trust, Du Cane Road, London, UK, W12 0HS and* [2]*School of Biosciences, University of Westminster, 115 New Cavendish Street, London, UK, W1W 6UW*

Hip fracture is one of the most severe consequences of falling in the elderly (Hayes *et al.* 1996), accounting for a high mortality rate (28% at 6 months; Magaziner *et al.* 1997), with fewer than half of those affected regaining their prefracture status (Morattoli *et al.* 1992). One clinical issue in elderly orthopaedic in-patients is insufficient energy and macro- and micronutrient intakes leading to sub-optimal nutritional status. Malnutrition has been postulated as a factor that increases the tendency to suffer falls (Lumbers *et al.* 2003) and increases the recovery time.

We conducted an observational study to evaluate the nutritional state and the nutritional support which was provided during their stay in hospital in the first twenty-five individuals to be admitted to an orthopedic ward with a fractured neck of femur between August 2003 and December 2003 at Charing Cross Hospital, London, UK. This provided us with baseline data to assist in the development of a prospective, randomised intervention trial to examine the benefits of dietary supplementation in this group of patients.

Data were collected from hospital records, nursing Cardex, care staff, dietitians, occupational therapists and physiotherapists. A group of variables corresponding to nutritional status (anthropometrics, biochemical indices, 3 d dietary intake) were collected. Patients were followed until discharge.

On admission patients were 85.3 (SD 7.4) years (92% aged more than 75 years), 64% were living alone, 72% were female, 96% of white ethnicity, and in 84% of cases the fracture was caused by a fall. This population had a significantly lower BMI compared with the mean BMI for sex and age in an elderly UK population (21.97 (SD 5.3) *v.* 26.73 (SD 4.2) kg/m^2; $P<0.005$) (Finch *et al.* 1998). Of the patients, 92% had a mid-arm circumference (MAC) less than the mean for sex and age in this population (249 (SD 48) *v.* 299 (SD 36) mm; $P<0.005$) (Finch *et al.* 1998). Median C-reactive protein (CRP), 83 (range 1–295) mg/l, was significantly higher than the normal range (<10 mg/l). Mean plasma albumin (34 (SD 4.5) g/l) on admission was at the lower limit of the normal range (33–55 g/l).

Energy intake was 47% less than the estimated average requirement (4.2 (SD 1.6) *v.* 7.9 (SD 0.6) MJ/d; 1005 (SD 372) *v.* 1896 (SD 151) kcal/d). Using the hospital's own nutritional risk assessment tool, 43% of patients were found to be at risk of malnutrition, yet only 28% were referred to a dietitian, and only 24% were given the hospital high-energy and high-protein menu. Based on this nutritional risk assessment tool, comparison between the at-risk group (*n* 11) and the not-at-risk group (*n* 14) suggests that the at-risk group had significantly lower MAC (228 (SD 45) *v.* 274 (SD 42) mm; $P<0.02$), lower weight (50.7 (SD 10.7) *v.* 65.1 (SD 14.3) kg; $P<0.02$), lower BMI (20 (SD 4.9) *v* 24.4 (SD 5.4) kg/m^2; NS), and higher CRP (120 (SD 102.1) *v.* 69.9 (SD 70.5) mg/l; NS). Energy and protein intakes were significantly lower in the at-risk group (3.3 (SD 1.2) *v.* 5 (SD 1.6) MJ/d (787 (SD 295) *v.* 1198 (SD 380) kcal/d), $P<0.016$; 116 (SD 43.5) *v.* 201 (SD 56.4) g protein/d, $P<0.002$). The mean length of stay in this group of surgical patients was 36 d. This is significantly longer than the average surgical patient admitted to this hospital of 5 d.

In summary this group of elderly patients with a fractured neck of femur are malnourished on admission, and catabolic during their extended stay in hospital. There is failure to meet energy needs by as much as 50% during their stay. Poor referral to a dietitian means that supplementation rates were also low. These results reinforce the need to understand the metabolic process, appetite regulation and feeding in this extremely high-risk group.

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Validation of a food-frequency questionnaire (FFQ) for assessment of bone health nutrients in a Swiss population: preliminary results. By E. WYNN[1], M.A. KRIEG[1], J. CORNUZ[1], D.R. WHITTAMORE[2], P. BURCKHARDT[1] and S.A. NEW[2], [1]*University Hospital (CHUV), 1011 Lausanne, Switzerland and* [2]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 5XH*

Assessment of dietary intake in specific populations is a critical component when determining the effect of nutritional factors on health. For the assessment of average long-term dietary intake in large numbers of individuals, food frequency questionnaires (FFQ) have emerged as particularly useful tools since they give a better approximation of usual long-term dietary intake than short-term records, can be self-administered and are relatively inexpensive to use. Before their use in studies, FFQ need to be validated in the population under investigation by assessing the level of agreement between dietary intakes using another method of dietary assessment.

As part of an ongoing study into osteoporosis in a Swiss elderly population (Swiss evaluation of measurement of risk of osteoporotic fracture; SEMOF), the influence of dietary intake on bone health indices will be examined in a total of 400 ambulatory women from Lausanne. Since few dietary methodologies exist for populations within Switzerland, it was considered imperative to develop a Swiss FFQ. The present paper presents the validation work in the development of the questionnaire.

In order to test the FFQ on a sample of the study population, the dietary intake of forty-five Swiss women (mean age 82 years; mean weight 61 kg) was assessed using a 4 d weighed record (WR) during Summer 2002 through to Winter 2002. A dietician, before starting the weighed record, trained each woman and regular contact was made during and after the recording period to ensure compliance and competency. Food portion sizes were estimated using the SU-VI-MAX photo manual and all components of dietary intake were measured using Prodi 4.5^{R} software. Women then completed an FFQ during Spring 2003. To validate the FFQ, the mean bone-health nutrients from the FQ were compared with the results from the WR. Analysis for forty-four women is shown in the Table.

	FFQ			WR			
Nutrient	Mean	SD	Range	Mean	SD	Range	*P* value
Energy (MJ)	7.1	2.3	2.98–11.54	6.5	1.4	3.77–9.58	0.225
Energy (kcal)	1701.5	555.5	711.1–2758.7	1545.3	334.6	901.2–2289.4	0.225
Protein (g)	72.5	23.7	33.8–114.1	65	17.4	34.1–116.5	0.199
Fat (g)	69.7	27	31.8–131.4	65	20.7	29.3–111.5	0.486
Carbohydrate (g)	186.9	65.7	62.2–315.3	165.6	44	96.9–277.8	0.176
Ca (mg)	1057.7	514.9	380.6–2492.2	956.6	467.4	471.7–2765.8	0.462
K (mg)	3054.6	1078.1	1268.4–5368.9	2691.6	783.4	1514.6–4596.2	0.171
Vitamin C (mg)	90.4	41.6	33.5–190.1	105.5	60.2	18.9–266.4	0.298

The FFQ was found to be in relative agreement with the nutrient results from the WR for key macro and micronutrients. In general, the FFQ tended to overestimate intakes by approximately 10% for the majority of nutrients studied (except vitamin C and fibre that were over-estimated by about 2%), but differences were not significant apart from protein. These findings are consistent with other FFQ validation studies, which in general have shown that FFQs tend to overestimate nutrient intakes (Cade *et al.* 2002).

Further studies to examine the FFQ's reproducibility are required to enable its use as a valuable dietary assessment tool for nutrition and bone-health studies in the Swiss population.

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Krieg MA, Cornuz J, Hartl F, *et al.* (2002) *Journal of Clinical Densitometry* **5**, 335–341.
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Experiences of Women with Chronic Radiation Enteritis indicate the need for dietary advice. By ABAYOMI, J.[1,2], KIRWAN, J.[1], HACKETT, A.[2] and BAGNALL, G.[2], [1]*Liverpool Women's Hospital, Crown street, Liverpool L8 7SS and* [2]*Liverpool John Moores University, Barkhill Road, Liverpool l17 6BD*

Cancer of the cervix remains a major world health problem with almost half a million women diagnosed each year and 200,000 fatalities (Pisani *et al.* 2000). Radiotherapy remains the standard treatment, especially for more advanced disease (stage 2 and above). Gastro-intestinal side effects are often experienced due to radiation-induced injury to the intestinal epithelial cells. This can result in shortening of the villi, which may cause malabsorption and fluid and electrolyte imbalances. Patients with these symptoms may benefit from a moderately low fat, low residue, low lactose diet (Walker & Masino, 1998). Due to the restrictive nature of the above diets, dietetic supervision is essential. This also allows the variety of foods in the diet to be decreased or increased depending on the gut response (Shaw, 2000).

Aim To investigate the experiences of women with chronic radiation enteritis.

Methodology Due to the sensitive nature of this study, a qualitative approach was adopted. Women who had been treated with radiotherapy for cervical cancer were identified from their medical records. Informed volunteers took part in one-to-one tape-recorded in-depth interviews to explore their experiences following treatment and including methods used to control side effects of treatment. The taped interviews were transcribed verbatim and analysed qualitatively using a software package: NUD*IST.

Results Ten women were interviewed aged 23–85 years. The majority reported diarrhoea either during or after radiotherapy. Sufferers reported that they were managing their symptoms with regular medication and/or self-imposed restricted or bland diets.

- "*Yeah fruit because I haven't any for a couple of days and it's not been as bad. So I think the fruit makes it worse*" (Frances aged 54).
- "*Some things now give me diarrhoea when I eat … cabbage nearly killed me one time so I haven't eaten that since.*" (Eileen aged 43).
- "*Well I don't eat veg. it's mainly potatoes, chips, eggs and meat*" (Julia aged 41).

On looking more closely at the data it became apparent that women are avoiding or attempting to avoid many of the so-called 'protective foods' (i.e. foods containing antioxidant vitamins) such as fruit and vegetables, which may prevent recurrence of cancer and other health problems later in life. Few of these women had ever sought professional help in dealing with their problems because of embarrassment or reluctance to complain.

- "*I haven't really told them about that one. It's embarrassing*" (Carol aged 54).

Conclusion CRE is a side effect of pelvic radiotherapy requiring dietary advice. Without appropriate advice sufferers may consume an unbalanced diet, free from fruit and vegetables. This study shows that some sufferers are reluctant to seek help and rely on self-restriction of diet in order to manage symptoms. Health professionals need to be more pro-active in identifying and caring for sufferers of CRE.

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Development of patient information leaflets for constipation using a range of cognitive interview techniques: LIFELAX. By A.A. LAKE, A.L. BROOKES, C. SPEED, S. CORBETT, P.J. MOYNIHAN and A.J. ADAMSON on behalf of the LIFELAX STUDY TEAM, *Human Nutrition Research Centre, University of Newcastle, Wellcome Research Laboratories, RVI, UK, NE1 4LP*

Constipation affects the quality of life in about 20% of older individuals (Cummings *et al.* 1992). Approximately £42 million is spent annually on prescribed laxatives in England (Department of Health, 2002). Although constipation is multi-factorial, diet and lifestyle have an important role in the management of constipation. The LIFELAX study is a randomised controlled trial (ISRCTN7388134) that aims to develop and evaluate a cost-effective intervention to promote a healthy diet and lifestyle for the treatment and management of chronic constipation for older individuals in primary care. The LIFELAX intervention involves a nutritionist delivering training on diet and lifestyle to primary care practitioners, who in turn deliver this advice to older adults with chronic constipation. The practitioners require educational resources to facilitate the intervention and to meet the patients' need for information. The present research reports on the first stage of the intervention process – the development and piloting of patient information leaflets.

Eight patient information leaflets were designed on the themes of: constipation, activity, bowel health, fruit and vegetables, fibre, fluid, alternative therapies and laxatives. These themes emerged following reviews of the relevant literature and qualitative interviews with practitioners and patients. Nine patients, ≥55 years old, who had received three or more prescriptions of laxatives in the last 12 months, were interviewed using a range of cognitive interview techniques (Collins, 2003). The technique enabled the interviewer to explore the respondents' understanding of words or terms used in the leaflets, check the clarity of the information and its accessibility to this patient group. Interviews were taped and analysed using content analysis (Krippendorf, 1980) in QSR NUD*IST N5 (QSR International Pty Ltd, Melbourne, Australia).

Twenty-three changes were made across the eight leaflets following the cognitive interview process, some of which are tabulated below.

Type of change	Before	After or action
Wording	Gut	Bowel
	Exercise	Activity
	Gut motility	Bowel movement
	Lactose intolerant	Dairy intolerance
	Probiotics	Friendly bacteria
	Bulking agents, osmotic agents, stimulants and softeners	Removed from leaflet and simplified
Examples given	Types of activity or exercise	Household activity given as an example

Examples of respondents' quotes included the following phrases:

'I don't know what normal is.';
'It's too involved for me dear, I think you should have had somebody that had a bit more brains than me.';
'I don't understand the different mechanisms . . . the mechanism what actually it achieves I wouldn't know.';
'I've never heard of these . . .'

The laxative leaflet and the bowel health leaflet raised most confusion and required thorough revision and simplification. The process of piloting and carrying out a cognitive test on the understanding of the information present is a valuable exercise. The cognitive interviews have resulted in significant changes to the prototype patient information which will go forward to be used in the LIFELAX intervention.

This work is funded by the NHS R&D Health Technology Assessment Programme (project number 01/10/04).

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Weight management: a multi-dimensional approach. By M.E. BARASI, G.S. MENDOZA and L.D. SANDERS, *Centre for Nutrition and Dietetics, UWIC, Western Avenue, Cardiff, UK, CF5 2YB*

Few studies have been carried out on the stages of change model in the field of weight management. Previous studies have been predominantly in a clinical setting on female subjects. There is a need to establish if individuals trying to lose weight go through various stages of change whilst achieving their goal and if criteria predicting success can be identified at the start. The multidimensional model proposed by Senekal *et al.* (1999) may be a way to tackle the problem of overweight and obesity in the community.

Data on dietary and activity habits were collected using questions from the Allied Dunbar National Fitness Survey (Fentem *et al.* 1994). Dimensions of decision making, considering the pros and cons of losing weight were assessed using the decisional balance inventory (DBI). Indivduals' self-efficacy regarding weight management was measured by the weight efficacy life-style questionnaire (WEL). To catergorise individuals into discrete stages, current activity and weight-loss intentions were measured by the stages of change algorithm (SCA). The stages construct was further explored using the University of Rhode Island change assessment scale (URICA). (Details of all psychometric tests can be found in Rossi *et al.* 1995.)

Fifty-six male volunteers were recruited; fifteen did not want to lose weight (NTLW), the rest were trying to lose weight (TLW). All were initially interviewed, with second interviews completed 4 months later; four subjects failed to complete the psychometric tests. The amount of weight loss was significantly correlated to activity levels ($P<0.05$), as was the DBI score (difference between pros and cons) ($P<0.05$) (see Table 1). Subjects with a larger difference in favour of pros lost more weight. Levels of self-efficacy were significantly correlated to weight loss ($P<0.05$). There was a significant correlation between the SCA (Table 2) and the URICA ($P<0.05$), suggesting these two tests may work well together in predicting a subject's stage of change. Three processes of change were significantly utilised more by the TLW group. These processes of change were: self liberation ($P<0.01$), consciousness raising ($P<0.05$) and helping relationships ($P<0.05$). Time available to exercise was seen as a major barrier to weight loss. Dietary intake between groups was not significantly different.

Table 1. DBI and WEL scores

	Group weight change (kg)		WEL score		DBI score	
Group	Mean	Range	Mean	Range	Mean	Range
NTLW	−0.45	−4.54 to +3.18	131 (NS)	84–159	7*	−7 to +23
TLW	−2.00	−15.42 to +6.80	125 $P<0.05$	71–180	10*	−15 to +32

* When TLW+NTLW considered together, DBI scores significantly correlated to weight loss ($P<0.05$).

Table 2. SCA scores

Group (SCA)	Current stage of change	Frequency	%
NTLW	Pre-contemplation	7	47
	Contemplation	4	27
	Action	4	27
	Total	15	100
TLW	Action	27	73
	Contemplation	8	22
	Maintenance	2	5
	Total	37	100

Assessing an individual's readiness to change in terms of decisional balance may improve the success rate of a weight-loss programme. Activity should be an important part of any programme and include assistance in overcoming perceived barriers to exercise. Dietary education is important as none of the subjects fully understood or could apply current healthy eating guidelines.

A multidimensional approach appears to offer useful indicators of effectiveness and will be developed further.

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Rossi JS, Rossi SR, Velicer WF & Prochaska JO (1995) In *Handbook of Assessment Methods for Eating Behaviors and Weight-related Problems: Measures, Theory, and Research*, pp. 387–430 [DB Allison, editor]. Thousand Oaks, CA: Sage Publications, Inc.
Senekal M, Albertse EC, Momberg DJ, Groenewald CJ & Visser EM (1999) *Journal of the American Dietetic Association* **99**, 1257–1264.

Evaluation of a pilot community-led weight management service within West Middlesbrough. By C.L. APPLETON[1], C.D. SUMMERBELL[1], V.J. WHITTAKER[1] and P. KELLY[2], [1]*School of Health and Social Care, University of Teesside, Middlesbrough, UK, TS1 3BA and* [2]*Middlesbrough Primary Care Trust, Middlesbrough, UK, TS2 1RH*

The concept of health inequalities is now widely accepted (Acheson, 1998). Policy makers and academics alike are in agreement on the need to tackle the issue (Whitehead, 1998, Shaw, 1999). The government is presently engaged in dealing objectively with the issue in light of the evidence base, through various initiatives including New Deal for Communities. The purpose of the present evaluation was to explore the effectiveness of community-led services funded through such initiatives, focusing primarily on a weight management group within a deprived area of Middlesbrough. A content analysis of the training package developed by the dietitians was performed, whereby all elements of the package and contained documentation were examined. Particular attention was given to the quality and quantity of the training provided by dietitians, and also the level of ongoing support.

A series of interviews were conducted to gather differing views and opinions on the service with all those involved. This included the first batch of users to have accessed the service for over a ten-week period (10 users in total), the dietitians, the group leaders, the nutritional support worker and the community health coordinator. Weight records belonging to users were also observed to assess the effectiveness of the service in terms of weight loss. New Deal for Communities (the funder of the service) was also contacted in order to carry out a cost assessment of the service.

On inspection, the training package presented a number of problems including a lack of structure and the deliverance of an excess of high-level and very theoretical material, thought to be inappropriate for the target audience. Despite this, interviews revealed that all involved perceived the service as being useful and effective with the main reasons being that it achieved weight loss, was cost effective, local and convenient. Mean weight loss over a 20-week period was 2.2 kg amounting to 0.1 kg/week, with overall weight loss ranging from 0.45–7.73 kg. The service proved to be extremely cost effective costing approximately £1832.00 per annum to run.

This community-led weight-management service demonstrated an excellent example of community development and indicated that such programmes possess the potential to relieve the pressures obesity creates within primary care. It is suggested that a standardised training pack be developed including appropriate resources for group leaders and users alike. Formal and measured evaluation techniques need to be employed when appraising such services in order to assess their success and impact both in the short and long term.

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Whitehead M (1998) *Lancet* **351**, 1279–1282.

Development of a multidisciplinary approach to the prevention and/or treatment of obesity within the Middlesbrough Primary Care Trust. By R. LANG[1], C.D. SUMMERBELL[1], H.J. MOORE[1], C.L. APPLETON[1] and J. SMITH[2], [1]*University of Teesside, Middlesbrough, UK, TS1 3BA and* [2]*Middlesbrough Primary Care Trust, UK, TS2 1RH*

The prevalence of obesity in the UK is rising in both adults (Department of Health, 2003) and children (Chinn & Rona, 2001), with highest levels observed in areas of social deprivation. The importance of a reduction in obesity levels has been identified in a number of government strategic documents (Department of Health, 2000*a*,*b*,*c*; National Audit Office, 2001). Work at a local level to develop and implement effective policies in obesity prevention and treatment has been recommended.

Middlesbrough has one of the highest obesity rates in the UK (Reilly *et al.* 2001). In March 2004, Middlesbrough PCT launched its obesity strategy. This included an extensive and comprehensive mapping of local services targeted at the prevention and/or treatment of obesity plus a summary of the evidence base from systematic reviews about strategies which are the most effective for the prevention and treatment of obesity and maintenance of weight loss. The outcome of the strategy is an action plan for Middlesbrough, which recognises that a strategic, whole-system approach with partnership across stakeholders is essential if the recommendations are to be translated into action. The action plan has been based on recommendations which were prioritised according to the strength of existing evidence and services already in place. For example, high priority recommendations were based on a good evidence base and existence of some services. Stakeholders include local schools, primary and secondary care teams, community-based initiatives and the PCT itself.

A number of equally important themes have emerged from the action plan which will provide an opportunity to be developed into business cases for funding in the coming financial year. First, training for both professionals and community groups was highlighted as a key theme across sectors and a programme is planned for development and dissemination during 2004. The University of Teesside and the Association for the Study of Obesity will take this forward with the aim of rolling this training programme out nationally. Second, appropriate data collection, protocols and frameworks were identified as necessary for audit and evaluation to ensure effective development and retention of efficient pathways of care. Third, the utilisation of community-based weight-management and physical activity initiatives within referral routes is to be clarified, preferably through a central point of coordination with appropriate sign posting.

The future implementation of the strategy-based action plan is reliant on a cross-sector partnership and sharing of best practice. The future health of Middlesbrough residents can only be enhanced by the development of a cohesive framework for the prevention and treatment of obesity which accommodates the challenges faced by social deprivation.

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Department of Health (2000*a*) *National Service Framework for Coronary Heart Disease*. London The Stationery Office.
Department of Health (2000*b*) *The NHS Cancer Plan*. London: The Stationery Office.
Department of Health (2000*c*) *The NHS Plan. A Plan for Investment. A Plan for Reform*. London: The Stationery Office.
Department of Health (2003) *Health Survey for England 2001*. London: The Stationery Office.
National Audit Office (2001) *Tackling Obesity in England*. London: The Stationery Office.
Reilly M, Ashton V, Eynon C, Lowson D & Heywood P (2001) *Health and Lifestyle Tees Area 2000*. Tees Health Authority, Middlesborough.

Preliminary results of the effectiveness of a commercial weight-loss programme compared with usual care in primary care. By C.L. APPLETON[1], C.D. SUMMERBELL[1], P. FINN[1], A. PLUMMER[2], A. HELMN[2], T. MITCHELL[3] and V. LAWRENCE[3], [1]*School of Health and Social Care, University of Teesside, Middlesbrough, UK, TS1 3BA,* [2]*Dr Chaudhry & partner, Oakfield Road, North Ormesby, Middlesbrough, UK, TS3 6EL and* [3]*Dr Mitchell & partners, Montpelier Medical Centre, Bristol, BS6 5PT*

The Department of Health (DOH) now appreciates that the National Health Service cannot tackle the level of obesity in the UK alone. The DOH has encouraged health professionals to work in partnership with other agencies (including the commercial sector) in order to meet the needs of the public. To this end, the DOH and the National Audit Office have asked that commercial agencies arrange for independent assessments of the efficacy of their products and programmes.

Overweight adult patients (BMI >27 kg/m^2) were identified within a primary care setting and randomly allocated to one of two weight-loss programmes; a commercial weight-loss programme (The Cambridge Health Plan Programme; CHP) provided free of charge OR usual care (UC).

If patients were randomised to the UC group they were dealt with by the practice, as they would "normally" deal with their overweight patients. For example; they could be referred to a dietitian, attend a weight management clinic or simply be given healthy eating advice. If patients were randomised to the commercial weight loss programme (CHP), they were given a diet composed of powdered meals, bars, soups and mousses ranging from approximately 500 to 1500 kcal (should be in kJ or MJ) per day.

Two primary care practices in two different geographical areas of the UK were recruited to the study. In total thirty-five patients were randomised to a commercial weight-loss programme (CHP) and thirty-one were randomised to UC. Randomisation was accomplished using sealed, opaque envelopes in blocks of ten. Patients measurements will be recorded at baseline, 3, 6, and 12 months. At each assessment patients will be also asked to complete a series of questionnaires relating to health and wellbeing. Process evaluation, outcome evaluation and an assessment of "willingness to pay" for the commercial weight-loss programme will be performed at the end of the study.

	Baseline	Weight at baseline (kg)		3 Months	3 Month change (kg)		6 Months	6 Month Change (kg)	
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Bristol (CHP)	16	100.9	21.5	14	−10.8	6.1	14	−15.4	9.5
Bristol (UC)	12	93.6	7.7	4	−3.1	0.9	4	−2.7	1.0
Teesside (CHP)	19	94.2	14.7	9	−6.7	4.3	9	−7.9	5.3
Teesside (UC)	19	93.9	14.8	18	−3.2	3.1	13	−4.1	3.8

At 6 months the data suggests that patients randomised to the commercial weight-loss programme achieved a greater weight loss than those randomised to UC.

It is anticipated that 12-month data will be collected by September 2004.

Gender differences in use of an Internet-based weight club. By J. JONASSON, M. HYLL, Y. LINNÉ and S. RÖSSNER, *Obesity Unit, Karolinska University Hospital, Huddinge Stockholm, Sweden*

The aim of the present study was to create an Internet-based weight club to reach the Swedish overweight and obese population at large and to examine gender differences in their use of this website.

Standard algorithms for calculating BMI, food energy content, exercise cost and energy balance (Stunkard & Messick, 1985) were presented via the website. A food diary where the members described their food intake and received immediate feedback in the form of energy, fat, carbohydrate and protein content is also available on the website. The members can interact with professional weight-loss staff in different ways. For example, they can submit personal questions and get answers on the website. Every week they also have the opportunity to participate in a chat room with some of the weight-loss staff. They received weekly recipes, recipe modification suggestions, exercise suggestions and exercise (pedometers etc) or cooking apparatus at reduced prices. Security cut-off rules were included to minimise the risk of subjects with eating problems or disease joining the club. Basic questions, taken from the standard questionnaire used by the Obesity Unit were included to form a database for scientific evaluation. Treatment success, attrition, use of the various components of the programme can now be monitored online prospectively. Data presented are from the first 9284 subjects, who have been in the programme for 3 months. Of the participants, 172 were excluded because of failure to answer key questions, missing data or invalid body-weight data.

	Males		Females	
	Mean	SD	Mean	SD
Sample size	1342		7770	
Age (years)	44	12	41	11
Initial BMI (kg/m^2)	31.3	4.6	29.8	5.3

	Males		Females		
	Mean	SD	Mean	SD	*P*
Weight club entries	58	75	56	71	NS
Frequency of weight report	6.8	5.1	6.9	4.8	NS
Desired weight loss (kg)	11.9	4.4	10.5	4.3	0.001
Time to lose desired amount (weeks)	14	3.8	13	4.1	0.001
Actual weight loss (kg)	3.6	4.6	2.2	11.4	0.008

Stepwise multiple regressions were performed to identify predictors of weight loss in the first 3 months of the Internet club. Use of the food intake diary was the biggest predictor.

Greater desired weight loss, male gender, higher initial BMI, more frequent weighing, being a smoker, more frequent log-ins, and older age were also significant predictors of weight loss.

Together these variables accounted for 14% of the variance.

The Internet-based weight club offers a number of advantages:

efficient alternative for those who cannot get help from other support systems;

possible to treat numerous participants cost-effectively;

provides unique database, which offers opportunity to evaluate factors of importance for maintenance of weight loss;

huge difference in proportion of men and women using the Internet club;

gender difference in weight loss in first 3 months such that males show greater weight loss than females.

Stunkard A & Messick S (1985) *Journal of Psychosomatic Research* **29**, 71–83.

Intensive lifestyle intervention combined with the choice of pharmacotherapy leads to weight loss and improvement in cardiac risk factors in the obese at 6 months and maintenance of weight loss at 1 year. By J. BOYLE and G. FROST, *Department of Nutrition and Dietetics, Charing Cross Hospital, Hammersmith Hospitals NHS Trust, Fulham Palace Road, London, UK, W6 8RF*

Over the last 20 years, the prevalence of obesity in the UK has nearly trebled (Joint Health Surveys Unit on behalf of the Department of Health, 2002). This rise has led to an increase in obesity-related conditions such as type 2 diabetes and CVD. Obese individuals who have successfully lost weight are prone to relapse after the end of a treatment programme (Garner & Wooley, 1991). Our aim was to investigate the effects of a 6-month lifestyle weight-loss programme on weight and cardiovascular risk factors and maintenance of weight loss at 1 year.

Patients with a BMI >30 kg/m^2 or BMI >28 kg/m^2 with one or more co-morbidities were referred to the lifestyle clinic from local general practitioners or from within Hammersmith Hospitals NHS Trust. The 6-month programme focused on dietary change, behaviour modification and increased physical activity. The aim was to achieve a 5–10% weight loss from the starting weight over 6 months, with or without the aid of pharmacotherapy (Orlistat; Roche Products Limited, Welwyn Garden City, Herts, UK). Exercise tolerance, blood pressure, fasting glucose, HbA1c and a full lipid profile were measured at the start and at the end of the 6-month programme. Patients who had successfully completed the programme were followed up at 12 months.

Patients were screened using a behaviour-change model, to ensure they were ready to commit to the intensive programme. A total of 289 patients were enrolled in the programme. Of these, 175 have been discharged without completing, predominantly for not attending without prior notification, and non-adherence to the clinic protocol. To date eighty-one patients have completed the 6-month programme. Results at 6 months are shown in the Table.

	Start		6 months			
	Median	IQ range	Median	IQ range	% Change	*P* value
Glucose (mmol/l)	5.50	5.0–7.0	5.30	4.85–6.3	−4.0	0.03
HbA1c (%)	6.2	5.4–7.0	5.8	5.2–6.5	−3.6	0.002
Triacylglycerols (mmol/l)	1.56	1.17–2.06	1.33	0.9–1.88	−15	<0.001
	Mean	SD	Mean	SD		
Total cholesterol:HDL-cholesterol	4.34	1.01	4.01	0.98	−6.6	<0.001
Shuttle test (m)	390	159	471	181	18.2	<0.001
Waist (mm)	1116	156	1035	141	−7.0	0.008
Weight (kg), all patients (*n* 81)	107.6	21.9	99.1	19.9	−7.7	<0.001
Weight (kg), advice+orlistat (*n* 23)	119.3	25.7	108.6	23.0	−8.9	<0.001
Weight (kg), advice only (*n* 58)	102.4	18.3	95.0	17.2	−7.0	<0.001

Forty-six patients have been followed up at 1 year. The mean weight change for those on orlistat (*n* 16) was 11.6 kg (−9.4% change; $P<0.001$), and for those who had advice only (*n* 30) was 10.9 kg (−10.5% change; $P<0.001$).

Weight-management clinics that provide intensive support with a structured programme can achieve significant weight loss with improvements in several cardiovascular risk factors. Following patients up at 1 year demonstrated that most of the weight that was lost in this 6-month period had been maintained. This is in contrast to other treatment programmes that often demonstrate weight re-gaining at 1 year. The weight loss achieved by the group on pharmacotherapy plus lifestyle advice was very similar to the weight loss achieved by the group who were given lifestyle advice alone, suggesting that the programme format was the critical factor for success.

Garner DM & Wooley SC (1991) *Clinical Psychological Reviews* **11**, 729–780.
Joint Health Surveys Unit on behalf of the Department of Health (2002) *Health Survey for England 2001*. London: The Stationery Office.

Dietary changes induced by the Atkins diet. By R. BOLEY[1], A. HERRIOT[1], A. DELOOY[2], K. FOX[3], M.P. BONHAM[4], I. MACDONALD[5], D.J. MILLWARD[1], L. MORGAN[1] and H. TRUBY[1], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH,* [2]*Queen Margaret's University College, Edinburgh, EH12 8TS, UK,* [3]*The University of Bristol, BS8 1TH, UK,* [4]*The University of Ulster, BT52 1SA, UK and* [5]*The University of Nottingham, NG7 2RD, UK*

Diet Trials was a 6-month, multi-centre, randomised controlled trial investigating the efficacy of popular commercially available weight-loss regimens in the UK, which included the Atkins diet (Atkins, 2003). Controversy surrounds the mechanism by which low-carbohydrate diets achieve weight loss, and about the potentially high intake of both protein (St Jeor *et al.* 2001) and saturated fat (Council on Foods and Nutrition, 1973). We report here the dietary data (measured using a 7 d food diary) collected from this trial. Under-reporters at baseline (70% of the subjects according to established criteria; Goldberg *et al.* 1991) were excluded leaving fifteen records likely to be valid. We report here intakes of energy and macronutrients measured in the baseline diet and after 2 months on the Atkins diet.

	Baseline (*n* 15)		2 Months (*n* 10)	
	Mean	SD	Mean	SD
Energy intake/kg (kJ/kg)	134.1	22.7	85.6*	22.3
Protein (g/kg)	1.2	0.2	1.4	0.2
Carbohydrate (g)	272.7	80.7	46.2*	31.9
NSP (g)	13.4	3.9	6.1†	3.1
Total fat (g)	122.9	26.3	108.3	28.5
Saturated fatty acids (g)	40.7	12.4	40.1	9.2
PUFA (g)	21.6	6.4	17.5	7.1

* Significantly different from baseline (paired-samples *t* test) ($P<0.001$).
† Significantly different from baseline (Wilcoxon signed ranks test) ($P<0.01$).

Energy intake (per kg body weight) decreased significantly after 2 months on the Atkins diet. Whilst the protein:energy ratio of the diet increased from 0.15 to 0.27 there was no significant increase in the absolute amount of protein consumed, although some individuals (four at 2 months) had intakes in excess of 200% RNI (1.5 g/kg). As would be expected, carbohydrate intake fell significantly, since the basis of the Atkins nutritional approach is a drastic reduction in carbohydrate intake (Atkins, 2003). Although the fat:energy ratio was significantly higher ($Z-2.80$; $P=0.005$) at 2 months (0.58) compared with baseline (0.39), there was no significant change in absolute fat intake.

It is clear that weight loss achieved on the Atkins diet results from an involuntary reduction in energy intake, presumably reflecting the effects of the protein-rich, more monotonous diet on appetite, although this requires further investigation. NSP levels were below recommendations at baseline (18 g/d) and decreased further on commencement of the diet. The nutritional quality of low-carbohydrate diets have been given the lowest 'healthy eating index' compared with high-carbohydrate diets, which have been rated as having the highest index (Kennedy *et al.* 2001). However, detrimental dietary changes induced by the Atkins diet (reduced NSP and carbohydrate intake) need to be considered in conjunction with the overall positive effects of decreased energy intakes which leads to weight loss and associated health benefits. However, longer-term health effects on bowel health have yet to be evaluated.

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Objectively measured physical activity and inactivity in rural Irish and urban Scottish children: a comparison study. By L.A. KELLY[1], J.J. REILLY[1], S. GRANT[2] and J.Y. PATON[3], [1]*University Department of Human Nutrition, Yorkhill NHS Trust, Glasgow, UK, G3 8SJ,* [2]*IBLS The University of Glasgow, Glasgow, UK, G12 8QQ and* [3]*Department of Child Health, Yorkhill NHS Trust, Glasgow, UK, G3 8SJ*

The recent obesity epidemic in children has led to an increased awareness of physical inactivity as a serious medical and public health concern (Reilly & Dorosty, 1999). However, to date there is a paucity of data on objectively measured physical activity and inactivity in contemporary populations of children. We hypothesised that young Irish children from rural areas might be expected to be more physically active than children from urban areas.

In the present study, we measured levels of activity and inactivity in a sample of contemporary rural Irish children and compared them with children from Glasgow. We studied forty-one pairs (*n* 82) from a broadly socio-economically representative sample of children aged 4–6 years old (mean age 5.3 (range 4.3–6.4) years) from County Carlow, Ireland and Glasgow, Scotland. Total physical activity (mean accelerometer counts/min; cpm), inactivity (% of monitored time below 1100 cpm (Reilly *et al.* 2003); and time spent in light activity, and moderate–vigorous physical activity (Puyau *et al.* 2002) were assessed over 7 consecutive days using the Actigraph accelerometer. The Actigraph accelerometer has been shown to be a valid and practical measurement of activity and inactivity in young children (Nilsson *et al.* 2002; Kelly *et al.* in press). Mann–Whitney *U* test, or Wilcoxon signed rank test where appropriate, was used to test the significance of any differences in anthropometric characteristics, engagement in physical activity or inactive behaviour between the Irish rural subjects and the Scottish urban subjects (see Table). The significance level was set at 5%.

	Irish children (*n* 41)		Scottish children (*n* 41)		
Variable	Median	Range	Median	Range	*P**
Total activity (cpm)	726	395–1287	762	477–1062	0.61
% Sedentary time (≤1100 cpm)	77.6	53.0–89.3	78.7	66.8–88.5	0.83
% Light activity (>1101–<3200 cpm)	18.8	9.9–39.9	18	3 10.0–25.8	0.83
% Moderate–vigorous activity (≥3200 cpm)	3.0	0.7–8.2	2.8	0.8–8.5	0.89

* *P* values obtained by Wilcoxon signed rank test.

We found no significant differences in any of the anthropometric measurements in the two settings. Irish and Scottish boys were significantly more active than the Irish and Scottish girls but there were no significant differences in total activity for each sex group in the two areas. Engagement in sedentary behaviour was high in both the Irish and Scottish children. We found no significant differences between the two sex groups for any aspect of physical activity or inactivity.

The present study has been the first to measure physical activity and inactivity in Irish rural children objectively. It shows that levels of physical activity and engagement in inactivity are similar in children in rural Ireland and in urban Scotland. The amount of inactivity in both settings at this young age is a major concern.

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Food consumption patterns associated with high intakes of cereal and dairy products in Irish adults. By S.J. BURKE, S.N. McCARTHY, N.A. O'DWYER and M.J. GIBNEY, *Department of Clinical Medicine, Trinity College Dublin, Republic of Ireland*

In Irish adults, a high consumption of reduced-fat milk and yoghurt has been found to be associated with better nutrient-quality diets than a low consumption of reduced-fat milk and yoghurt, or indeed a high consumption of other dairy products (Burke *et al.* 2003). This is also the case with a high consumption of wholemeal bread and breakfast cereals. The present study was carried out to determine if high intakes of these foods were associated with food choices that result in better nutrient quality overall.

The North/South Ireland Food Consumption Survey database (*n* 958) was used to further examine the dietary patterns of those in the highest tertile of consumption of these foods. A cross-tabulation was carried out to examine whether the high or low consumption of other food groups may have contributed to the better nutrient-quality diets observed in the high consumers of wholemeal bread, breakfast cereals, reduced-fat milk and yoghurt. The results of the analysis for the food groups of most interest are shown in the Table.

	Wholemeal bread	Breakfast cereals	Reduced-fat milk	Yoghurt
Fruit and vegetables	+	+	NS	+
Potatoes (boiled, mashed and baked)	+	+	+	NS
Chips and processed potatoes	–	–	NS	NS
Savoury snacks (including nuts)	–	–	NS	NS
Alcoholic beverages	–	–	NS	–
White bread	–	–	NS	–f
Breakfast cereals	+m	NA	NS	+
Wholemeal bread	NA	+	+	+
Total cereals	+	+	NS	NS
Yoghurt	+f	+f, –m	+	NA
Total dairy	NS	+	+	+

+, High consumers of wholemeal bread, breakfast cereals, reduced-fat milk and yoghurt are high consumers of the listed food groups; –, high consumers of wholemeal bread, breakfast cereals, reduced-fat milk and yoghurt are low consumers of the listed food groups; f/m, trend for females/males only; NA, not applicable; NS, no significant interaction.

For the high consumers of wholemeal bread consumption, a large proportion were found to be high consumers of butter and spreads (men only), low-fat spreads, fruit and vegetables, potatoes, fish and fish dishes, breakfast cereals (men only), total cereals, reduced-fat milk (men only) and yoghurt (women only). They were also found to be low consumers of chips, savoury snacks, alcoholic beverages and white bread. High consumers of breakfast cereals appear to be high consumers of fruit and vegetables, potatoes, wholemeal bread, cakes, pastries and buns (men only), total cereals and total dairy. They were found to be low consumers of chips, meat products, savoury snacks, alcoholic beverages and white bread. High consumers of reduced-fat milk were found to be high consumers of potatoes, wholemeal bread, yoghurt and total dairy, and low consumers of poultry (men only), full-fat milk and cheese (men only). High consumers of yoghurt were observed to be high consumers of fruit and vegetables, wholemeal bread, breakfast cereals, reduced-fat milk and total dairy, and low consumers of alcoholic beverages and white bread (women only).

The present results imply that it is not only the high consumption of wholemeal bread, breakfast cereals, reduced-fat milk and yoghurt that leads to a better nutrient-quality diet, but rather the overall dietary pattern the high consumers of these food groups follow, for example, the high consumption of fruit and vegetables and the low consumption of savoury snacks.

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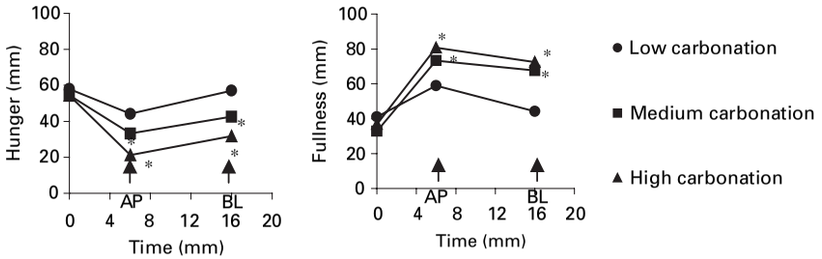
Level of carbonation in a beverage preload increases satiety and decreases subsequent intake. By S.A. MOORHEAD[1], R.W. WELCH[1], M.B.E. LIVINGSTONE[1], T.A. McCALL[1], and A. DUNNE[2], [1]*Northern Ireland Centre for Food and Health (NICHE), University of Ulster, Coleraine, BT52 1SA and* [2]*Statistics Department, University College Dublin, Dublin 4, Ireland*

Obesity, which is an increasing problem, occurs when energy intake is greater than energy expenditure. Strategies to prevent or alleviate obesity comprise increasing energy expenditure and/or decreasing energy intake (National Audit Office, 2001). A previous study has shown that gas (air) incorporated into a preload (yoghurt-based milkshakes) to make a foam with increased total volume, increased satiety and decreased energy intake at the next meal (Rolls *et al.* 2000). The aim of the present study was to evaluate the effects of varying levels of dissolved gas (CO_2) in carbonated beverages served as a preload on satiety and subsequent intakes.

The study was a repeated measures, randomised, within-subject crossover design. Men and women (*n* 30) participated on 3 occasions, 1 week apart. On each occasion the subjects consumed a standard breakfast (25% estimated energy intake). A preload was served 180 min later, which was an orange flavoured beverage (400 ml; 639 kJ), served with 3 levels of carbonation, low, medium and high (338, 494, 728 mg CO_2/100 ml respectively). All 3 beverages had identical energy and macronutrient contents. Time taken to drink was equalised at 6 mins. An *ad libitum* lunch meal was served 10 mins after completion of the preload, at which intakes were assessed. Satiety was measured by subjective assessment using visual analogue scales completed immediately before and after the preload and before the lunch meal. Subjects completed food diaries for the remainder of the day.

The subjects were significantly ($P<0.05$) less hungry, and felt fuller after consuming the beverages with medium and high carbonation compared to the low carbonation beverage, up to 16 min post consumption (immediately before lunch).



AP, after preload; BL, before lunch; * significantly different from low carbonation ($P<0.05$).

At the *ad libitum* lunch meal, weight of food, and energy and macronutrient intakes decreased in the order low >medium >high carbonation ($P<0.05$). However there were no significant differences in intakes for the remainder of the day, indicating that these effects were short-term. The table shows intakes at the *ad libitum* lunch meal following the carbonated beverage preloads.

	Level of carbonation					
	Low (*n* 30)		Medium (*n* 30)		High (*n* 30)	
	Mean	SD	Mean	SD	Mean	SD
Weight of food (g)	857.7[a]	202.0	764.6[b]	235.6	681.9[c]	184.6
Weight of drink (g)	330.0[a]	195.7	312.6[a]	136.0	301.5[a]	172.3
Energy (kJ)	4345.9[a]	1450.6	3939.4[ab]	1174.0	3621.1[b]	1022.5
Protein (g)	54.1[a]	16.2	50.4[b]	18.1	47.4[b]	14.7
Fat (g)	60.6[a]	20.4	54.6[b]	20.8	50.1[b]	16.1
Carbohydrate (g)	74.0[a]	18.2	66.4[b]	13.2	60.1[b]	17.9

Means in the same rows with same superscript letters are not significantly different ($P<0.05$).

Taken with previous results (Rolls *et al.* 2000), this study indicates that dissolved gas, as well as gas incorporated as a foam, can increase satiety and decrease subsequent intakes, at least in the short-term.

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The nutrient intake of older adults living in sheltered housing accommodation. By C.E. WOOD[1], R. LANG[1], V. ZOHOURI[1], A.J. ADAMSON[2], C.J. SEAL[2], J.C. MATHERS[2] and P.J. MOYNIHAN[1], [1]*School of Dental Sciences, University of Newcastle upon Tyne, UK, NE2 4BW and* [2]*Human Nutrition Research Centre, Wellcome Research Laboratories, RVI, Queen Victoria Road, Newcastle upon Tyne, UK, NE1 4LP*

Few data exist on the intake of nutrients in older adults from lower income groups. The National Diet and Nutrition Survey of people aged 65 years and over showed those with lower socio-economic status had lower intakes of energy, protein, carbohydrate, fibre and some vitamins, notably vitamin C, and minerals compared with the rest of the sample population (Finch *et al.* 1998).

As part of a larger nutrition intervention study, the aim of the present research was to obtain baseline information on the nutrient intakes of older adults living in sheltered accommodation in socially deprived areas of Tyne and Wear.

Subjects (*n* 288) were asked to complete two 3 d estimated food diaries, each consisting of 2 weekdays and 1 weekend day, with 2 weeks between each recording period. On completion of each food diary subjects were interviewed at their home to clarify and expand on recorded food intake and, with the help of a photographic atlas (Nelson *et al.* 1997), to determine the portion size. All foods and drinks consumed were coded using supplements to McCance and Widdowson's *The Composition of Foods* and data were entered into a purpose-designed Microsoft Access database and subsequently analysed using SPSS.

Both food diaries were completed by 201 subjects (thirty-one males, 170 females; 69.8% completion rate). The mean age of the participants was 76.4 (SD 5.5, range 61–90) years. The Table shows the mean daily nutrient intake of the sample population, by gender. Values for fat, saturated fat, carbohydrate and non-milk extrinsic sugars are presented in the Table as a percentage of the total food energy. The mean physical activity level was calculated and was 1.1 (95% CI 1.1, 1.2); for males it was 1.3 (95% CI 1.2, 1.4) and for females it was 1.1 (95% CI 1.0, 1.1).

	Male		Female		Total	
Nutrient	Mean	SD	Mean	SD	Mean	SD
Energy (MJ)	8.1	1.98	5.9	1.45	6.3	1.73
% Food energy from fat	37.5	6.60	35.0	6.97	35.4	6.96
% Food energy from saturated fat	13.2	3.47	13.4	4.82	13.4	4.63
% Food energy from carbohydrate	45.1	3.47	47.9	6.40	47.5	6.60
% Food energy from non-milk extrinsic sugars	9.2	5.51	9.8	5.40	9.7	5.41
Total fat (g)	82.8	25.52	56.9	19.63	60.9	22.62
Saturated fat (g)	28.9	10.60	21.9	10.58	23.0	10.86
Carbohydrate (g)	229.5	69.35	177.7	48.86	185.7	55.59
Non-milk extrinsic sugars (g)	48.8	31.17	37.2	23.56	38.9	25.12
NSP (g)	13.6	7.06	10.6	4.30	11.1	4.93
Protein (g)	81.7	22.38	57.9	14.33	61.6	17.97
Ca (mg)	835.8	242.85	647.9	186.93	676.9	207.37
Folate (μg)	271.8	103.07	190.6	59.70	203.1	73.97
Fe (mg)	11.8	5.39	8.3	3.26	8.9	3.86
Vitamin C (mg)	70.6	54.97	58.0	39.36	59.9	42.23
Vitamin D (μg)	4.0	4.08	2.3	2.42	2.6	2.80

Intake of fat, in particular saturated fat, was above the dietary reference value (DRV) whilst intakes of fibre and vitamin D were below the DRV (Department of Health, 1991). On the other hand, intakes of non-milk extrinsic sugars were below and within the range of the DRV. In summary, as a population group, older adults could benefit from dietary interventions that aim to reduce saturated fat and increase fibre and vitamin D intakes.

The present study was funded by the Food Standards Agency contract N09015. The views expressed are the authors' own.

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Enteral tube feeding: do patients receive their full prescription of formula? By L. HILL[1], K. WHELAN[1], P.A. JUDD[2], V.R. PREEDY[1] and M.A. TAYLOR[3], [1]*Department of Nutrition and Dietetics, King's College London, London, UK, SE1 9NN,* [2]*Lancashire School of Health and Postgraduate Medicine, University of Central Lancashire, Preston, UK, PR1 2HE and* [3]*School of Biomedical Sciences, University of Nottingham, Nottingham, UK, NG7 2UH*

Enteral tube feeding (ETF) is a common method of nutritional support for patients in both hospital and community settings. In patients receiving ETF on the intensive care unit, tube-related and gastrointestinal complications can occur that impede the delivery of enteral formula (Adam & Batson, 1997). However, little is known regarding the volume of formula delivered to patients receiving ETF on general wards. The aim of the present study was to measure the volume of formula delivered to patients starting ETF on general wards, comparing days on which patients did, and did not, remove their nasogastric tube (NGT) or have diarrhoea.

Patients starting ETF with a fibre-free enteral formula via an NGT were prospectively recruited from general wards at St George's Hospital, London. The patients' medical, nursing and dietetic notes were monitored daily for 14 d or until the introduction of oral diet. The volume of enteral formula prescribed and delivered was taken daily from the dietetic prescription sheets and nursing fluid balance charts, respectively, and the percentage delivery calculated. The removal and re-insertion of the NGT was noted from patient inspection and from nursing records. The incidence of diarrhoea was recorded daily by nursing staff using a validated chart (Whelan *et al.* 2004).

Twenty-eight patients (seven males, twenty-one females, mean age 75 years 8 months) were monitored for a total of 306 patient days. The mean volume of enteral formula prescribed was 1464 ml/d; however the mean volume delivered was only 1282 ml/d (87.6%). Only eleven patients (39%) maintained an NGT *in situ* for the entire monitoring period. The non-elective removal of an NGT complicated ETF on a total of 51 patient days (17%), with one patient removing thirteen tubes on 8 different days. Patients experienced diarrhoea on 12% of feeding days. The percentage volume of formula delivered was compared on days when these complications (NGT removed; diarrhoea) were present or absent.

Days when patients …	Removed the NGT					Had diarrhoea				
	No (*n* 255 d)		Yes (*n* 51 d)			No (*n* 268 d)		Yes (*n* 38 d)		
	Mean	SEM	Mean	SEM	*P* value*	Mean	SEM	Mean	SEM	*P* value*
Formula delivered *v.* prescribed (%)	95.8	0.8	46.4	4.2	<0.0005	88.6	1.5	80.0	4.2	0.673
Marginal mean delivered (%)†	93.0	1.6	47.8	3.0		71.1	1.4	69.7	3.2	

*Two-way ANOVA to test two variables independent of each other.
†Estimated marginal mean adjusts for the remaining term in the model.

Patients on general hospital wards do not all receive their full prescription of enteral formula, which will result in their calculated energy, nutrient and fluid requirements not being achieved. Many factors may affect the percentage delivery of enteral formula; however, in this cohort, NGT removal was very common and was a major factor impeding delivery. Patients who experienced diarrhoea received only 80% of their formula prescription, although this was not significantly different from those without diarrhoea. Health professionals involved in ETF should take prompt action when a patient removes an NGT, since a major reduction in the delivery of formula may ensue. The efficacy, and the ethics, of alternative modes and routes of feeding may need to be considered.

This research was part of a study of the faecal microflora during ETF, and was partially funded by Nestlé UK.

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Gut hormone changes may explain appetite and glycaemic control effects of bariatric surgery. By C.W. LE ROUX[1], S.J.B. AYLWIN[2], S. SHUREY[4], V. PRASAD[1], F. COYLE[2], P. DARROCH[2], M.A. GHATEI[1], A.G. PATEL[3] and S.R. BLOOM[1], [1]*Department of Metabolic Medicine, Hammersmith Hospital, Imperial College London, London, UK, Departments of* [2]*Endocrinology,* [3]*Surgery, King's College Hospital, London, UK and* [4]*Department of Experimental Surgery, Northwick Park Hospital, Imperial College London, London, UK*

The beneficial effect of bariatric surgery on glycaemic control and reduced appetite is established, but the mechanism remains unclear. Gut hormones have been reported to physiologically reduce appetite and may also act as incretins. We aimed to investigate gut hormone responses following bariatric surgery. In the present paper we show the inhibition of gut hormones and the effect on weight gain; also the present study investigated the putative satiety gut hormone peptide YY (PYY) in a rodent model of bariatric surgery.

The gut hormone response to a 1757 kJ (420 kcal) standard meal in twelve patients following gastric bypass or gastric banding surgery was compared. In a separate study we investigated a single individual who had reactive hypoglycaemia following a Bilroth II partial gastrectomy; an operation with a different indication but similar post-operative anatomy. The gut hormone response was compared before and after treatment with somatostatin. A rodent model of bariatric surgery was used to evaluate the contribution of PYY to appetite reduction.

Obese patients treated surgically by gastric bypass were found to have an increased satiety and increased PYY, glucagon-like peptide (GLP)−1 and insulin responses ($P<0.01$) compared with gastric banding as well as lean and obese controls. In the patient following Bilroth II gastrectomy an oral glucose tolerance test (OGTT) revealed an exaggerated PYY, GLP-1 and insulin response. Treatment with somatostatin alleviated symptoms and suppressed the PYY response completely ($P<0.01$), while also attenuating GLP-1 ($P<0.01$) and insulin ($P<0.01$) responses to an OGTT. While on somatostatin, the patient gained 6 kg of weight. Following intestinal bypass, rats consumed less food and maintained a lower body weight, while having PYY levels three times higher than sham-operated rats ($P<0.01$). There was no evidence of malabsorption or inflammation in either group. Blocking PYY with a specific PYY antibody or somatostatin resulted in increased food intake in the bypass rats ($P<0.05$).

To conclude, significant changes in gut hormones are observed following gastric bypass but not banding surgery. Somatostatin suppressed PYY, GLP-1 and insulin and was associated with weight gain in one patient. Intestinal bypass in rats was associated with reduced food intake, decreased weight and elevated PYY levels. Changes in gut hormones may partly explain the beneficial effect of bariatric surgery on glycaemic control and appetite.

Can differences in filament length explain the effect of mycoprotein on glucose diffusion *in vitro*?

By L.I. MARKS[1], A.M. GALLAGHER[1], J.J. STRAIN[1], G. RODGER[2] and R.W. WELCH[1], [1]*Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine, UK, BT52 1SA and* [2]*Marlow Foods Ltd, Stokesley, North Yorkshire, UK, TS9 7AB*

Mycoprotein is a food product derived by continuous fermentation of a filamentous fungus, *Fusarium venenatum*. It is a high-fibre, high-protein, low-fat alternative to meat (Rodger, 2001). Previous studies have demonstrated beneficial effects of mycoprotein on blood lipids (Turnbull *et al.* 1992), appetite variables (Turnbull *et al.* 1993) and glycaemic responses (Turnbull & Ward, 1995). The mechanisms by which mycoprotein modulates these effects are not fully understood. It is possible that altering the physical characteristics, in particular modulation of the filament length, may explain how mycoprotein lowers glycaemia. The present study investigated the effect of altering filament length, by homogenisation, on glucose diffusion *in vitro*.

A simple *in vitro* model was developed to evaluate the effect of varying mycoprotein filament length on glucose diffusion across a dialysis membrane (8000MWCO). Mycoprotein filament length (unhomogenised range, 400–700 µm, Marlow Foods) was modified by homogenisation for 1 min, 3 min, 5 min, 10 min, and 30 min (longest to shortest filament length). The filament length was measured by phase contrast microscopy (see Table). Glucose The effect of mycoprotein (0.1 g/ml) on glucose movement over 360 min (expressed as area under the curve; AUC), was compared with guar which is known to decrease glucose absorption *in vivo* (0.5% w/v, 220 mM glucose) and control (220 mM glucose).

	Concentration		Filament length (µm)		Glucose movement AUC (mmol/l per 360 min)			
			Range	Median	Mean	SD	% Decrease	*P*
Control	Glucose	220 mM	–	–	7.51	0.07	–	
	Guar	0.5%	–	–	6.12	0.02	19	**
Homogenisation time	1 min	10% (w/v)	193.6–371.9	282.8	3.97	0.02	47	***
	3 min	10% (w/v)	126.7–247.8	187.3	4.54	0.01	40	***
	5 min	10% (w/v)	43.3–129.9	86.6	4.62	0.02	39	***
	10 min	10% (w/v)	35.2–71.8	53.5	5.03	0.02	33	***
	30 min	10% (w/v)	12.6–32.6	22.6	5.98	0.09	20	**

Significantly different from glucose control: ** $P<0.01$, *** $P<0.001$.

Mycoprotein homogenised for 1, 3, 5, and 10 min reduced glucose movement by 47, 40, 39, and 33% respectively as compared with 220 mM-glucose control ($P<0.001$). The longest filament length (1 min) was 57% more effective at reducing glucose diffusion compared with the shortest filament length (30 min). However, the decrease (20%) exerted by the shortest filament length was significantly different ($P<0.01$) as compared with the glucose control. This was similar to the effect exerted by 0.5% guar solution.

The present study suggests that the filamentous nature of mycoprotein may be a contributing factor to the mechanisms by which mycoprotein elicits beneficial glycaemic responses. Furthermore these *in vitro* data suggest that the mycoprotein experiencing minimal structural changes (for instance chopping) during manufacturing may have the best potential benefit on glycaemic responses in consumers.

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Can differences in filament length of mycoprotein explain the effects of mycoprotein on glycaemic responses, appetite variables, and gastric emptying in healthy subjects?

By L.I. MARKS[1], A.M. GALLAGHER[1], J.J. STRAIN[1], G. RODGER[2] and R.W. WELCH[1], [1]*Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine, UK, BT52 1SA and* [2]*Marlow Foods Ltd, Stokesley, North Yorkshire, UK, TS9 7AB*

Mycoprotein is a food product derived from a filamentous fungus, *Fusarium venenatum*. Previous studies in healthy subjects have shown a beneficial effect of mycoprotein on blood lipids (Turnbull *et al.* 1992), appetite variables (Turnbull *et al.* 1993), and glycaemic responses (Turnbull & Ward, 1995). The mechanisms by which mycoprotein lowers glycaemia are not fully understood. It is possible that altering the physical characteristics, in particular modulation of the filament length, may explain how mycoprotein lowers glycaemia. The present *in vivo* study aimed to extend previous *in vitro* work, which showed that filament length contributed to differences in glucose diffusion across a dialysis membrane.

In the present 3-week randomised cross-over study, twelve healthy fasting subjects (five male and seven female) consumed one of two test drinks, or a control drink. All drinks contained 50 g carbohydrate (glucose) and 1 g paracetamol (as a marker of the rate of gastric emptying). In addition to this, test drinks contained 90 g wet weight mycoprotein (22 g dry weight) of long or short filament length. Mycoprotein was homogenised for 1 or 30 min to produce mycoprotein of long filament length (MFL) or short filament length (MFS) respectively. Filament length was measured by phase contrast microscopy. MFL (homogenised for 1 min) had a median length of 293 (range 217.5–369.1) µm. MFS (homogenised for 30 min) had a median length of 27 (range 15.2–38.4) µm. Fasting and postprandial blood samples were collected at timed intervals (30, 60, 90 and 120 min) to determine serum insulin and paracetamol and plasma glucose and glucagon-like peptide 1 (GLP-1). Visual analogue scales (VAS) were completed immediately pre-meal and at timed intervals (30, 60, 90, 120 min), to determine satiety ratings.

Both MFL and MFS significantly decreased postprandial blood concentrations of insulin ($P<0.05$) and GLP-1 ($P<0.05$) at 30 min, and paracetamol ($P<0.01$) at 30 and 60 min as compared with control. There were no significant differences between the effects of MFL and MFS on postprandial insulin or GLP-1 responses. Postprandial paracetamol was significantly decreased ($P<0.05$) following the consumption of MFL as compared with MFS at 60 and 90 min, thus suggesting that MFL slows the rate of gastric emptying. Overall differences in postprandial glucose responses were not significant after the consumption of MFL or MFS when as compared with control. Area under the curve at 30 min for postprandial glucose responses, although not significant, was decreased by 8.1% by MFL and 6.3% by MFS. Subjects reported significantly greater feelings of satiety, on the VAS, following the consumption of MFL and MFS, as compared with control ($P<0.01$). However, no significant differences in satiety ratings between MFL and MFS were reported indicating that changes to the morphology of mycoprotein did not further modulate its effects on satiety.

The results of the present study suggest that the beneficial glycaemic responses *in vivo* may be attributed at least in part to the filament length. It is possible that other contributing factors during the digestion of mycoprotein may take precedence over the effects of filament length on glycaemic responses and satiety.

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The interaction between smoking status and body mass index exaggerates cardiovascular risk factors. By M. AKBARTABARTOORI, C.R. HANKEY and M.E.J. LEAN, *Human Nutrition at Glasgow, Division of Developmental Medicine, University of Glasgow, Glasgow Royal Infirmary, Glasgow, UK, G31 2ER*

CVD is a major cause of death worldwide. Multiple risk factors for CVD have been identified, and each of them may have either a separate or a synergistic effect. Cigarette smoking, overweight and obesity are major risk factors for CVD, which are also associated with other risk factors including raised plasma lipids, coagulation factors and measures of inflammation. Cardiovascular risks increase when smoking is combined with overweight or obesity. The aim of the present study was to define the interaction of smoking status and overweight or obesity with some cardiovascular risk factors in adults aged 16–74 years who participated in the cross-sectional Scottish Health Survey 1998 (Shaw *et al.* 2000). Of the total 9047 adults who participated in the survey, blood samples were obtained by a nurse from 84% of men and 80% of women. For the present study four measured cardiovascular risk factors were used as dichotomous dependent variables in logistic regression analysis. Cigarette smoking status and BMI were combined into nine categories and non-smokers with BMI below 25 kg/m^2 were defined as the reference category.

	Cholesterol >5.4 mmol/l (*n* 5924)		HDL-cholesterol <1 mmol/l (*n* 5891)		Fibrinogen >3 g/l (*n* 5460)		C-reactive protein>3 mg/l (*n* 5988)	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Non-smokers BMI<25 kg/m^2	1		1		1		1	
Ex-smokers BMI<25 kg/m^2	0.85	0.60, 1.20	1.50	0.82, 2.76	1.44†	1.05, 1.98	1.60†	1.13, 2.26
Current smokers BMI<25 kg/m^2	1.21	0.93, 1.60	1.96†	1.26, 3.04	2.29*	1.81, 2.90	2.20*	1.70, 2.84
Non-smokers BMI 25–30 kg/m^2	1.76*	1.39, 2.24	2.45*	1.62, 3.70	1.57*	1.24, 1.99	1.92*	1.49, 2.48
Ex-smokers BMI 25–30 kg/m^2	1.70*	1.30, 2.20	2.45*	1.54, 3.88	1.39†	1.06, 1.82	2.28*	1.72, 3.03
Current smokers BMI 25–30 kg/m^2	2.31*	1.77, 3.00	4.52*	2.96, 6.91	3.30*	2.54, 4.25	4.08*	3.13, 5.32
Non-smokers BMI≥30 kg/m^2	2.31*	1.77, 3.01	4.60*	2.99, 7.09	2.61*	2.00, 3.40	5.69*	4.35, 7.44
Ex-smokers BMI≥30 kg/m^2	1.89*	1.39, 2.56	6.90*	4.38, 10.8	2.52*	1.85, 3.44	6.45*	4.76, 8.73
Current smokers BMI≥30 kg/m^2	2.70*	1.98, 3.68	9.47*	6.06, 14.8	4.66*	3.42, 6.35	9.11*	6.67, 12.5

The results were adjusted for social class, physical activity, age, gender, alcohol consumption and fruit and vegetables. Significantly different from the reference category: *P<0.0001, †P<0.03.

Smoking, overweight and obesity were significantly associated with greater odds ratios (OR) of having elevated cholesterol, C-reactive protein, fibrinogen and lower levels of HDL-cholesterol than non-smokers with normal BMI (P<0.03). Obese current smokers had the greatest OR for all the risk factors, particularly for low HDL-cholesterol and C-reactive protein (P<0.001). The OR of having HDL-cholesterol below 1 mmol/l and C-reactive protein above 3 mg/l, the high-risk category, was almost nine times more than the reference category. Ex-smokers had lower OR for the risk factors compared with current smokers in different BMI categories.

The results show that the combination of smoking and obesity exaggerates the risks of CVD synergistically, and indicate a need to tackle both of these issues simultaneously.

Shaw A, McMunn A & Field J (2000) *The Scottish Health Survey*, vol. 1, 2. HMSO: London.

Obesity is a risk factor for adenocarcinoma of the oesophagus. By A. RYAN, S. ROWLEY, P. FLOOD and J.V. REYNOLDS, *Departments of Clinical Nutrition and Surgery, St James's Hospital, Trinity College, Dublin, Republic of Ireland*

Recent evidence suggests that obesity may be responsible for the rising incidence of oesophageal adenocarcinoma. No link with squamous cell carcinoma (SCC) has been found. Ireland has seen a 67% increase in the incidence of obesity in the last decade (Irish Universities Nutrition Alliance, 2001). Ireland has also one of the highest age-standardised rates of oesophageal cancer in the Western world, with the incidence of adenocarcinoma increasing dramatically over the past 20 years. The mechanism linking obesity to oesophageal adenocarcinoma may relate to either an increase in the frequency of acid reflux brought about by increasing abdominal adiposity, or may relate to the metabolic syndrome of obesity.

The aims of the present study were to (1) address the hypothesis that obesity is related to oesophageal cancer in an Irish population, (2) to compare adenocarcinoma to SCC and (3) compare cancer cases to healthy controls.

All histologically confirmed cases of oesophageal cancer diagnosed between 1989 and 2004 at our institution were included in the study. Nutritional data on 806 patients (475 adenocarcinoma and 331 SCC) were available. Pre-illness BMI was calculated from reported pre-illness weight. Data were compared with 1531 age-matched healthy controls. Logistic regression analysis was used to calculate the odds ratio (OR) of developing adenocarcinoma or SCC when above the normal BMI category.

Based on pre-illness BMI, 78% of adenocarcinoma patients were overweight or obese *v.* 27% of SCC (P<0.001). Adenocarcinoma patients had a pre-illness BMI that was significantly greater than healthy controls (28 *v.* 26.5 kg/m^2; P<0.001). SCC patients were significantly lighter than controls (24 *v.* 26.5 kg/m^2; P<0.001). The odds of adenocarcinoma increased significantly with increasing BMI (OR 4.1 for highest quartile *v.* lowest quartile; P<0.001). A significant inverse relationship between BMI and SCC was found (OR 0.13 highest BMI quartile *v.* lowest; P<0.001).

To conclude, the present study is the second largest case–control study of obesity and oesophageal cancer. We have shown that BMI and oesophageal adenocarcinoma are directly related in an Irish population with the OR for oesophageal adenocarcinoma rising significantly with increasing BMI but not for SCC. The prevalence of obesity in Ireland, and in Western countries, could be important in understanding the increasing incidence of this tumour.

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Prenatal exposure to a low-protein diet programmes a preference for high-fat foods in the rat. By L. BELLINGER, C. LILLEY and S.C. LANGLEY-EVANS, *Centre for Reproduction and Early Life, School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, UK, LE12 5RD*

Epidemiological associations between intra-uterine growth restriction and disease in later life have prompted research to determine the relationship between nutritional insults to the fetus and metabolic disorders in adult life. Studies of animal models suggest that undernutrition in pregnancy may predispose the offspring to develop obesity and associated metabolic conditions in later, adult life (Vickers *et al.* 2000). One question which has arisen from previous research is whether prenatal undernutrition may lead to obesity through changes in appetite, food preferences, or an alteration in the way the energy is metabolised.

The present study aimed to assess appetite, feeding behaviour and body composition in male and female rats subjected to protein restriction *in utero*. Pregnant Wistar rats were fed either a control diet (180 g casein/kg diet; *n* 5), or a low-protein (LP) diet (90 g casein/kg diet; *n* 5) throughout the entire pregnancy, as previously described (Langley-Evans *et al.* 1994). The resulting offspring were allocated to a self-selection diet protocol to assess appetite and food preferences at either 12 or 30 weeks of age. All rats were individually caged and had free access to three different foods; high-fat (HF; 40 g fat/100 g), high-carbohydrate (HC; 62 g carbohydrate/100 g) and high-protein (HP; 48.5 g protein/100 g).

		Food intake (g/kg body weight per d) for five to twelve animals per group											
		12 weeks						30 weeks					
		HF		HC		HP		HF		HC		HP	
Maternal diet	Sex	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	M	44.01	4.72	28.98	4.84	2.15	0.81	38.26	1.90	8.13	1.28	2.81	1.10
LP	M	54.47	6.39	18.83*	6.55	0.98	1.10	40.90	4.37	10.15	3.66	1.47	0.83
Control	F	54.13	5.22	35.86	5.35	0.92	0.90	49.64	1.90	13.97	3.64	1.43	0.68
LP	F	86.69*	7.00	12.97*	7.17	3.68	1.20	53.77	7.35	14.47	3.14	2.16	1.22

* $P<0.05$ for control *v.* LP of same sex.

Studies of 12-week-old rats indicated that the prenatal environment influenced feeding behaviour, with both male and female LP offspring consuming significantly more of the HF ($P<0.001$) and significantly less of the HC ($P<0.02$) food source than the control animals (Table). However, male LP-exposed rats appeared able to regulate their overall energy and macronutrient intake and there was no significant difference between actual nutrient intakes, or total energy intake between groups. The female LP-exposed rats failed to adjust food intake to maintain a constant energy intake and had higher fat ($P<0.005$) and energy intakes ($P<0.05$) than control females. At 30 weeks the pattern of self-selection that was noted at 12 weeks had disappeared, with no significant differences in food preferences or nutrient intake between the two groups. Analysis of total food intake at both 12 and 30 weeks showed no significant differences between maternal groups. However, when 12-week-old rats were fed standard laboratory chow, the control rats consumed significantly more then the LP-exposed offspring ($P<0.05$).

The present study suggests that LP exposure *in utero* changes food preferences of young adult, but not older animals. These programmed changes may relate to altered structure or function of hypothalamic centres that are involved in appetite regulation. Long-term consumption of a high-fat diet may drive the development of obesity in these animals.

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Quantification of the dietary acidity characteristics of the Atkins diet: estimates of net endogenous non-carbonic acid production (NEAP) and potential renal acid load (PRAL). By R.H.T. GANNON[1], R. BOLEY[1], S.A. NEW[1], A. DELOOY[2], K. FOX[3], J.M.C. WALLACE[4], I. MACDONALD[5], D.J. MILLWARD[1], L. MORGAN[1] and H. TRUBY[1], [1]*School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH,* [2]*Queen Margaret's University College, Edinburgh, EH12 8TS,* [3]*University of Bristol, BS8 1TH,* [4]*University of Ulster, BT52 1SA and* [5]*University of Nottingham, NG7 2RD*

Dietary imbalances of key macro- and/or micronutrients may place an individual at risk of the development of a variety of diseases, including osteoporosis. A lot of media attention has been given to the use of very-high-protein, very-low-carbohydrate weight-loss diets such as the Atkins diet. This regimen is designed to induce ketosis, which may perturb acid–base homeostasis, causing metabolic acidosis. Even mild acidosis can have potentially deleterious consequences in the longer term, leading to hypophosphataemia, increased urinary Ca loss from bone (Kurtz *et al.* 1983), and hence increased propensity to osteoporosis (Reddy *et al.* 2002). The aim of the present study was to quantify the dietary acidity characteristics of the Atkins diet by estimating PRAL and NEAP. These values were calculated using $\sum((\text{protein } (SO_4)+P) - (Mg+K+Ca))$ and protein:K ratio respectively (Frassetto *et al.* 1998; Remer *et al.* 2003).

Subjects for the present study were participants of the Diet Trials Investigation, details of which have been reported elsewhere (Boley *et al.* 2004). Baseline food diaries were analysed by dietitians from the study centres using Windiets. In order to verify their validity, a cut-off value using Goldberg's equation was used to determine under-reporting (Goldberg *et al.* 1991). Nutrient data from subjects not found to be under-reporting were used for the present study (*n* 22).

	Atkins						Control					
	Baseline (*n* 15)			6 months (*n* 8)			Baseline (*n* 7)			6 months (*n* 5)		
	Mean	SEM	95% CI	Mean	SEM	95% CI	Mean	SEM	95% CI	Mean	SEM	95% CI
PRAL (mEq/d)	11.0[a]	3.3	−9.6, 37.5	40.2[a]	5.1	28, 71.6	7.3[a]	7.0	−7.5, 43.3	0.9[b]	4.5	−9.6, 17.4
NEAP (g/mEq per d)	48.7[a]	3.0	32.2, 74.1	83.5[a]	5.5	55, 102.3	44.9[a]	4.2	33.2, 61.4	40.1[b]	3.5	33.1, 53.5
Protein (g)	107.4[a]	3.7	77.5, 129.8	111.3[a]	9.4	88.2, 172.6	94.2[b]	3.9	81.3, 112.2	75.2[b]	5.4	57, 90.3
Ca (mg)	1014[a]	78.9	650, 1672	847[a]	123.4	576, 1403	1674[a]	591	519, 4972	764[a]	115	455, 984
K (mg)	3956[a]	455	3028, 4705	2705[a]	242	2141, 4030	3712[a]	220	3085, 4534	3185[a]	304	2575, 4302

Mean values with unlike superscript letters are significantly different between both groups. Independent-samples *t* test, paired-samples *t* test, Mann–Whitney U test and Wilcoxon signed rank test were used for the analyses.

As shown in the Table, PRAL and NEAP values at baseline for both groups were not significant respectively. However, when the above variables were compared at 6 months, there were significant differences ($P<0.001$) with intakes of PRAL and NEAP being much higher in the Atkins group compared with the controls. Intakes of protein were also found to be significantly higher in the Atkins group, with significant differences between the Atkins and control group ($P<0.05$). K intake decreased significantly from baseline values in the Atkins group at 6 months ($P<0.05$). No significant associations were found for K intake at baseline or 6 months between groups. There were no significant differences between Ca intake between baseline and 6 months in the Atkins group, or between Atkins and the control group. However, there were significant differences ($P<0.05$) in Ca intakes in the control group.

These preliminary findings indicate that the acid-generating potential of the Atkins diet is high, reflective of the high protein and low K contents of the diet, which may, in turn, be detrimental to the skeleton (New *et al.* 2004). However subject numbers are small and require investigation in a larger study group. Further research is currently underway to determine the effects of the Atkins diet on indices of bone health.

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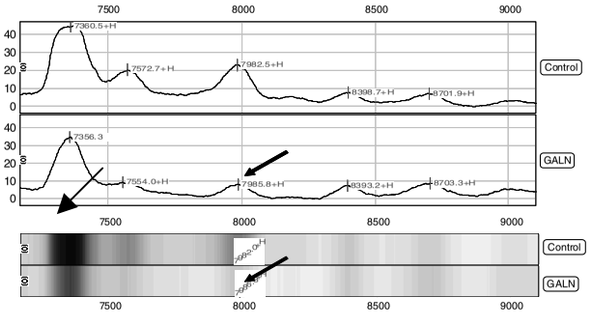
Identification of putative biomarkers of oxidative stress: an analysis of rat plasma by surface-enhanced laser desorption ionisation (SELDI) time-of-flight mass spectrometry (TOF-MS). By M.C.Y. WONG, S. DRAYTON, R. RAJENDRAM, M. BAILEY, P.W. EMERY, V.R. PREEDY and H. WISEMAN, *Nutritional Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9NN*

Oxidative stress and imbalance is an important component of many nutritional disorders, leading to a spectrum of abnormalities ranging from disruptions in the cellular milieu to overt membrane and organ pathology. Studies in this area have been facilitated by the use of a variety of biomarkers, such as isoprostanes and aldehydes and are often used to assess the efficacy of antioxidant strategies (for example, see Wiseman *et al.* 2000). We hypothesised, however, that there may also be hitherto uncharacterised markers of oxidative stress and/or organ damage. To test this, we used surface-enhanced laser desorption ionisation (SELDI) time-of-flight mass spectrometry (TOF-MS) to identify changes in the plasma of rats treated with D-galactosamine (GALN), an oxidative perturbant (Sun *et al.* 2003).

Male Wistar rats (about 0.1 kg body weight) were injected with GALN (1 g/kg body weight) dissolved in saline (0.9 mol NaCl/l). Controls were injected with an identical volume of saline. Food was withdrawn after injections and rats were killed by decapitation after 24 h. Blood was immediately collected for subsequent extraction of plasma. Plasma was analysed using SAX2 chips with cationic surfaces (ProteinChip system; Ciphergen Biosystems). Binding reactions of proteins to the chip surface were carried out at pH 8 after which surfaces were coated with sinapinic acid, an energy absorbing material (EAM). Positions 40–80 were scanned using the maximal (i.e. 280 setting) laser intensity and optimised data were acquired from proteins with a molecular weight of 0.5 to 250 kDa. There were five transients per position and the detector sensitivity was fixed at setting 9. Peaks were detected with the Biomarker Wizard using default parameters. All data were then exported to SPSS (version 11.0 for Windows) and differences between means were analysed by two-way ANOVA followed by least significant differences test using the pooled estimate of variance (which also included data not presented here). Differences were considered to be significant at $P<0.05$.

We determined that peaks below 3 kDa may be artificial, due to either cross-contamination between samples or the presence of 'ghost' proteins in the EAM. We also showed that a total of 138 protein peaks with good discriminatory indices were identified in each sample. The relative abundance of thirty-eight proteins was significantly altered by GALN with P ranging from <0.05 to <0.001; seven increased and thirty-one decreased. Presently the identities of these proteins remain un-elucidated and it is not known whether they relate to reactive oxygen species *per se* or organ damage as a consequence of reactive oxygen species. However, the complete analysis of thirty-two separate plasma samples took less than 1 d and strategies are now in place to identity putative biomarkers in target organs such as the liver and brain. We conclude that SELDI TOF-MS is a powerful screening technique for detecting alteration in plasma and a preliminary tool for identifying putative biomarkers.



The upper figure shows a selected area (7 kDa–9 kDa) of the averaged spectra from the two different groups. The lower figure shows 'gel views' of the same section of the spectra. The protein peak at molecular weight 7989 Da was significantly reduced by GALN treatment. Mean peak intensities were (n 17 control, n 15 GALN): control 25.48 (SEM 2.30); GALN 13.72 (SEM 2.47); $P<0.01$).

Sun F, Hamagawa E, Tsutsui C, Sakaguchi N, Kakuta Y, Tokumaru S & Kojo S (2003) *Biochemical Pharmacology* **65**, 101–107.
Wiseman H, O'Reilly JD, Adlercreutz H, Mallet AI, Bowey EA, Rowland IR & Sanders TAB (2000) *American Journal of Clinical Nutrition* **72**, 395–400.

Is the ferric reducing antioxidant power assay a good marker of oxidative stress in laboratory animals? By M.C.Y. WONG, E. LYNN, R. HUNTER, R. SRIRAJASKANTHAN, V.R. PREEDY and H. WISEMAN, *Nutritional Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9NN*

Studies in laboratory animals have suggested that the ferric reducing antioxidant power (FRAP) assay is a suitable method for evaluating oxidative stress and investigating the efficacy of novel agents or treatment regimens.

We hypothesised that the FRAP assay would also be useful in determining antioxidant power as a consequence of starvation, ethanol (EtOH) administration and treatment with a phyto-oestrogen (i.e. daidzein). To test this, male Wistar rats (0.1 kg body weight; BW) were ranked and assigned to eight groups (n 6 rats per group). They were subjected to a 'pre-treatment' for 2 d followed by a 'treatment' for 2.5 or 24 h as follows (all as pre-treatment+treatment): group A, carrier+saline 2.5 h; group B, daidzein+saline 2.5 h; group C, carrier+EtOH 2.5 h; group D, daidzein+EtOH 2.5 h; group E, carrier+saline 24 h; group F, daidzein+saline 24 h; group G, carrier+EtOH 24 h; group H, daidzein+EtOH 24 h. The carrier was Intralipid, and daidzein was mixed with this emulsion before administration at a dose of 100 mg/kg BW. EtOH was injected as a dose of 75 mmol/kg BW; all doses were injected intraperitoneally. Rats treated for 24 h were fasted before termination of the study (groups E to H) while rats treated for 2.5 h were allowed to feed *ad libitum* (groups A to D). All rats were killed by decapitation and blood collected in heparinised tubes for subsequent analysis by the FRAP assay.

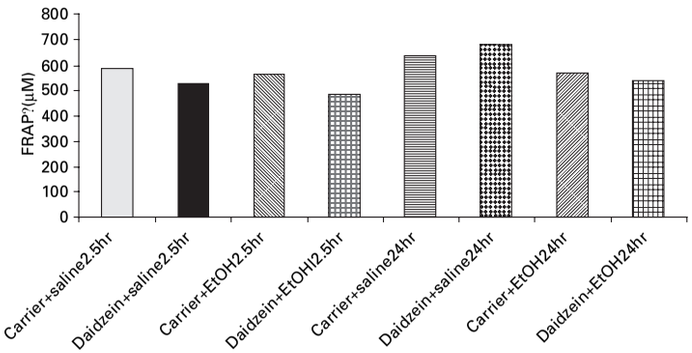


Fig. 1. Data are expressed as means (n 6 in all groups except n 5 in groups C and G and n 2 in group D). Data were analysed using a 2×2×2-way ANOVA. There was a significant difference due both EtOH and time ($P<0.01$) but no significant difference between either the daidzein treatment or the interactions between these three independent factors.

There was an overall increase in FRAP value in starvation (+9%; $P<0.01$) and a decrease in EtOH dosage (−10%; $P<0.01$). Although we predicted an amelioration of some of these effects with daidzein, there was no significant effect with this phyto-oestrogen ($P>0.05$). However, in general, the changes in antioxidant power were relatively small compared with other markers of oxidative stress shown to alter in alcohol toxicity (for example, Preedy *et al.* 2002) and the results showing an increase in FRAP in starvation (represented by time of treatment) were unexpected. Thus the FRAP assay may be of limited use in animal models of oxidative imbalance. Furthermore, consideration should be given to other suitable biomarkers of oxidative stress (for example, Wiseman *et al.* 2000).

We wish to thank Hangzhou FST, Republic of China, for their generous gift of phyto-oestrogen.

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Wiseman H, O'Reilly JD, Adlercreutz H, Mallet AI, Bowey EA, Rowland IR & Sanders TAB (2000) *American Journal of Clinical Nutrition* **72**, 395–400.

Weight loss decreases lipopolysaccharide-stimulated peripheral blood mononuclear cell tumour-necrosis factor-α, but not interleukin-6, concentrations in overweight women. By L.M. BROWNING, J.D. KREBS, M.A. O'CONNELL and S.A. JEBB, *MRC Human Nutrition Research, Fulbourn Road, Cambridge, UK, CB1 9NL*

Excess weight is associated with an increased risk of a number of diseases, including CVD and type 2 diabetes, while weight loss reduces the risk of these diseases. Obesity is associated with increases in the circulating cytokines IL-6 and TNF-α (Dandona *et al.* 1998; Kern *et al.* 2001), which are reduced with weight loss (Ziccardi *et al.* 2002). The increase in inflammatory mediators in obesity may in part mediate the relationship between obesity and disease. While circulating and adipose tissue cytokine production have been studied in obesity and weight loss, little is known about the cytokine production from other tissues. Peripheral blood mononuclear cells (PBMC) cytokine production contributes to the inflammatory status of an individual and their response to weight loss has not been demonstrated.

The present preliminary study investigated whether PBMC cytokine production is reduced with weight loss. Nine overweight women (BMI>25 kg/m^2), aged 29–69 years were recruited to the 12-week weight-loss study. The programme initially consisted of a 3.8 MJ/d milk-based energy-restricted diet, with gradual reintroduction of food. Blood samples were collected at baseline and after weight loss. PBMC were isolated from heparinised whole blood by density gradient centrifugation and suspended in supplemented media. Cells (1×10^6 cells/ml) were stimulated with lipopolysaccharide (LPS; 10 μg/ml) and incubated for 24 h, at 37 °C, 5% CO_2. After 24 h, cells were removed, centrifuged and the supernatant fraction stored at −80 °C. TNF-α and IL-6 in supernatant fractions were measured by ELISA. Serum C-reactive protein (CRP) concentrations were determined by a high sensitivity enhanced turbidimetric immunoassay.

Women lost between 4.1 and 20.1 kg (4.1 and 20.5%). Serum CRP and LPS-stimulated PBMC TNF-α production were significantly lower at 12 weeks, than at baseline ($P<0.05$), while IL-6 concentrations were not significantly different (Table). However, the change in TNF-α production did not correlate with the change in CRP, weight or waist circumference.

	Baseline			12 weeks			
	Mean	SD	Range	Mean	SD	Range	
Weight (kg)	97.1	18.4	66.6–124.4	87.6	17.1	61.8–112.9	***
BMI (kg/m^2)	35.3	6.1	25.7–44.1	31.8	6.2	23.6–41.2	***
Waist circumference (mm)†	1040	112	810–1220	980	120	800–1180	**
TNF-α (pg/ml)	5389	1344	3314–7076	2663	1856	1073–7271	*
IL-6 (pg/ml)	2765	95	2637–2903	2770	130	2659–3036	
CRP (mg/l)†	35.5	33.6	11.5–112.2	20.4	16.3	3.1–53.8	**

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ (paired *t* tests).
† Log_e-transformed, geometric mean.

The present study provides preliminary evidence that PBMC TNF-α production is reduced by a weight-loss intervention, while IL-6 is not. This may reflect the differing roles of TNF-α and IL-6, as paracrine and endocrine factors respectively. The present study does not confirm that the reduction in PBMC TNF-α production either contributes to the reduction in circulating inflammatory markers (indicated by CRP), or links to improvements in metabolic risk, but was underpowered to detect this effect. While the present study lacked a 'control' group, a contemporary 12-week study in our laboratory of weight-stable subjects showed no changes in PBMC TNF-α and IL-6 production. A randomised trial in a larger population is now needed.

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***Cis*-9, *trans*-11 and *trans*-10, *cis*-12 conjugated linoleic acid-induced changes in progression of atherosclerosis, plasma lipids and liver proteome in apolipoprotein E-knockout mice.** By B. DE ROOS[1], G. RUCKLIDGE[1], M. REID[1], K. PICKARD[1], J. OSADA[2], E. NOONE[3] and H.M. ROCHE[3], [1]*Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 9SB,* [2]*Department of Biochemistry, Veterinary School, University of Zaragoza, Miguel Servet 177, E-50013, Zaragoza, Spain and* [3]*Nutrigenomics Research Group, Department of Clinical Medicine, Institute of Molecular Medicine, St James's Hospital, Dublin 8, Republic of Ireland*

Conjugated linoleic acids (CLA) refers to a group of conjugated dieonic isomers of linoleic acid that are present as minor constituents of the lipid fraction of meat, milk, and dairy products or other foods derived from ruminant animals. We recently showed that a CLA-rich diet promoted the regression of established atherosclerosis in the apo E-knockout model of diet-induced atherogenesis (Toomey *et al.* 2003). The present study determined the isomer-specific effect of CLA on the progression of atherosclerosis in apo E-knockout mice. 3-Month-old male mice were randomly assigned to receive 1% (w/w) *cis*-9, *trans*-11 CLA, 1% (w/w) *trans*-10, *cis*-12 CLA or 1% (w/w) linoleic acid (control group) diets, enriched with cholesterol (0.15%) for 28 d. *Cis*-9, *trans*-11 CLA significantly reduced the atherosclerotic aortic root face lesion size compared with the control group (10.19 (SD 1.13) and 16.44 (SD 2.48), respectively; values refer to the percentage of area covered by lesion) ($P<0.05$). *Trans*-10, *cis*-12 CLA also reduced lesion size (12.52 (SD 1.92) % area), but this was not significantly different from the control group. Furthermore, the increase in plasma total cholesterol levels during the intervention period was 30% lower in the *cis*-9, *trans*-11 CLA group compared with the control group ($P<0.001$), and *cis*-9, *trans*-11 CLA significantly decreased plasma triacylglycerol (TAG) levels by more than 50% ($P<0.01$) compared with the control group. In contrast, *trans*-10, *cis*-12 CLA significantly increased plasma TAG by over 300% compared with the control group ($P<0.005$).

Proteomics and principle component analysis were used to elucidate a wide range of liver cytoplasm and membrane proteins that were significantly up or down regulated in the *cis*-9, *trans*-11 CLA or *trans*-10, *cis*-12 CLA group compared with the control group. Such proteins could possibly be involved in an (anti)-atherogenic mechanism of either fatty acid and would potentially deliver novel proteins that could be used as a biomarker in future studies. We were able to identify 113 cytoplasm proteins that were significantly and up or down regulated by either *cis*-9, *trans*-11 CLA or *trans*-10, *cis*-12 CLA compared with linoleic acid. We were able to identify 84% of these proteins with MALDI-TOF MS. Principle component analysis of the log-transformed liver cytoplasm protein spot density values revealed that the *trans*-10, *cis*-12 CLA isomer exhibited the largest treatment effect on the first principle component, whereas the *cis*-9, *trans*-11 CLA isomer exhibited the largest treatment effect on the second principle component. A similar result was observed for the membrane protein fraction. Proteins that provided the largest positive and negative contribution to the calculated differences towards the treatment effects of both CLA isomers were involved in several pathways; those being glycolysis and gluconeogenesis, protein turnover, oxidative stress, and receptor uptake.

The present study successfully exploited a powerful combination of proteomics with physiological outcome parameters to aid the identification of mechanisms that could explain changes in markers for the development of atherosclerosis upon treatment with different CLA isomers in the diet.

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Toomey S, Roche H, Fitzgerald D & Belton O (2003) *Biochemical Society Transactions* **31**, 1075–1079.

Plasma biokinetics of natural and synthetic α-tocopherol in healthy apolipoprotein-E4 male smokers and non-smokers. By A.R. PROTEGGENTE[1], R. TURNER[2], C. ROTA[2], J. MAJEWICZ[2], A.-M. MINIHANE[2], G. RIMBACH[2], K. KRÄMER[3] and J.K. LODGE[1], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH,* [2]*Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, University of Reading, Reading, UK, RG6 6AP and* [3]*BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany*

Recent studies have highlighted that diseases associated with oxidative damage, such as CHD, are more common in carriers of the E4 isoform of the apo E protein. Furthermore, research has shown that apo-E4 smokers have an increased risk of CHD, due to increased oxidative stress between other factors, compared with non-smokers. As the major lipophilic antioxidant, requirements and utilisation of α-tocopherol may be higher in smokers and therefore antioxidant supplementation could be beneficial in this population. However, still very little is known on the *in vivo* bioavailability of α-tocopherol and particularly on the differences between uptake of natural (*RRR*-) and synthetic (*all-rac*) α-tocopherol. In previous studies, where the natural and synthetic forms were compared by competitive uptake, the *RRR*:*all-rac* ratio, and therefore the relative bioavailability of natural and synthetic vitamin E, was 2:1, although the currently accepted ratio is 1.36:1.

By using a non-competitive model, we have studied the plasma biokinetics of α-tocopherol in seventeen healthy male subjects (seven smokers, ten non-smokers) of similar age, BMI and cholesterol and triacylglycerols levels, who carry an apo-E4 genotype. The study design was a randomised cross-over, comprising of two 4-week treatments with 400 mg/d (either *RRR*-α-tocopheryl or *all-rac*-α-tocopheryl acetates) with a 12-week wash-out period between treatments. Before and after each treatment period the subjects underwent a 48 h biokinetic protocol, starting with the consumption with a standard breakfast of a capsule containing 150 mg ^{2}H-labelled α-tocopheryl acetate in either the *RRR*-(d6) or *all-rac* (d3) form. Blood was collected before and at 3, 6, 9, 12, 24 and 48 h following capsule ingestion. Tocopherols were analysed using our recently developed LC–MS method.

There was a significant change with time on the plasma levels of either labelled d6- or d3-α-tocopherol in both smokers and non-smokers but no significant difference between the biokinetic profiles obtained before and after the 4-week supplementation period with either *RRR*- or *all-rac*-α-tocopherol in the two groups. Interestingly, plasma levels of d6- and d3-α-tocopherol reached peak concentrations at 9 and 6 h, respectively, in the smokers group compared with 12 h in the non-smokers group. Furthermore, in non-smokers the area under the curve (AUC) for the d3-α-tocopherol profile obtained after 4 weeks' supplementation with *all-rac*-α-tocopherol was significantly lower (P=0.03) than that for the d6-α-tocopherol profile following 4 weeks' supplementation with *RRR*-α-tocopherol. The *RRR*:*all-rac* ratio ranged from 0.91 (SD 0.33) to 1.46 (SD 0.57): 1 in the smokers and from 1.45 (SD 0.56) to 1.73 (SD 1.00): 1 in the non-smokers. In parallel with the uptake of labelled α-tocopherols, plasma unlabelled α-tocopherol (d0) significantly decreased with time in both groups on each biokinetic assessment. Baseline plasma levels of d0-α-tocopherol were significantly increased on supplementation with either *RRR*- or *all-rac*-α-tocopherol in both smokers (P=0.04 and P<0.01, respectively) and non-smokers (P<0.01 and P=0.02, respectively). Similarly to the findings for the ^{2}H-labelled forms, in non-smokers the AUC for the d0-α-tocopherol profile obtained after 4 weeks' supplementation with *all-rac*-α-tocopherol was significantly lower (P=0.03) than that for the profile obtained following 4 weeks' supplementation with *RRR*-α-tocopherol.

These data suggest that absorption of α-tocopherol may be faster in smokers than in non-smokers, potentially due to faster utilisation, although the total amount absorbed does not differ between the two groups. Plasma saturation with either *RRR*- or *all-rac*-α-tocopherol does not seem to influence the bioavailability of α-tocopherol in both groups; however synthetic α-tocopherol appears to be less well absorbed than natural α-tocopherol in non-smokers. Nonetheless, using a non-competitive uptake approach, the relative bioavailability of natural and synthetic α-tocopherol is closer to the generally accepted ratio of 1.36:1 than to the more recently proposed ratio of 2:1. Overall, these results expand the understanding of the bioavailability of α-tocopherol, necessary for understanding the implications of α-tocopherol intervention as a mean of preventing CHD, although further research is required.

The effects of isoflavone supplementation on plasma lipid concentrations in postmenopausal women: the ISOHEART study. By W.L. HALL, A. VAFEIADOU, C.M. WILLIAMS and The Isoheart Consortium, *Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, University of Reading, Reading, UK, RG6 6AP*

The increase in CHD incidence following the menopause is attributed to dyslipidaemia and arterial dysfunction, associated with oestrogen loss. There is convincing evidence from epidemiological, cross-sectional and intervention studies that dietary soya has blood lipid-lowering and hypotensive properties (Beaglehole, 1990; Nagata *et al.* 1998; Vigna *et al.* 2000). However, it is unclear whether the oestrogenic compounds found in soya, isoflavones, are the active component because the few studies conducted to date using pure isoflavone extracts have lacked adequate statistical power. In the present study, a total of 133 postmenopausal women were recruited in the UK, Germany, Denmark and Italy, providing sufficient statistical power to investigate the effects of isoflavones on a range of biomarkers for cardiovascular health, including atherogenic lipoproteins. The large sample size also allowed investigation of responsiveness to isoflavone supplementation according to common polymorphisms in cardiovascular risk-related genes.

The study was a placebo-controlled, 2×8 week randomised cross-over trial, with an 8-week wash-out period. Subjects consumed two cereal bars per d providing 50 mg isoflavones/d (genistein:daidzein ratio 2:1) or the same quantity of cereal bars containing no added isoflavones (placebo). Blood samples were taken at week 0, 4 and 8 of each arm. Subjects were recruited according to specified inclusion criteria, including: age 45–70 years; BMI 20–32 kg/m^2; total cholesterol <8 mmol/l; triacylglycerols (TAG) <3 mmol/l; blood pressure <160/90 mmHg. Subjects who had menstruated within the last 12 months, had medical conditions, or who were taking hormone-replacement therapy were excluded from the study. At screening, subjects were aged 46 to 70 years (mean 58 (SD 5) years), with a mean BMI of 25 (SD 3) kg/m^2, plasma cholesterol 5.9 (SD 0.9) mmol/l, plasma HDL-cholesterol 1.78 (SD 0.38) mmol/l, plasma LDL 3.6 (SD 0.8) mmol/l, plasma TAG 1.10 (SD 0.47) mmol/l, systolic blood pressure 120 (SD 15) mmHg and diastolic blood pressure 76 (SD 8)mmHg.

One hundred and seventeen subjects completed the 6-month intervention, giving a drop-out rate of 12%. No significant differences were shown between plasma responses to isoflavone treatment or placebo for cholesterol, TAG, HDL-cholesterol, LDL-cholesterol, or systolic and diastolic blood pressure. Plasma HDL-cholesterol increased over the 8-week intervention period following both isoflavone treatment and placebo (ANOVA treatment×time interaction; P<0.000). There were no differences in blood lipid response to isoflavone supplementation between the major apo E genotypes (ε3/ε3, ε3/ε4, and ε2/ε3), or oestrogen receptor-α genotypes (XbaI: xx, Xx, XX; PvuII: pp, Pp, PP). There were no differences in blood lipid response to isoflavone supplementation between normo- and hypercholesterolaemic subjects (<6 and >6 mmol/l respectively), or between urinary equol producers and non-producers. Analysis is currently ongoing to investigate the effects of isoflavone on LDL subclasses and lipoprotein (a).

In conclusion, isoflavone supplementation had no effect on blood pressure, plasma total, HDL-, or LDL-cholesterol or TAG in a large cohort of postmenopausal women. No significant responses to isoflavones were observed when the cohort was subdivided according to apo E or oestrogen receptor-α genotypes, plasma cholesterol status at screening, or to urinary equol status. HDL-cholesterol concentrations increased with the treatment and placebo arms of the intervention, which may be due to the cereal bars or dietary change. Isoflavone supplementation has no beneficial effect on blood lipid risk factors for CVD in postmenopausal women. Hypolipidaemic effects reported for soya supplementation may be due to components of soya other than isoflavones.

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Homocysteine and cardiovascular risk: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). By J.A. TROUGHTON[1], J.V. WOODSIDE[1], I.S. YOUNG[1], C.C. PATTERSON[1], D. ARVEILER[2], P. AMOUYEL[3], J. FERRIERES[4], P. DUCIMETRIERE[5], J.W.G. YARNELL[1] and A. EVANS[1], on behalf of the PRIME Investigators, [1]*School of Medicine, Queen's University Belfast, Grosvenor Road, Belfast, UK, BT12 6BJ,* [2]*The Strasbourg MONICA Project, Strasbourg, France,* [3]*The Lille Monica Project, Lille, France,* [4]*The Toulouse MONICA Project, Toulouse, France and* [5]*The Coordinating Centre, Villejuif, France*

Classical CHD risk factors fail to explain most of the large CHD incidence gradient between Northern Ireland and France. The Prospective Epidemiological Study of Myocardial Infarction (PRIME) is a multicentre prospective, nested case–control study which aims to investigate the role of novel risk factors in CHD incidence in these populations. One potential risk factor is increased homocysteine concentration, which is an S-containing amino acid that is an intermediary product of methionine metabolism. The aim of the present study was to prospectively test the hypothesis that elevated homocysteine concentrations are associated with increased CHD risk.

In total, 9758 men aged 50–59 years were recruited between 1991 and 1993 and examined for evidence of CHD at baseline. Subjects were followed annually by questionnaire and a 5-year follow-up was completed. There were 323 new CHD cases identified. Each case was matched to two controls who were study participants of the same age (±3 years), recruited in the same centre on the same day (±2 d) as the corresponding case and were free of CHD on the date of the ischaemic event of the case. The present analysis therefore included 323 cases and 638 controls. Total homocysteine (tHcy) was measured by HPLC with fluorescence detection according to Ubbink *et al.* (1991), while serum folate and cobalamin concentration were assessed by radioassay (ICN Pharmaceuticals).

There were no significant differences in tHcy, folate or cobalamin concentration between cases and controls. The tHcy concentration (geometric mean) for cases was 9.27 (interquartile range (IQR) 7.49, 11.06) µmol/l compared with 8.97 (IQR 7.26, 10.91) µmol/l for controls (P=0.18). The folate concentration (geometric mean) for cases was 7.34 (IQR 5.19, 10.81) ng/ml compared with 7.25 (IQR 5.09, 10.67) ng/ml for controls (P=0.95). The cobalamin concentration (geometric mean) for cases was 553 (IQR 407, 758) pg/ml compared with 518 (IQR 379, 726) pg/ml for controls (P=0.07). However tHcy was significantly higher in current smokers compared with non-smokers (geometric mean of 9.45 (IQR 7.43, 11.75) µmol/l *v.* 8.90 (IQR 7.32, 10.70) µmol/l for non-smokers; P=0.007). After adjustment for other cardiovascular risk factors (case–control triplet, waist:hip ratio, diastolic blood pressure, total cholesterol, HDL-cholesterol, triacylglycerols, glucose, alcohol, physical activity, apo B, fibrinogen, glutathione peroxidase, cobalamin, folate, lipoprotein (a) and cystatin C), tHcy was a significant predictor of CHD risk only in current smokers for increase in risk per step increase in tHcy quintile in current smokers (odds ratio 1.30 (95% CI 1.05, 1.60); P=0.015).

Therefore the findings of the present study suggest that elevated tHcy is a risk factor for CHD, but only in current smokers.

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Retinal vein occlusion, homocysteine and methylene tetrahydrofolate reductase genotype. By J.V. WOODSIDE, S.J. McGIMPSEY, L. BAMFORD, R. GRAYDON, S.E.C.M. GILCHRIST, G. McKEEMAN, C.C. PATTERSON, I.S. YOUNG, A. HUGHES, D. McGIBBON, U. CHAKRAVARTHY, *School of Medicine, Queen's University Belfast, Grosvenor Road, Belfast, UK, BT12 6BJ*

Homocysteine has been proposed to play a role in retinal vein occlusion (RVO), but its exact role is still unresolved. The aim of the present retrospective case–control study was to investigate the relationship between plasma total homocysteine (tHcy), 5,10 methylene tetrahydrofolate reductase (MTHFR) C677T genotype, folate and vitamin B_{12} status, and RVO.

RVO patients were recruited from both out-patient and in-patient sources, while controls were selected to have a similar age and sex distribution to cases. Two hundred and four subjects gave informed written consent and entered the study (106 cases and ninety-eight controls). Age, sex, smoking habit and cardiovascular status were noted and dilated fundal examination performed on both eyes. Blood samples were collected, separated and stored at −80 °C until analysis. Plasma tHcy was measured by HPLC with fluorescence detection according to Ubbink *et al.* (1991). Serum cobalamin and folate were measured by radioassay (ICN Pharmaceuticals). MTHFR genotype was determined following PCR amplification by polyacrylamide gel electrophoresis of the amplified product according to Frosst *et al.* (1995).

Age and sex were well matched between cases and controls (age of cases 67.9 (SD 14.2) years, age of controls 68.4 (SD 13.8) years, P=0.21; 52% male in cases, 46% male in controls, χ^2 test, P=0.39). A history of hypertension was more frequently recorded in cases compared with controls (49% cases *v.* 26% controls, χ^2 test; P=0.001). Cases and controls were similar in terms of smoking status, cardiovascular history, history of thrombosis, prevalence of diabetes and use of anticoagulant medication. There was no significant difference in plasma tHcy or thermolabile MTHFR allele frequency between cases and controls. Plasma tHcy (geometric mean) was 10.8 (interquartile range (IQR) 8.3–14.3) µmol/l for cases and 9.5 (IQR 6.8–14.9) µmol/l for controls (P=0.12); the frequency of the TT allele was 37.4% in cases *v.* 35.1% in controls. Similarly there was no significant difference in folate (geometric mean of 6.25 (IQR 4.24–8.49) ng/ml for cases *v.* 6.9 (IQR 4.84–8.71) ng/ml for controls; P=0.24) or vitamin B_{12} (geometric mean of 415 (IQR 298–558) pg/ml for cases *v.* 372 (IQR 264–494) pg/ml for controls; P=0.15) status between cases and controls. MTHFR genotype did not affect folate or vitamin B_{12} concentrations in either cases or controls. However, tHcy was significantly higher in thermolabile homozygotes than in non-thermolabile homozygotes (ratio of geometric means 1.35 (95%CI 1.04, 1.74)).

Hyperhomocysteinaemia, the MTHFR C677T mutation and folate and vitamin B_{12} status are not important risk factors for RVO in this population.

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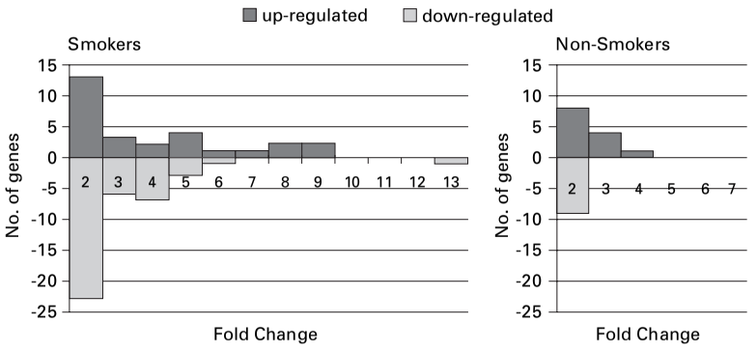
The effect of vitamin C supplementation on gene expression in primary human monocytes: a human intervention study. By J. MAJEWICZ[1], C. ROTA[1], R. TURNER[1], A.-M. MINIHANE[1], J. LODGE[2], A.R. PROTEGGENTE[2], K. KRAMER[3] and G.H. RIMBACH[1], [1]*Hugh Sinclair Unit of Nutrition, School of Food Biosciences, The University of Reading, Whiteknights, Reading, UK, RG6 6AP,* [2]*School of Biomedical and Molecular Sciences, University of Surrey, Guilford, UK, Surrey GU2 7XH and* [3]*BASF, Aktiengesellschaft, Fine Chemicals, Ludwigshafen, Germany*

CHD remains one of the leading causes of death in the Western world, with 30% of all CHD deaths associated with cigarette smoking. It has been demonstrated that smokers have decreased levels of plasma vitamin C (Mezzetti *et al.* 1995) and increased concentrations of F_2-isoprostanes (Morrow *et al.* 1995), a specific lipid oxidation product. A recent study demonstrated that subjects who smoked and carried the apo-E4 genotype have a non-cholesterol-linked increased risk of CHD events of almost 3-fold compared with non-E4, non-smokers (Humphries *et al.* 2001). It was suggested that this increased risk was due to greater oxidative stress in this subgroup, which is a major determinant of LDL oxidation, endothelial dysfunction and the functionality of the monocytes and macrophages within the intima. The present study examines the impact of vitamin C supplementation on monocyte gene expression in apo-E4 smokers and non-smokers.

Twenty-two male subjects (age 41 years, BMI 26.3 kg/m^2), ten smokers and eleven non-smokers, with an apo-E4 genotype (E3/E4 or E4/E4) were recruited and supplemented with 60 mg vitamin C/d for 4 weeks. Monocytes were isolated before and after supplementation and cultured in the presence of phorbol 12-myristate 13-acetate (100 ng/ml) for 24 h to differentiate them into macrophages, after which they were treated with lipopolysaccharide (0.5 μg/ml) for 6 h and the RNA extracted. To examine the transcriptional response to vitamin C, Atlas cytokine/receptor cDNA arrays were used. The data were normalised to ubiquitin, GAPDH and tubulin α. Genes with a 2-fold change in expression were considered significant and examined for further investigation. Out of 256 genes, vitamin C caused a 2-fold or greater decrease in nine genes and an increase in thirteen genes in non-smokers and a decrease in forty-three genes and an increase in twenty-eight genes in smokers. The Figure shows the effect of vitamin C on the gene expression of inflammatory genes in human primary monocytes.



Vitamin C had very little effect on the expression levels of inflammatory cytokines in non-smokers. In smokers the expression levels of cytokines and receptors was greatly altered, with changes in inflammatory genes which have been shown to be important in the development of many chronic diseases, including atherosclerosis, rheumatoid arthritis and Alzheimer's disease. The results suggest that the monocyte expression of inflammatory markers in individuals in a state of oxidative stress, for example, apo-E4 smokers, can be improved by vitamin C supplementation.The cytokine–receptor array provided an important platform in contributing to our understanding of the impact of dietary factors on complex events leading to the pathogenesis of inflammatory diseases.

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Curcumin induction of antioxidant response element-regulated genes in primary human monocytes. By S.A. RUSHWORTH, C.A. CHARALAMBOS, R.M. OGBORNE and M.A. O'CONNELL, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL*

Curcumin, a yellow pigment from *Curcuma longa*, is a major component of turmeric and is commonly used as a spice and food-colouring material. It exhibits antioxidant, anti-inflammatory and anti-tumour properties (Lin *et al.* 2000). Curcumin supplementation has been reported to have cytoprotective effects in animal models of cerebral ischaemia and myocardial infarction (Nirmala & Puvanakrishnan, 1996; Thiyagarajan & Sharma, 2004).

Many cytoprotective and detoxification genes, including haeme oxygenase 1 (HO-1), NADP(H) quinone oxidoreductase (NQO1), ferritin and glutamine cysteine ligase modulator (GCLM), contain an antioxidant response element (ARE) or ARE-like transcriptional regulatory sequence in their promoters. The transcription factor Nrf2 has recently emerged as a key player in the transcriptional activation of ARE-mediated gene expression (Ishii *et al.* 2000). Dietary antioxidants including isothiocyanates and green tea polyphenols induce ARE-mediated reporter activity (Owuor & Kong, 2002). Recently, curcumin has been reported to induce HO-1 expression in bovine endothelial cells (Balogun *et al.* 2003). The aim of the present study was to investigate the effects of curcumin on ARE-mediated gene expression in human monocytic cells.

Primary human monocytes were left unstimulated or incubated with curcumin (0–15 μM) for various times. Following this, mRNA was extracted from the monocytes and real-time PCR carried out to determine relative quantitative expression of HO-1, NQO1, ferritin and GCLM. Gene expression was normalised to 18 ribosomal subunit mRNA levels. Nuclear extracts were prepared from curcumin-treated monocytic cells and analysed for Nrf2 protein expression using Western blot analysis.

Curcumin stimulated HO-1, NQO1, GCLM and ferritin mRNA expression in primary human monocytes, measured by real-time PCR analysis. Curcumin dose-dependently increased HO-1 expression, which peaked at 4 h (15 μM: 38 (SD 1.62)-fold above control; three independent experiments) and decreased by 8 h. Curcumin also stimulated HO-1 protein expression in primary monocytes. Following curcumin stimulation, ferritin mRNA expression increased by 4 h and continued to increase to 24 h in primary monocytes (15 μM: 5.2 (SD 0.54)-fold above control; three independent experiments). Curcumin dose-dependently increased NQO1 mRNA expression with a maximum at 8 h (15 μM: 32 (SD 2.54)-fold above control; three independent experiments) and GCLM mRNA expression with a maximum expression at 4 h (15 μM: 25 (SD 2.15)-fold above control; three independent experiments). In response to curcumin, Nrf2 translocated into the nucleus in primary human monocytes and THP-1 cells, a human monocytic cell line.

These results demonstrate that the dietary antioxidant curcumin up regulates ARE-mediated gene expression in human monocytic cells and suggest that this occurs via the activation of the transcription factor Nrf2. In animal models of cerebral ischaemia and myocardial infarction, the cytoprotective effects seen during curcumin supplementation may be due to up regulation of these ARE-mediated genes in monocytes.

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α-Lipoic acid induces haem oxygenase-1 and ferritin expression in human monocytic cells. By R.M. OGBORNE, S.A. RUSHWORTH, C.A. CHARALAMBOS and M.A. O'CONNELL, *MRC Human Nutrition Research, Fulbourn Road, Cambridge, UK, CB1 9NL*

α-Lipoic acid (ALA) is a powerful antioxidant found at high concentrations in spinach, broccoli and tomatoes (Wollin & Jones, 2003). ALA has been reported to have anti-inflammatory effects on vascular cells *in vitro*, including inhibiting cytokine and NO production in monocytes (Kiemer *et al.* 2002). In addition, ALA supplementation prevents the development of hypertension and hyperglycaemia in animal models (Midaoui *et al.* 2003).

Haem oxygenase-1 (HO-1) is a stress-inducible enzyme, catalysing the degradation of haem, producing free Fe, CO and biliverdin. Biliverdin is subsequently converted to bilirubin, a powerful antioxidant, by biliverdin reductase. Free Fe, a pro-oxidant, is sequestered by ferritin. HO-1 plays a key cytoprotective role, defending the cell from oxidative insults through the antioxidant activities of its metabolites. Recently, it has also been found to exert anti-inflammatory effects by inhibiting cytokine production in murine macrophages (Lee *et al.* 2003). HO-1 deficiency has led to detrimental effects in various types of vascular disease animal models, including atherosclerosis and hypertension, while HO-1 over-expression plays a protective role in ischaemia-reperfusion injury (Perrella & Yet, 2003).

The HO-1 and ferritin promoters contain antioxidant response element (ARE). The ARE is a transcription regulatory element found in specific genes associated with cytoprotection and detoxification. The recently identified transcription factor Nrf2 has been reported to bind to this element in response to various stimuli, including antioxidants. HO-1 is up regulated by antioxidants, including *tert*-butyl hydroquinone and some dietary polyphenols. The aim of the present study was to determine if ALA induced HO-1 and ferritin expression in human monocytic cells and to investigate if Nrf2 may be involved in this response.

THP-1 is a human monocytic cell line. THP-1 cells were left unstimulated or treated with 0.25–1 mM-ALA for various times and whole cell extracts or nuclear extracts prepared or RNA isolated. HO-1 protein expression was examined by Western blot analysis. HO-1 and ferritin mRNA expression were measured by real-time PCR and normalised against 18 s ribosomal subunit mRNA using the comparative cycle threshold method. Nrf2 nuclear accumulation was examined by Western blot analysis and densitometry performed.

ALA induced a dose-dependent increase in HO-1 mRNA expression in THP-1 cells, with a peak induction at 4 h (1 mM-ALA: 40 (SD 2.65)-fold above control; three independent experiments) and returning to baseline by 24 h. This correlated with an increase in HO-1 protein expression at 8 h, detected by Western blotting. ALA also induced ferritin mRNA expression in a dose-dependent manner with maximal induction at 8 h (1 mM-ALA: 8.5 (SD 0.42)-fold above control; three independent experiments) that remained elevated at 24 h (1 mM-ALA: 7.2 (SD 0.48)-fold above control; three independent experiments). In response to ALA, Nrf2 accumulated in the nucleus of THP-1 cells and was detected within 30 min (2.4-fold increase above control), peaked at 2 h (3.4-fold above control) and returned towards baseline levels by 4 h.

These results demonstrate that the dietary antioxidant ALA induces HO-1 and ferritin expression in THP-1 cells and suggest that this may be regulated by the transcription factor Nrf2. The potential beneficial effects of ALA and other dietary antioxidants in protection against vascular disease may be, at least in part, due to the up regulation of the cytoprotective genes, HO-1 and ferritin, in human monocytes. Further work is required to confirm the role of Nrf2 in regulating ALA-induced HO-1 and ferritin expression in THP-1 cells.

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Carotenoid intakes and food sources in 18–64-year-old Irish adults. By LUCEY and M. KIELY, *Department of Food and Nutritional Sciences, University College, Cork, Republic of Ireland*

Increased intakes of lycopene are associated with a reduction in the risk of prostrate cancer (Giovannucci, 1999), α- and β-carotene with a reduced risk of coronary artery disease (Osganian *et al.* 2003), and there is evidence that lutein and zeaxanthin may protect against age-related macular degeneration (AMD) (Mares-Perlman *et al.* 2002). We measured intakes and food contributors to carotenoids in a nationally representative sample of the Irish adult population. We used the North/South Ireland Food Consumption Survey (NSIFCS) database (Irish Universities Nutrition Alliance, 2001), which estimated food and beverage intake using a 7 d food diary in 1379 Irish adults, aged 18–64 years, and a recently established carotenoid composition database (O'Neill *et al.* 2001) which was updated with published literature data.

	36–50 years		36–50 years		51–64 years		All ages	
Carotenoids (mg/10 MJ)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Men: *n*	253		236		173		662	
β-Carotene	2.85[a]**	2.7	4.02[b]	2.9	3.83[b]**	3.7	3.52**	3.1
Lycopene	1.50**	1.4	1.60*	1.5	1.70	1.9	1.57**	1.6
Lutein	0.87[a]**	0.5	1.02[b]*	0.6	1.13[b]	0.9	0.99**	0.7
α-Carotene	0.83*[a]	0.9	1.18[b]	1.0	1.12[b]**	1.2	1.03**	1.0
β-Cryptoxanthin	0.31**	0.5	0.40	0.7	0.33*	0.6	0.35**	0.6
Women: *n*	269		286		162		717	
β-Carotene	3.78[a]	3.0	4.82[b]	3.4	5.93[b]	4.6	4.68	3.7
Lycopene	2.12	1.9	2.11	2.2	1.82	1.7	2.05	2.0
Lutein	1.05[a]	0.6	1.17[b]	0.7	1.27[ab]	0.8	1.15	0.7
α-Carotene	1.05[a]	1.0	1.34[b]	1.1	1.72[b]	1.5	1.32	1.2
β-Cryptoxanthin	0.48	0.6	0.47	0.7	0.55	0.7	0.50	0.7

[a,b] Mean values within a row, for the three age categories, with unlike superscript letters were significantly different ($P<0.05$). Mean values within an age category were significantly different between men and women: * $P<0.01$, ** $P<0.001$.

Of the 3060 foods recorded in the NSIFCS, 1418 foods contained carotenoids. The most significant contributors to carotenoid intakes were carrots (24%), beef dishes (12%), salad vegetables (11%), peas and beans (9%), soups and sauces (9%), vegetable dishes (5%), and fruit juices (4%). Total and individual carotenoid intakes (mg/10 MJ) were significantly higher ($P<0.001$) in women than men overall. In both men and women, mean daily intakes (mg/10 MJ) of β-carotene ($P<0.001$), lutein ($P<0.05$), α-carotene ($P<0.01$) and total carotenoids ($P<0.01$) were significantly lower in 18–35-year-olds than in the older age categories. The current α- and β-carotene and β-cryptoxanthin intakes were substantially higher than recently reported data from nationally representative German and UK studies (Pelz *et al.* 1998; Food Standards Agency, 2003). Lycopene intakes were similar and lutein intakes were roughly half of those reported by Pelz *et al.* (1998). These data reflect the relatively higher intakes of carrots, fruit juice and tomatoes than leafy green vegetables in the Irish adult population (O'Brien *et al.* 2003). Recent recommendations to increase the consumption of leafy green vegetables to boost lutein intakes for the possible prevention of AMD (Mozaffarieh *et al.* 2003) should be implemented.

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Dietary intake and sources of phylloquinone (vitamin K_1) according to age of British adults from a recent national survey. By C.W. THANE and W.A. COWARD, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL*

Emerging evidence suggests that a low intake of vitamin K, present mainly in the diet as phylloquinone (vitamin K_1), may lead to poor vitamin K status and contribute to the development of osteoporosis and increased risk of fracture (Shearer, 2000). Sub-optimal vitamin K status has also been associated with atherogenesis and vascular calcification (Shearer, 2000). Reliable estimates of vitamin K_1 intake are required in order to establish normal ranges of intake and determine factors that influence its variation. This understanding may then be useful for relating to biochemical markers of vitamin K status and health outcomes, and for the setting of dietary recommendations.

Dietary vitamin K_1 intake was estimated in a nationally representative sample of British adults aged 19–64 years who participated in the cross-sectional National Diet and Nutrition Survey of 2000–1 (Henderson *et al.* 2002). After excluding those who reported being unwell with eating habits affected, 7 d weighed dietary records were analysed for 1504 participants (702 men, 802 women). Dietary vitamin K_1 intake was estimated by assigning content values (Food Standards Agency, 2002; C Bolton-Smith and M Shearer, unpublished results) for a comprehensive range of over 4000 foods consumed in the survey. Dietary intake and food sources of vitamin K_1 were examined according to age, sex, region, season, occupational social class and cigarette smoking habit. Differences by age group are reported here, with adjustment for the other socio-demographic and lifestyle factors.

Dietary vitamin K_1 intakes were positively skewed with an overall geometric mean and 95% CI of 67 (65, 69) μg/d (men, 70 (68, 73); women, 64 (61, 66) μg/d; $P<0.001$). Sixty-six percent of men and 52% of women reported vitamin K_1 intakes below 1 μg/kg body weight per d ($P<0.001$). Vegetables and vegetable dishes contributed most to vitamin K_1 intake (60% overall), with cooked leafy green vegetables (LGV) alone providing around 19% (broccoli>cabbage>Brussels sprouts). Overall, the food groups of cereals, meat, and fat spreads contributed 11, 8 and 4% respectively of vitamin K_1 intake.

Whether expressed in terms of μg/d or μg/kg body weight per d, vitamin K_1 intakes rose significantly with age. Food sources of vitamin K_1 intake also varied significantly, as shown in the Table.

	19–34 years (*n* 401–426)		35–44 years (*n* 410–426)		45–54 years (*n* 337–354)		55–64 years (*n* 281–298)		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	*P**
K_1 intake (μg/d)†	54[a]	51, 57	65[b]	61, 68	75[c]	71, 80	81[c]	76, 87	<0.001
K_1 intake from vegetables (%)‡	55[a]	53, 57	60[b]	58, 62	62[bc]	60, 64	64[c]	62, 66	<0.001
of which: cooked LGV (%)‡	12[a]	10, 14	18[b]	16, 20	22[bc]	20, 25	26[c]	23, 29	<0.001
of which: potatoes and potato products‡	13[a]	12, 14	9[b]	8, 10	7[c]	6, 8	6[c]	5, 7	<0.001
K_1 intake <l μg/kg body weight per d (%)	73[a]		60[b]		53[b]		43[c]		<0.001

[a,b,c] Mean values within a row were significantly different ($P<0.05$; Scheffé test for continuous variables, following ANOVA; χ^2 test for discontinuous proportions, following multiple logistic regression).
* Adjusted for other socio-demographic and lifestyle factors.
† Geometric means obtained from antilog of $\log_e$-transformed data.
‡ Arithmetic means.

These data show distinct differences in vitamin K_1 intake by age group, that applied in both sexes and remained after excluding likely under-reporters (energy intake: estimated BMR <1.1). If these clearly observed lower intakes and different sources of vitamin K_1 in younger adulthood are reflected in vitamin K status they may be pertinent to the development of osteoporosis and possibly cardiovascular disease that become manifest in later adulthood.

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Intra-uterine programming of hepatic glutathione in relation to oxidative injury and ageing. By D.V. SCULLEY and S.C. LANGLEY-EVANS, *Centre for Reproduction and Early Life, School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, UK, LE12 5RD*

The offspring of rats fed a low-protein (LP) diet in pregnancy exhibit a number of physiological and metabolic disorders, including hypertension, renal impairments and bone abnormalities (Langley-Evans, 2004). Such animals also appear to have a significantly shorter lifespan, although the causes of premature death appear unrelated to specific physiological disorders or disease states (Jennings *et al.* 1999; Sayer *et al.* 2001). This raises the possibility that mechanisms of ageing may be subject to intra-uterine programming influences. It has been previously reported that rats exposed to LP *in utero* exhibit altered metabolic processing of glutathione and increased susceptibility to tissue injury on exposure to an oxidant challenge (Langley-Evans *et al.* 1997). The present study investigated the relationship between hepatic glutathione status and tissue protein oxidation as a preliminary assessment of the likely contribution of oxidative processes to ageing and reduced lifespan.

Pregnant female Wistar rats (200–225 g) were fed a control diet (180 g casein/kg; *n* 7) or an LP diet (90 g casein/kg diet; *n* 7) *ad libitum*, throughout gestation. At delivery litter size was standardised to eight pups and all animals were transferred to standard laboratory chow diet. Thereafter one male and one female from each litter were culled at 4, 16, 30 or 44 weeks of age. Hepatic glutathione was determined in fresh tissue and protein carbonyl concentrations were determined in snap-frozen tissue as a marker of ongoing oxidative injury. Size at birth was unaffected by LP feeding. LP neonates had lower hepatic superoxide dismutase activity but showed no evidence of increased oxidative injury to the tissue.

(*n* 9–14 observations)	Hepatic GSH (μmol/g tissue)				Protein carbonyls (nmol/mg protein)			
	Control		LP		Control		LP	
Age (weeks)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
4	6.06	0.86	5.03	0.26	2267	294	3561*	477
16	6.88	0.31	6.14*	0.33	2843	363	3504*	498
30	6.71	0.53	7.37	0.37	2066	272	1774	230
44	7.13	0.33	8.80	1.02	1448	131	1407	136

* Mean value was significantly different to control ($P<0.05$).

Animals exposed to LP *in utero* tended to have lower hepatic glutathione concentrations up to 16 weeks of age (Table), an effect that was more pronounced in male than in female animals. Similarly levels of oxidative injury were greater in the LP group than in controls up to 16 weeks, but thereafter there was no significant difference. Again, the impact of prenatal diet upon the level of protein oxidation appeared to be greater in male than in female animals. Hepatic glutathione concentrations were significantly correlated with the concentration of protein carbonyls in the tissue ($R-0.337$; $P=0.001$). No significant differences between groups were noted for the hepatic activities of the antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase. Effects of age and prenatal dietary manipulations upon hepatic glutathione were not noted in the lung, suggesting that the transport of the tripeptide to extra-hepatic tissues may be maintained at the expense of oxidative injury to the liver. Indeed the finding that oxidative damage to the eye, as indicated by protein carbonyl concentration, was present at a lesser level in rats exposed to LP *in utero* ($P=0.018$).

The data lend support to the hypothesis that oxidative injury as a consequence of programmed down regulation of glutathione synthesis may enhance the rate of ageing in prenatally undernourished rats. Increased oxidative injury in key tissues is proposed to act as a trigger for apoptosis, culminating in loss of key functions, premature organ failure and death. Ongoing studies will evaluate these processes in greater detail in the liver and in major tissues including the heart and kidneys, where there is some evidence of down regulation of expression of genes encoding rate-limiting enzymes in glutathione synthesis.

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Antioxidant vitamins and their relationship to health parameters in an adolescent population. By S.E.C.M. GILCHRIST[1], J.V. WOODSIDE[1], D.C. WATKINS[1], G. CRAN[1], C.A.G. BOREHAM[2], A. BINGHAM[1], J.M. SAVAGE[1] and I.S. YOUNG[1], [1]*School of Medicine, Queen's University Belfast, Grosvenor Road, Belfast, UK, BT12 6BJ and* [2]*School of Applied Medical Sciences and Sports Studies, University of Ulster, UK, BT37 0QB*

It is now accepted that atherosclerotic lesions are identifiable as early as the second decade of life, and that the development of CHD is a lifelong process. Epidemiological data show Northern Ireland inhabitants to be amongst those with the highest risk of death from CHD worldwide, and The Northern Ireland Young Hearts Project has established a high prevalence of CHD risk factors in adolescence. Strong associations have been shown between CHD risk factors and the extent of postmortem coronary atherosclerosis in young adolescents.

The present study aimed to establish vitamin A, C, and E status in a sample of Northern Irish 15-year-olds, and to determine whether antioxidant status is associated with cardiovascular risk markers. A total of 225 males and 230 females were recruited, representing approximately a 2% random sample of this age-group population in Northern Ireland. Nutritional data were collected by diet history method with open-ended interview, using a photographic atlas to determine portion sizes. Smoking behaviour was ascertained by categorised response in a self-report questionnaire, while socio-economic status was assessed according to parental occupation. Height and weight were measured, while habitual physical activity, including sports participation, was assessed by a self-report questionnaire, from which a standardised activity score out of 100 was calculated. Non-fasting venous blood was collected and stored at −80 °C until analysis. Vitamin A and E concentrations were assessed in serum by HPLC. Plasma vitamin C and serum cholesterol were assessed by automated enzyme assays.

Vitamin C concentrations were significantly lower in alcohol drinkers *v.* non-drinkers in males ($P<0.05$), and in those in social class IIIb-V *v.* social class I-IIIa in both males and females (both $P<0.05$). There were no significant differences in either vitamin A or vitamin E in either males or females between smokers and non-smokers, alcohol drinkers and non-drinkers, and those in social class I-IIIa *v.* social class IIIb-V. Vitamin C in males showed a significant inverse correlation with the number of cigarettes smoked per d (r −0.15; $P=0.03$), BMI (r −0.17; $P=0.02$) and social class considered as a continuous variable (r −0.20; $P=0.01$), and a positive correlation with exercise (r 0.17; $P=0.01$). In females, vitamin C was significantly inversely correlated with low social class (r −0.32; $P<0.001$) and positively correlated with exercise (r 0.14; $P=0.05$). Vitamin A showed a positive correlation with smoking in males (r 0.15; $P=0.03$), and with BMI in both sexes (males, r 0.38, $P<0.001$; females, r 0.14, $P=0.04$). Correlations between vitamin E and the above parameters were non-significant. Using multivariate analysis, the relationships between vitamin C and both BMI and smoking remained significant ($P<0.05$) in males in the presence of the other variables. Similarly the relationships between vitamin A and both BMI (in both sexes) and smoking (in males) remained statistically significant in the presence of the other variables.

These results suggest that vitamin C in this age group has some predictive value in evaluating overall cardiovascular health status, and that low vitamin C status may be a risk marker of the presence of other modifiable risk factors for atherosclerosis, especially in males. The association of vitamin A status with a less healthy cardiovascular profile, namely higher BMI and number of cigarettes smoked, needs to be further examined.

Barriers to the consumption of wholegrain foods. By A.R. JONES[1], S. KUZNESOF[1], D.P. RICHARDSON[2] and C.J. SEAL[1], [1]*Human Nutrition Research Centre, School of Agriculture, Food and Rural Development, University of Newcastle, Newcastle upon Tyne, UK, NE1 7RU and* [2]*DPR Nutrition Limited, 34 Grimwade Avenue, Croydon, Surrey, UK, CR0 5DG*

Wholegrain foods (WGF) are an important source of nutrients and phytoprotective substances which are in short supply in the Western diet (Slavin, 2004). Increased consumption of WGF is associated with a reduced risk of several chronic diseases such as heart disease, certain cancers and type 2 diabetes (Smith *et al.* 2003). Current US dietary guidelines advise the consumption of at least three servings of WGF daily. In contrast, the UK does not have any specific recommendations relating to the consumption of WGF. Furthermore, recent findings suggest intakes within the UK are extremely low with less than 5% of British adults consuming three or more servings daily and more than 30% consuming none (Lang *et al.* 2003). The present study explores those factors influencing WGF intake in order to further understand current consumption patterns.

To explore barriers to consumption of WGF seven semi-structured focus group discussions were undertaken with a total of thirty-four respondents (nineteen males and fifteen females). Discussants were grouped by age and sex; the group profiles are reported in the Table. Focus groups were held in a permissive and non-threatening environment to optimise free-flowing discussion and participant interaction. Respondents discussed the topic of healthy eating and were then prompted to discuss specific topics relating to WGF. Focus group data were transcribed and analysed using QSR NVivo. Those factors identified by subjects as influencing WGF consumption are summarised in the Table for each group.

Focus group number ...	1 (*n* 5)	2 (*n* 5)	3 (*n* 5)	4 (*n* 4)	5 (*n* 5)	6 (*n* 5)	7 (*n* 5)
Sex	Female	Female	Female	Male	Male	Male	Male
Age group (years)	18–25	26–45	46–65	18–25	18–25	26–45	46–65
Factors influencing WGF intake							
Access to WGF			✓	✓		✓	✓
Access to WGF when eating out		✓					
Consumption patterns of friends/family		✓	✓		✓		✓
Cost of WGF			✓	✓			
Difficult to digest							✓
Taste	✓	✓				✓	✓
Texture	✓	✓					
Time required for preparation/cooking	✓	✓	✓	✓			✓

Although responses varied for males and females and between age groups, results suggest that there are several key factors influencing WGF consumption. The taste of and time required to prepare and cook WGF were cited as barriers to consumption in most groups. Subjects also reported that friends and/or family members (particularly children) were also influential when consuming WGF and that it would be uneconomical to purchase WGF when shopping for the family. The limited availability of WGF (for example, rice and pasta), both in local food stores and when eating out, was also suggested as being a barrier to greater consumption. In addition WGF were described as having a 'gritty' texture and in some instances difficult to digest. Consumer ability to identify WGF and their benefits was also limited.

The findings from the present study are similar to those reported by Adams & Engstrom (2000). To improve current consumption patterns food manufacturers should be encouraged to develop convenient and acceptable WGF for consumption in the home and when eating out. Furthermore, a coordinated education programme from the government, health professionals, manufacturers, retailers and restaurants is essential to help consumers understand the importance and benefits of WGF. Simple educational messages such as 'Three are Key' in particular would provide positive and realistic advice.

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An investigation of the toxic effects of cholesterol oxide mixtures towards human U937 cells. By E. RYAN, S.M. O'SULLIVAN and N.M. O'BRIEN, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Cholesterol oxidation products, termed oxysterols, may be formed in the diet during the processing of cholesterol-rich foods or synthesised endogenously by auto- or enzymic oxidation. Oxysterols, including 7β-hydroxycholesterol (7β-OH), have been shown to be cytotoxic to a number of cell lines. The mode of toxicity in certain studies has been identified as apoptosis. The objective of the present study was to investigate the cytotoxicity of mixtures of oxysterols, representative of those found *in vivo*, towards a human monocytic cell line.

U937 cells were grown in RPMI 1640 medium supplemented with 2.5% fetal bovine serum. Oxysterols mixtures were added to the tissue culture medium to give a final concentration of 30 μM or 60 μM and incubated for 24 h at 37 °C and 5% CO_2. The oxysterols used were 7β-OH, 25-hydroxycholesterol (25-OH), cholesterol-5α,6α-epoxide (α-epoxide), cholesterol-5β,6β-epoxide (β-epoxide), 19-hydroxycholesterol (19-OH), and 7-ketocholesterol (7-keto). All oxysterols were administered in ethanol. Control cells were treated with an equal volume of ethanol (treatment 1). Treatment 2 included supplementation with all six oxysterols, each at a concentration of 10 μM. Treatment 3 involved supplementation with 30 μM-7β-OH alone. To investigate if α-epoxide or 25-OH affected the 7β-OH-induced toxicity, treatments 4 and 5 involved combinations as follows: α-epoxide+7β-OH and 25-OH+7β-OH, each oxysterol added at a concentration of 30 μM. Viability was assessed by the fluorescein diacetate–ethidium bromide assay and apoptotic nuclei were quantified following staining with Hoechst 33342. Cellular glutathione levels were measured as an indicator of oxidative stress.

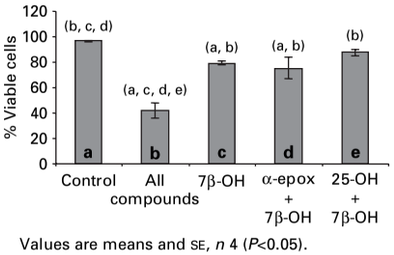


Fig. 1. Viability of U937 cells.

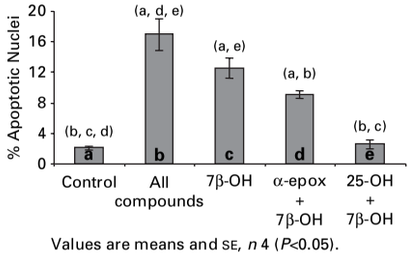


Fig. 2. % Apoptotic nuclei of U937 cells.

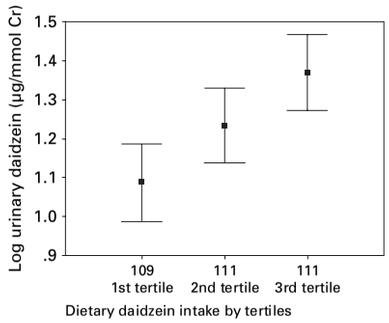
Exposure to the 60 μM mix of the six oxysterols decreased the viability to 40% of control (Fig. 1). This coincided with an 8-fold increase in apoptotic nuclei to almost 17% (Fig. 2). Following exposure to 30 μM-7β-OH alone, viability decreased to 80% whilst apoptotic nuclei increased to 12.5%. The addition of 30 μM-α-epoxide did not significantly protect against the 7β-OH-induced toxicity. However, supplementation with 25-OH significantly ($P<0.05$) protected against the apoptosis caused by 7β-OH. Similarly 25-OH significantly ($P<0.05$) protected against the decrease in glutathione caused by 7β-OH (data not shown), suggesting that the mechanism by which 25-OH protects is through the prevention of oxidative stress. In conclusion, the level of apoptosis obtained by a simultaneous treatment with 7β-OH and 25-OH appears to be significantly lower than that induced by 7β-OH on its own indicating that certain oxysterols may act antagonistically to one another.

Phyto-oestrogen concentrations in serum and spot urine as biomarkers for dietary phyto-oestrogen intake among free-living women in EPIC-Norfolk. By Y.L. LOW[1], P.B. GRACE[1], J.I. TAYLOR[1], N.P. BOTTING[2] and S.A. BINGHAM[1], [1]*MRC Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge, UK, CB2 2XY and* [2]*School of Chemistry, University of St Andrews, Fife, UK, KY16 9ST*

Epidemiological studies investigating phyto-oestrogen intake and cancer risk have been inconclusive, partly due to difficulties in quantifying intake. Measuring phyto-oestrogens in urine and serum as biomarkers provides an alternative. However, studies on the validity of such biomarkers were often done in populations with high soya intakes and typically used extensive urine collections. The present cross-sectional study aimed to investigate whether phyto-oestrogen concentrations in serum and untimed spot urine can serve as biomarkers of dietary phyto-oestrogen intake in 332 free-living women (aged 45–75 years) in the EPIC-Norfolk cohort (Day *et al.* 1999), a population with a low habitual soya consumption. EPIC-Norfolk forms part of the European Prospective Investigation of Cancer (EPIC), which is a large multi-centre study looking at the connection between diet and cancer.

Dietary information was collected using 7 d food diaries and isoflavone intakes computed using an in-house database. Seven phyto-oestrogen concentrations (daidzein, genistein, glycitein, O-desmethylangolensin (O-DMA), equol, enterodiol and enterolactone) in spot urines and serums were analysed using newly developed rapid and sensitive GC–MS and liquid chromatography–tandem MS methods respectively, incorporating $^{13}C_3$ standards (Grace *et al.* 2003*a*,*b*). Pearson correlations and trend tests were performed on log-transformed data.

Phyto-oestrogen concentrations in spot urine (adjusted for urinary creatinine) correlated strongly with that in serum (*r* 0.81–0.94) (see Table). Trend tests showed significant dose-response shifts in the distributions of urinary and serum isoflavone concentrations ($P<0.02$) across increasing tertiles of intakes (see Figure). We conclude that phyto-oestrogen concentrations in untimed spot urine and serum can serve as biomarkers of phyto-oestrogen intake in epidemiological studies, even in free-living populations with a low soya intake.



	Pearson correlation coefficient between urinary and serum phyto-oestrogens ($P<0.001$)
Isoflavones	0.85
Daidzein	0.91
Genistein	0.89
Glycitein	0.81
O-DMA	0.82
Equol	0.81
Lignans	0.93
Enterodiol	0.83
Enterolactone	0.94

This work is supported by UK Medical Research Council, UK Food Standards Agency, Cancer Research UK, US Department of Army and Material Command DAMD – 97 – 1 7028 and the Agency for Science, Technology and Research, Singapore.

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Consumption of wholegrain wheat, rye and rice by laboratory animals is associated with elevated plasma concentrations, urinary excretion and faecal output of enterolactone. By A.M.S. NAGAH[1], T. NURMI[2] and C.J. SEAL[1], [1]*Human Nutrition Research Centre, School of Agriculture, Food and Rural Development, University of Newcastle, Newcastle upon Tyne, UK, NE1 7RU and* [2]*Research Institute of Public Health, University of Kuopio, PO Box 1627, Fin-70211, Kuopio, Finland*

The mammalian lignans enterolactone (ENL) and enterodiol, commonly found in human plasma and urine, are phyto-oestrogens that may contribute to the prevention of hormone-related cancers and CHD. They are formed by the conversion of dietary precursors such as secoisolariciresinol and matairesinol as well as recently identified pinoresinol and lariciresinol by the colonic microflora. These precursors are present in bran; thus wholegrain cereals may contribute to lignan intake. Rye bread, commonly consumed in Finland is a good source of lignans (Hallmans *et al.* 1997), but the potential of other whole grains more commonly consumed in the UK as sources of lignans is unclear. The present study was carried out to compare the effect of feeding rats diets supplemented with wholegrain rye, wheat flour and brown rice on circulating ENL concentrations and ENL excretion in urine and faeces.

Groups of six male, Wistar strain rats, initial weight 110 g, were housed individually in plastic metabolism cages in a controlled environment with *ad libitum* access to drinking water. Control animals (C) were fed a nutritionally complete semi-synthetic diet containing 50% maize starch (Seal & Mathers, 2001). Test animals were fed the same diet in which the starch had been replaced with wholegrain wheat flour (WW), rye flour (WR) or brown rice flour (BR). The animals were fed 25 g of each diet for 3 weeks, the last week of which included a complete collection of urine and faeces. At the end of the experiment the animals were killed by exsanguination from the heart and plasma was separated from the blood for later analysis. Mammalian lignans in plasma (Nurmi & Adlercreutz, 1999), urine (Nurmi *et al.* 2003) and faecal samples (Heinonen *et al.* 2001) were measured by HPLC with coulometric array detection as described.

Diet ...	C		BR		WR		WW	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma ENL (nmol/l)	0.97	2.368	62.57	12.002	112.6	59.016	58.9	35.208
Urinary ENL (nmol/7 d)	4.7	3.85	81.7	31.43	124.4	135.65	225.3	249.55
Faecal ENL (nmol/7 d)	21.9	16.22	1202.1	172.98	1488.9	966.45	809.0	572.85

There was no detectable enterodiol in most of the plasma, urine and faeces samples analysed. In contrast, for animals fed wholegrain flours, plasma ENL concentrations were significantly higher than control animals ($P<0.05$). The highest concentration of ENL was seen in plasma in the WR group, although due to the variability in the data this was not significantly different from BR ($P=0.094$) or WW ($P=0.066$). Urinary ENL was significantly higher in animals fed wholegrain flours, although due to variability between animals there was no difference between the different flours. Likewise for faecal ENL output, this was significantly higher in animals fed wholegrain flours but was not different between flours.

Since there was no enterodiol or plant lignans in urine or faeces, this indicates that the amount of administered plant lignans did not exceed the metabolic capacity of the animals. The high ENL levels seen for rye-fed animals were expected from data in human subjects (Hallmans *et al.* 1997). However, the high values seen for animals fed brown rice suggest that this may also be a good source of lignans in the human diet and requires further investigation.

A.M.S.N. is in receipt of a Libyan Government postgraduate studentship.

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Oral ferrous sulfate leads to a marked increase in circulating levels of pro-oxidant non-transferrin bound iron. By C. HUTCHINSON[1], W. AL-ASHGAR[1], D.Y. LIU[2], R.C. HIDER[2], J.J. POWELL[1,3] and C.A. GEISSLER[1], [1]*Departments of* [1]*Nutrition and Dietetics,* [2]*Pharmacy, Franklin-Wilkins Building, King's College London, 150 Stamford Street, London, UK, SE1 9NN and* [3]*MRC Human Nutrition Research, Fulbourn Road, Cambridge, UK, CB1 9NL*

Serum non-transferrin bound Fe (NTBI) is a potential catalyst for the production of reactive oxygen species, contributing to tissue damage. As there is evidence of increased oxidative damage during $FeSO_4$ treatment (Tuomainen *et al.* 1999) and the rate of transfer of Fe from the duodenal enterocyte to the plasma is increased in Fe-deficiency anaemia (IDA), we aimed to determine whether oral $FeSO_4$ results in NTBI in seven anaemic women (age 18–45 years). On the first occasion, a 200 mg $FeSO_4$ tablet was ingested with two slices of toasted white bread and a glass of dilute cordial. On a second occasion, 200 mg $FeSO_4$ was taken with an identical drink but without food. Serial blood samples were drawn for 4 h after $FeSO_4$, for measurement of total serum Fe, total Fe-binding capacity (TIBC) and serum NTBI. NTBI was determined using a method described elsewhere (Gosriwatana *et al.* 1999). The rise in NTBI following 200 mg $FeSO_4$ was statistically significant (Fig. 1; $P<0.001$) and was positively correlated with an increase in transferrin saturation ($P<0.0001$). However, NTBI occurred even in the presence of unsaturated transferrin (Fig. 2), presumably because the rate of Fe influx exceeded the rate of uptake of Fe by transferrin.

Fig. 1.

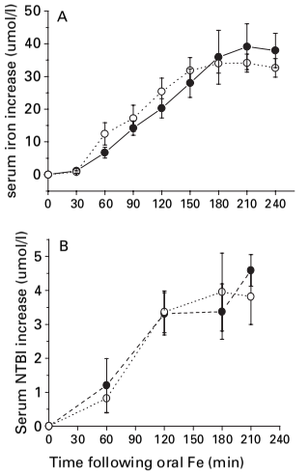

Fig. 1. Serum Fe (A) and serum NTBI (B) post 200 mg $FeSO_4$ with (●) and without (○) food.

Fig. 2.

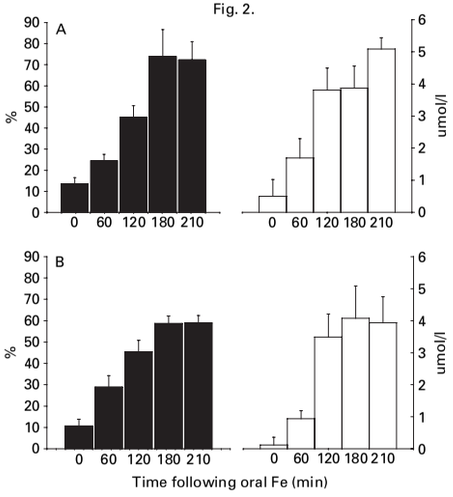

Fig. 2. Transferrin saturation (■) and NTBI (□) post 200 mg $FeSO_4$ with (A) and without (B) food.

Oral $FeSO_4$ containing 65 mg Fe resulted in a significant rise in pro-oxidant NTBI, which may provide an explanation for reports of Fe-induced oxidative stress during $FeSO_4$ supplementation (Tuomainen *et al.* 1999). This finding deserves further investigation in light of the potentially toxic effects of NTBI, and widespread use of $FeSO_4$ for treatment of IDA in vulnerable groups, such as pregnant women.

We gratefully acknowledge the help of Wendy Williams for phlebotomy, and the Department of Clinical Biochemistry (KCL Hospital) for determining total serum Fe and TIBC.

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Physiological responsiveness to low-dose fish oil intake. The impact of sex, genotype, age and geography: an introduction to the Fingen study. By C.K. ARMAH[1], B.M. KOFLER[1], P. CURTIS[2], J. STANNARD[2], L. FARRELL[3], F. NAPPER[4], J.P. GREW[1], J. LUFF[1], E. MILES[4], G. LIETZ[2], M. CASLAKE[3], C. PACKARD[3], P. CALDER[4], J. MATHERS[2], C.M. WILLIAMS[1] and A.M. MINIHANE[1], [1]*The University of Reading, Reading, UK,* [2]*School of Clinical Medical Sciences, University of Newcastle, Agriculture Building, Kings Road, Newcastle upon Tyne, NE1 7RU UK,* [3]*Department of Vascular Biology, University of Glasgow, Glasgow Royal Infirmary, Glasgow, G31 2ER UK and* [4]*Department of Nutrition, School of Medicine, University of Southampton, Southampton, UK*

The benefits of long-chain *n*-3 PUFA, EPA and docosahexaenoic acid (DHA), on cardiovascular health have been repeatedly demonstrated. However, a variable response to fish oil intervention is often observed. In particular, a highly variable (0–20%) increase in LDL-cholesterol (LDLC) is evident following fish oil treatment and a previous study conducted in our unit suggested that increases in LDLC may predominantly occur in individuals with an apo-E4 genotype (approximately 25% UK population) (Minihane *et al.* 2000). However, this study administered relatively high doses of fish oils, to insulin-resistant middle-aged males, and LDLC-genotypes associations were based on retrospective genotyping of a small group number. The impact of genotype, age and sex to more modest, dietary achievable, intakes of EPA+DHA remain unknown and will be the subject of the present investigation. Furthermore, in addition to lipid risk factors the study aims to examine a range of novel CHD risk indicators such as inflammatory markers, including cell adhesion molecules, vascular tone, homocysteine levels and markers of oxidative stress.

The Fingen study (*n* 330) is one of the largest fish oil intervention trials ongoing in the UK and involves the Universities of Glasgow, Newcastle, Southampton and Reading. Between 2003 and 2005 all participants will undergo a three-arm cross-over trial comparing the effects of 0.8 g EPA+DHA/d *v.* 1.6 g EPA+DHA/d *v.* a placebo on an array of outcomes. Amongst the four centres we hope to recruit 330 men and women in total on the basis of their apo-E genotype (110 apo-E2, 110 apo-E3, 110 apo-E4). Our population set will include five age sub-groups with fifty-five participants recruited from each of the five decades of the 20–70 year age range, to ensure the study group recruited represents that of the UK population. To date 193 individuals (E2 (*n* 42), E3 (*n* 92), E4 (*n* 59)) are participating in cohort 1, due to be completed in November 2004. It is anticipated that over 1000 individuals will need to be screened for cohort 2 in order to achieve the desired number of apo-E2 individuals who represent only 12–15% of the populations in Northern Europe. The basic screening data for cohort 1 is given in the Table.

	Sex		TAG (mmol/l)		TC (mmol/l)		Glucose (mmol/l)		BMI (kg/m^2)	
	Male	Female	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Group (*n* 193)	87	106	1.21	0.58	5.20	0.99	4.96	0.64	25.29	3.20
E2 (*n* 42)	17	25	1.28[a]	0.09	4.79[a]	0.13	5.05	0.08	26.20	0.47
E3 (*n* 92)	42	50	1.09[b]	0.05	5.22[b]	1.05	4.87	0.08	25.10	0.34
E4 (*n* 59)	28	31	1.30[a]	0.09	5.45[b]	0.12	5.03	0.06	25.00	0.41

[a,b] Mean values with unlike superscript letters suggest a significant inter-genotype group difference.

Apo-E3 triacylglycerol (TAG) was found to be significantly lower than both the apo-E2 (P=0.049) and apo-E4 (P=0.01) TAG, with apo-E2 individuals having significantly lower total cholesterol (TC) than both the apo-E3 (P=0.019) and apo-E4 (P<0.001) groups. The outcome data for all 330 participants will be available in 2005.

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Effects of the dietary *n*-6:*n*-3 polyunsaturated fatty acid ratio on plasma lipids and lipoprotein subfractions: the 'OPTILIP' study. By I.G. DAVIES[1], M.D. GRIFFIN[1], F. LEWIS[2], S. SLAUGHTER[2], D.J. MILLWARD[1], T.A.B. SANDERS[2] and B.A. GRIFFIN[1], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH and* [2]*Nutrition Food and Health Research Centre, King's College London, UK, SE1 9NN*

Prospective epidemiological and intervention studies suggest that the *n*-6:*n*-3 fatty acids ratio is associated with the risk of IHD. Dietary PUFA of the *n*-3 series, linolenate (18:3) but especially the longer-chain fatty acids in fish oil, EPA and docosahexaenoic acid (long-chain (LC) *n*-3; 20:5 and 22:6), exert favourable effects on blood lipids, lowering plasma triacylglycerol (TAG) and redistributing subfractions of LDL and HDL. The extent to which these effects can be optimised through dietary intakes on *n*-3 PUFA, and potentially offset by a high intake of dietary *n*-6 PUFA (linoleic acid), is unknown. A randomly controlled, parallel study was designed to determine the optimal intake of dietary *n*-6 and *n*-3 fatty acids (OPTILIP) with respect to blood lipids and lipoproteins. This was achieved by comparing four diets with varying *n*-6:*n*-3 ratios (3:1–5:1); one containing additional LC *n*-3 (from fish, notably salmon, and a LC *n*-3-enriched spread), two with additional linolenate (from a rapeseed oil-based spread and cooking oil) and one with a combination of LC *n*-3 and linolenate, with a control based on an estimated UK diet (10:1). Healthy males (*n* 141) and postmenopausal females (*n* 111) aged 45–70 years were randomly allocated to one of the five diets for 6 months. Dietary intakes were assessed by 7 d weighed food intake, food diaries and 3 d duplicate diet collections. Blood lipids and lipoproteins were measured by commercially available assays in overnight fasted plasma samples, with LDL and HDL subfractions measured by iodixanol density centrifugation and 4–30% gradient gel electrophoresis respectively. The dietary interventions were well accepted and successful in achieving the target intake ratios of *n*-6:*n*-3 fatty acids. There were no significant effects of the diets on total plasma cholesterol, LDL- and HDL-cholesterol, and apo B. In contrast, there were consistent dietary effects on plasma TAG, small, dense LDL (sdLDL) and the larger, lipid-rich HDL_2 subfraction.

	Diets	% Change		
n-6:*n*-3	PUFA	TAG	sdLDL	HDL_2
10.9:1	Control (*n* 40)	−7.4	−4	−2.1
4.7:1	Increased linolenate (*n* 49)	−0.1	0	−0.1
4.0:1	Increased linolenate (*n* 52)	−3.1	3	3.7
3.1:1	Increased LC *n*-3 (*n* 60)	−13.8	−4	4
2.6:1	Increased linolenate+LC *n*-3 (*n* 51)	−11	−13	8.6
P (ANCOVA)		0.02	NS	0.02

All data were log-transformed. Percentage changes are differences in geometric means (post–pre-diet) adjusted for baseline values, age, BMI and sex.

There was a highly significant diet–sex interaction for the changes in plasma TAG (P=0.009). Subsequent analysis by sex showed no dietary effects in females. However, in males there were overall significant decreases in TAG (P=0.015) and sdLDL (P=0.031) that were pronounced in the two diets containing LC *n*-3; i.e. TAG fell by 17.6% (LC *n*-3) and by 12.3% (linolenate+LC *n*-3). Combination of dietary groups into males with and without increased intakes of LC *n*-3 revealed highly significant decreases in both plasma TAG (with LC, 1.40 to 1.18 mmol/l *v.* without LC, 1.38 to 1.37 mmol/l; P=0.002), and sdLDL (with LC, 31.5 to 27.6% *v.* without LC 32.6 to 32.8%; P=0.002).

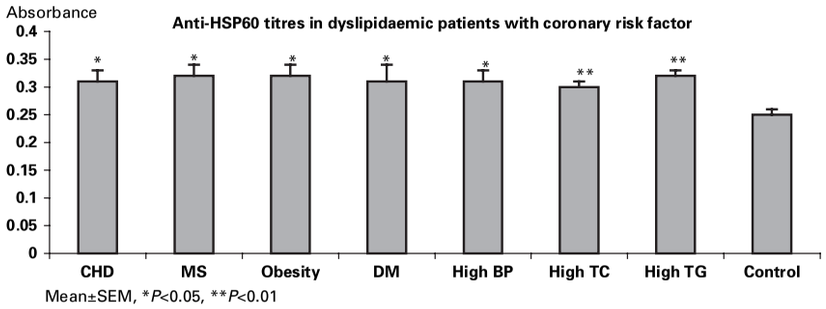
In conclusion, the two diets enriched with LC *n*-3 PUFA produced changes in plasma TAG and lipoprotein subfractions in males consistent with previous favourable effects of fish-oil supplementation on blood lipids. However, overall, the diet containing the mixture of LC *n*-3 and shorter-chain linolenate was the most effective in modifying these biomarkers of cardiovascular risk.

Impact of dietary intake on plasma antibody titres to heat shock protein 60. By M. GHAYOUR-MOBARHAN[1], S.A. NEW[1], D. LOVELL[2], D. LAMB[1], C. LIVINGSTONE[1,3], T. WANG[1,3] and G.A. FERNS[1,3], [1]*University of Surrey, Guildford, UK, GU2 7XH,* [2]*Postgraduate Medical School, University of Surrey, Guildford, UK, GU2 7DJ and* [3]*The Royal Surrey County Hospital, Guildford, UK, GU2 7XX*

The heat shock proteins (HSP) are a family of protein chaperones that may elicit an autoimmune response during atherogenesis. HSP60 appears to be an important antigen that may be responsible for immunoactivation during atherogenesis (Lamb *et al.* 2003). We have investigated the relationship between dietary vitamin E and vitamin C intake on plasma anti-HSP60 titres in dyslipidaemic patients and controls.

Patients were recruited from the lipid clinics at the Royal Surrey County Hospital (RSCH) (*n* 238). Of these patients, eighty-two were obese (BMI>30 kg/m^2), forty-two were diabetic (DM), fifty-five had established CHD, seventy-six were hypertensive (high BP), 217 were hypercholesterolaemic (high TC), 176 were hypertriacylglycerolaemic (high TG), and 142 had metabolic syndrome (MS) as defined using the ATP III criteria (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). Controls were recruited from employees at the University of Surrey and the RSCH (*n* 188). Of these controls, thirty-three subjects were obese, nine had MS, and fifteen were on medication. These fifty-seven controls were excluded. A food-frequency questionnaire (FFQ) was used for estimating the dietary intake of vitamins E, C, and fat. This FFQ was previously validated against 7 d weighed records and biochemical markers of antioxidant status, and its short- and long-term reproducibility has been tested (New & Bolton Smith, 1993; Bodner *et al.* 1998). Plasma anti-HSP60 titres were determined by ELISA. ANOVA was used for comparing plasma anti-HSP60 titres in each group, and stepwise multiple regression analysis was used to explore the relationship between dietary antioxidant intake and anti-HSP60 titres.

Compared with controls, plasma anti-HSP60 was significantly raised in patients with obesity (28%), DM (24%), CHD (24%), hypercholesterolaemia (20%), hypertriacylglycerolaemia (28%), and MS (28%).



The dietary determinants explaining most of the variability in anti-HSP60 titres in the multiple regression were vitamin C (P=0.005), vitamin E (P=0.04), and monounsaturated fat (P=0.009).

The increased antibody titres to HSP60 in patients with coronary risk factors may be related to the immunoactivation during atherogenesis in these groups. It appears that dietary constituents may modulate this response in subjects with dyslipidaemia.

Financial support from the Iranian government is gratefully acknowledged.

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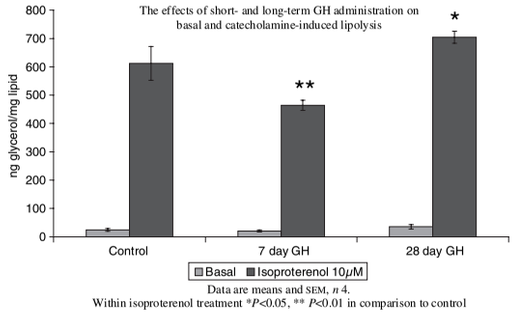
Short- and long-term effects of growth hormone on basal and catecholamine-induced lipolysis in pigs. By K.R. HEADLAND[1], P.L. SENSKY[1], P.J. BUTTERY[1], K. DICKINSON[2] and J.M. BRAMELD[1], [1]*Division of Nutritional Biochemistry, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, UK, LE12 5RD and* [2]*RenaSci, Biocity, Nottingham, UK, NG1 1GF*

Growth hormone (GH) has remarkable metabolic effects, acting chronically as a repartitioning agent to not only increase lean body mass, but also decreasing fat levels. Most of the growth-regulatory effects of GH are via insulin-like growth factor (IGF)-1. However, direct effects are observed in adipose tissue in all species studied. GH acts chronically to decrease adiposity by inhibiting lipogenesis (synthesis of triacylglycerol), antagonising insulin action, and facilitating lipolysis, particularly catecholamine-induced lipolysis. Despite the effects of chronic administration being well documented, there is very little work comparing the short- and long-term effects of GH on lipolysis. The study of both short- and long-term effects of GH on lipolysis will be of use in determining potential obesity treatments. The pig provides a good model for man due to the many similarities of their nutrition and endocrine axes. The present study aims to demonstrate the effects of short- and long-term GH administration (*in vivo*) on basal and catecholamine-induced lipolysis (*ex vivo*).

Thirty-two intact male Large White pigs with an average initial body weight of 35 kg were fed a diet consisting of 18% crude protein (14 MJ/kg) *ad libitum* for 28 d. The pigs were split into three groups: controls (*n* 10); GH administered for the final 7 d (*n* 11); GH administered for 28 d (*n* 11). Over the 28 d the pigs received a daily intramuscular injection of either porcine GH (5 mg Reporcin in 1 ml sterile water; Alpharma, Australia) or vehicle (sterile water). On day 28 the pigs were slaughtered and plasma was taken for IGF-1 quantification. Peri-renal adipose tissue was taken from four pigs from each group, chopped in M199 media and fat explants were incubated in Krebs–Ringer bicarbonate buffer ±10 μM isoproterenol for 3 h. Lipolysis was quantified using an end-point assay for free glycerol (Sigma), which was corrected for lipid content measured by Folch extraction.



Neither short- nor long-term GH administration resulted in a change to basal lipolysis. However, long-term GH treatment resulted in an increase in catecholamine-stimulated lipolysis (P<0.01). This is consistent with findings by Watt *et al.* (1991) in sheep. In contrast, a decrease was seen in the 7 d-treated pigs (P<0.001). This is suggestive of differential regulation of short- and long-term effects.

Catecholamines stimulate lipolysis via β-receptors and inhibit lipolysis by α_2-receptors. However, the β-receptor-mediated lipolytic action dominates. Previous work in sheep (Doris *et al.* 1996) has shown an increase in the number of β-receptors with 7 d treatment of GH with no effect on the number of α_2-receptors and an increase in isoproterenol-stimulated lipolysis. This is not consistent with the decrease in lipolysis observed in our 7 d-treated animals, but may explain the increase with 28 d treatment as the dose of GH given was twice that used in the present study. We therefore suggest that further investigation is required to determine the mechanisms for the differences observed.

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Watt PW, Finley SC, Clegg RA & Vernon RG (1991) *Biochemical Journal* **273**, 39–42.

Cross-sectional associations of diet and insulin-like growth factor levels in 7–8-year-old children.

By I.S. ROGERS[1], D.J. GUNNELL[2], P.M. EMMETT[1], L.R. GLYNN[1], D.B. DUNGER[3], J.M. HOLLY[4] and THE ALSPAC STUDY TEAM, [1]*Unit of Paediatric and Perinatal Epidemiology, Division of Community Medicine, University of Bristol, 24 Tyndall Avenue, UK, BS8 1TQ,* [2]*Department of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Bristol, UK, BS8 1TQ,* [3]*Department of Paediatrics, University of Cambridge, Addenbrookes Hospital, Level 8, Box 116, Cambridge, UK, CB2 2QQ and* [4]*Divison of Surgery, University of Bristol, Bristol, UK, BS8 2HW*

The insulin-like growth factors (IGF) are polypeptide hormones which stimulate cell growth and inhibit apoptosis and have a number of potentially opposing roles on overall health. Higher plasma levels of IGF-I are associated with a reduced risk of osteoporosis, heart disease (Juul *et al.* 2002) and diabetes. However, several studies suggest that higher concentrations of IGF-I and lower levels of its main binding protein IGFBP-3 are associated with an increased risk of a number of cancers, including breast (Hankinson *et al.* 1998), prostate (Chan *et al.* 1998) and colon cancer. The dietary determinants of circulating levels of components of the IGF system are of interest, as these may mediate some of the effects of diet on later health. However, few studies have examined the relationship between diet and IGF levels in children. The objective of the present study was to investigate the associations between nutrient and food intakes and IGF-I and IGFBP-3 levels in a group of 7–8-year-old children in the Bristol area participating in the Avon Longitudinal Study of Parents and Children (ALSPAC website).

Diet was assessed using a 3 d unweighed food diary. Confounding variables considered were maternal education, housing tenure, birth weight and BMI. Complete information on dietary intakes, IGF levels and all confounding variables were available for 521 children (287 boys). In multivariable analyses, IGF-I was positively associated with intakes of protein, Mg, Zn, Ca, K and P, and IGFBP-3 was positively associated with energy. The IGF-I:IGFBP-3 ratio was positively associated with protein, Zn and P. There was some evidence that the dietary determinants of the IGF system differed between the sexes. Among girls but not boys there were significant inverse associations between IGF-I and IGFBP-3 and total and monounsaturated fat intake. The Table shows geometric mean age-adjusted IGF-I and IGFBP-3 levels according to tertile of energy-adjusted nutrient intake.

		Tertile of energy-adjusted nutrient intake			
	Sample	1	2	3	*P**
IGF-I (ng/ml)					
Protein	All children	127.7	139.5	149.2	<0.001
Zn	All children	132.4	139.6	143.8	0.005
P	All children	131.9	135.8	148.7	0.001
Total fat	Girls only	155.1	146.5	131.7	0.003
Monounsaturated fat	Girls only	157.1	148.1	128.6	0.001
IGFBP-3 (ng/ml)					
Energy	All children	4472	4674	4811	0.009
Total fat	Girls only	5062	4847	4385	0.017
Monounsaturated fat	Girls only	5138	4799	4363	0.001

* *P* value by regression for continuous nutrient variables, adjusting for maternal education, housing tenure, BMI and birth weight for gestational age.

None of the foods examined were strongly associated with IGF levels; in particular there was no association with red meat or vegetable intakes. These associations between diet and measures of the IGF system may provide a mechanism whereby childhood diet could have a long-term effect on the risk of chronic disease.

ALSPAC website: http://www.alspac.bris.ac.uk
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Juul A, Scheike F, Davidsen M, Gyllenborg J & Jorgensen T (2002) *Circulation* **106**, 939–944.
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Laying foundations for health: an investigation into the food provided to under-5s in day care.

By P. NELSON[1], J. MARSHALL[1], M. COOPER[2], H. ZAMBAS[2], K. BREWSTER[3], K. ATKIN[1] and H. MOORE[1], [1]*Centre for Research in Primary Care, University of Leeds, UK, LS2 9PL,* [2]*Department of Nutrition and Dietetics, St Mary's Hospital, Leeds, UK, LS12 3QE and* [3]*Leeds City Council, Merrion Centre, Leeds, UK, LS2 8DT*

The increase in obesity and in early onset of diseases such as diabetes among the nation's children has prompted growing concern (Royal College of Physicians Working Party, 2004) and has implications for health in later life (Law, 2000). Today's children consume a diet richer in fat and sugar and poorer in fruits and vegetables than previous generations. As a result of government policy, growing numbers of preschool children spend most of their day, and therefore consume most of their food, in day care, yet little is known about the quality of food served there, or its contribution to a child's overall diet.

The present study used quantitative and qualitative methods to investigate food delivered by childcare providers and to understand the context in which it is delivered. A stratified random sample of 345 providers, drawn from five local authorities (LA) received a questionnaire exploring the type of food provided, menu planning, and providers' attitudes toward promoting healthy eating. Respondents also submitted a week's menu. A food-frequency checklist was used to count the number of portions of the main food groups, types of drinks available, and the frequency on the menu of fatty and sugary foods.

Qualitative interviews were carried out with a purposive sample of eighteen childcare providers drawn from consenting questionnaire respondents, and seven members of Early Years Service staff involved with training or supporting providers. The interviews explored: food policies and practices; links between providers, parents and policy makers; sources of guidance regarding nutrition; the role of diet in health; and the question of responsibility for establishing healthy eating patterns in children.

Of 194 responses, 168 (49%) met eligibility criteria. Forty three percent of carers offered a full range of meals and snacks. One-quarter of childminders served only food provided by parents, as did 8% of independent nurseries. Most nurseries made menus available to parents; few childminders did so. Only one-fifth of all providers involved parents in menu planning. The Table shows the percentage serving portions from the four main food groups at lunch every day.

	Meat, fish, alternatives	Starchy food	Fruit or vegetables	Milk and dairy
Childminders (%)	47	87	23	21
Nurseries (%)	52	92	46	14
P value of difference	0.40	0.59	0.01	0.29

Interviews revealed that few childcare providers had training in nutrition. Those who had were able to discuss components such as 'carbohydrates, vitamins and minerals'; most spoke less specifically. Providers knew of the need to promote fruit and vegetable intake, but few felt they had had useful nutritional guidance from the LA. OFSTED inspections drew attention to food safety rather than nutrition, and may be prompting a trend among childminders to require parents to provide all food.

Childcare workers recognise their influence on food intake and eating behaviour, and many saw the positive potential in this: encouraging children to try new foods, or to sit down at a table and share a meal together. All types of providers and LA staff felt that food provision is a low priority for parents, and many providers felt responsible for moderating unhealthy food practices in the child's home.

The potential strength of the childcare worker's influence must, however, prompt a move toward improved training and support for them in this role. The area of nutrition within the childcare sector as a whole has been neglected in terms of policy; in particular, the present minimum standards of quality must be clarified and expanded if they are to prove a useful measure for training or assessment.

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Royal College of Physicians Working Party (2004) *Storing up Problems: the Medical Case for a Slimmer Nation.* London: Royal College of Physicians.

Best practices for the prevention of overweight and obesity in children with a focus on immigrants new to industrialised countries: a synthesis review. By D.A. McNEIL, M.A.T. FLYNN and S.C. TOUGH, *Calgary Health Region and University of Calgary, 29th St NW, Calgary, Alberta, Canada*

Few systematic reviews of obesity-related treatment or prevention interventions have included studies that were not randomised controlled trials or have searched beyond common library databases, while none have reviewed studies from a population health perspective. The present synthesis review has taken a broader perspective in search, selection and critical appraisal approaches to formulate best practice recommendations useful for 'real world' situations. The primary aim was to identify best practices for promoting healthy weights in children and youth to prevent chronic disease associated with obesity with a particular focus on children of immigrants new to industrialised countries. An advisory panel, with expertise in the areas of medical paediatrics and obesity, population health strategies and immigrant populations, exercise physiology, nutrition; behavioural sciences and child health policy provided guidance to the investigative team.

A search strategy that included the Internet, in addition to eighteen library databases, yielded 13 158 hits (includes duplicates) and 450 websites. Based on inclusion criteria, 500 programme reports were selected for appraisal, which occurred on four levels; programme evaluation, scientific rigour, population health and immigrant health, using scoring systems specifically designed for the present review. Within each of these appraisal levels, programmes were categorised as 'high', 'medium' or 'low' based on relevant standards. Any of the programmes categorised as 'high' in the four appraisal levels were included for synthesis to identify best practice. According to these methods, a total of 177 reports and articles were included for synthesis, thirty-three (19%) of which were categorised as 'high' in more than one appraisal level, all of which overlapped with programme evaluation. As shown in the Table, the majority was included based on programme evaluation (*n* 176; 99%), followed by scientific rigor (*n* 29; 16%), immigrant health (*n* 11; 6%) and population health (*n* 1; <1%) perspectives.

	Population health		Programme evaluation		Scientific rigour Quantitative and Qualitative		Immigrant health	
	n	%	*n*	%	*n*	%	*n*	%
Low or not applicable	395	79	85	17	328	65	475	95
Medium	104	21	239	48	143	29	14	3
High	1	<1	176	35	29	6	11	2

During synthesis, programmes were grouped based on intervention setting. Recommendations for best practice were based primarily on outcomes considering interventions (diet, physical activity, and mental health), programme strategies (education, behavioural change, activities, family involvement, environmental modification), intensity, duration and culture. These programme reports were reviewed with particular emphasis on understanding the outcomes in terms of the primary objectives and comparing and contrasting these findings within the context of all four appraisal levels. This enabled the development of different levels of recommendations such that those performing well on scientific appraisal were recognised as being more evidence based. Alternatively programmes performing well on the other appraisal levels provided information on important aspects such as acceptability, feasibility, propriety, (programme evaluation), applicability for public health (population health) and for marginalised population groups (immigrant health).

Effective public health strategies are critical in the face of the escalating childhood obesity epidemic and overwhelming prospect of chronic disease. The present synthesis research provides a novel approach to the development of best practice recommendations for the prevention and treatment of childhood obesity amenable for the complex and diverse reality of public health.

Prevalence of overweight and obesity in Aberdeen primary schoolchildren: a retrospective longitudinal study. By C.M. McDOUGALL[1] and J.E. CRUM[2], [1]*Department of Child Health, University of Aberdeen, Royal Aberdeen Children's Hospital and* [2]*Department of Community Child Health, Royal Aberdeen Children's Hospital, Westburn Road, Aberdeen, UK, AB25 2ZG*

The current epidemic of childhood obesity is a growing public health concern, with increasing recognition of the adverse physical and psychosocial effects both during childhood and in the long term. The limited published Scottish data have suggested that the prevalence of obesity is higher in Scotland than in England (Chinn & Rona, 2001). Estimates of the prevalence are necessary so that the need for preventative measures can be assessed and secular trends monitored. The aims of the present study were, first, to establish the current prevalence of overweight and obesity in Aberdeen primary schoolchildren and, second, to investigate how these children changed during primary school years.

Data were extracted from the school nurse records of all children currently in primary year 7 in nineteen city primary schools. These children had their heights (to 1 mm with a portable stadiometer) and weights (to 0.1 kg with Seca scales, in light indoor clothing) measured in primary year 1 (P1), in 1997/98, and again in primary year 6 or 7 (P6/7), in 2002/03, as part of routine child health surveillance. BMI was calculated and data converted to BMI centiles and SD scores using the UK 1990 reference data for BMI in childhood (Cole *et al.* 1995). Overweight was defined as BMI ≥85th centile (SD score ≥1.04) and obesity as BMI ≥95th centile (SD score ≥1.64) (Barlow & Dietz, 1998).

Paired P1 and P6/7 data were available for 367 children (191 boys). The mean age was 5.6 years in P1 and 10.9 years in P6/7. The prevalences of overweight and obesity did not change significantly between P1 and P6/7 but at both ages exceeded the expected prevalences of 15% for overweight and 5% for obesity by 2- to 3-fold.

	% Overweight			% Obese			Mean BMI SD score		
Year	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total
P1 (1997/98)	33.5	25.6	29.7	17.3	12.5	15.0	0.59	0.39	0.49
P6/7 (2002/03)	30.4	29.0	29.7	17.8	16.5	17.2	0.42*	0.30	0.36**

Mean scores were significantly different from those in P1 (paired *t* test): * $P<0.05$, ** $P<0.01$.

Differences in prevalence between boys and girls were not statistically significant. The mean BMI SD score significantly decreased during primary school in boys but not in girls. Overall, the BMI centiles were significantly lower in P6/7 compared with P1 ($P<0.001$). For boys the median centile decreased from the 72nd to the 66th whereas in girls the median BMI centile was the 61st in P1 and the 62nd in P6/7. Thirty-nine (10.6%) children became overweight, seventy (19.1%) remained overweight, thirty-nine (10.6%) had a BMI below the 85th centile in P6/7 despite being overweight in P1 and 219 (59.7%) children had a BMI below the 85th centile in both P1 and P6/7.

It is concluded that overweight and obesity are major problems in Aberdeen primary schoolchildren and they are essentially established before school entry. Public health interventions are needed urgently and, in particular, should target the families of preschool children. The present cohort study has not shown an increase in prevalence with increasing age. Indeed, it is encouraging that the mean BMI SD score decreased during primary school. Children, particularly boys, may be benefiting from increased physical activity at school. Further studies are required to follow these trends in this population.

Barlow SE & Dietz WH (1998) *Pediatrics* **102**, e29.
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Cole TJ, Freeman JV & Preece MA (1995) *Archives of Disease in Childhood* **73**, 25–29.

The development and evaluation of portion size assessment tools for use with children. By E. FOSTER[1], J.N.S. MATTHEWS[2] and A.J. ADAMSON[1], [1]*Human Nutrition Research Centre and* [2]*School of Mathematics and Statistics, University of Newcastle, Newcastle upon Tyne, UK, NE1 7RU*

For intakes of food to be converted into nutrient intakes, a measure or estimate of the amount of each food consumed is required. Weighing all foods consumed is an onerous task which often results in individuals changing what they eat (Macdiarmid & Blundell, 1997). Obtaining weighed records for young children is particularly difficult when the child spends a large part of the day away from their parents. Three portion size assessment tools were developed as an aid for estimating portion size with children. These were food photographs, food models and a computer-based interactive portion size assessment system (IPSAS). All three tools were based on age-appropriate portion sizes for four age groups; 4 to 6, 7 to 10, 11 to 14, and 15 to 16 years (Gregory & Lowe, 2000).

Children were provided with known portions of twenty-two foods commonly consumed by children (Gregory & Lowe, 2000). Any leftover food was weighed. The following day children were asked to estimate the portion size of foods they had been served and had consumed. Each child estimated the amount of each of the foods using each of the portion size assessment tools at six separate interviews.

The accuracy and precision of the tools were assessed by calculating the ratio of the child's estimate of the amount of food served (or that they consumed) to the actual amount of food served (or consumed). Therefore, a value greater than one indicates over-reporting and a value of less than one indicates under-reporting. The distribution of the ratios was generally skewed and the analysis was therefore performed on the logarithms of the ratios (base 10) and the results presented in terms of geometric means (that is the antilog of the mean of the log-transformed data).

Fig. 1 (*n*, number of estimates)

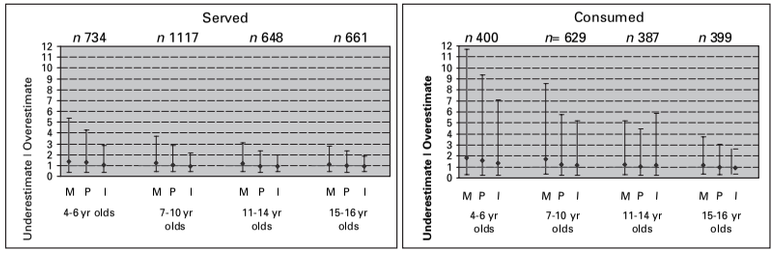

Figure 1 Key, M=Food models, P=Food photographs, I=IPSAS

Fig. 1 shows the geometric mean of the ratio of children's estimates of portion size to the actual portion size of foods served and consumed, plus and minus 2 SD, for each tool for each age group. Accuracy and precision increased with age for all tools. The IPSAS performed well with children of all ages for estimating both food served and consumed. The food photographs performed well with the older children but were poor in comparison with the IPSAS for the youngest age group. Accuracy and precision were better for estimates of the amount of food served compared with the amount consumed, particularly for the younger children. There may be a problem with reporting of leftovers in this age group.

The IPSAS has shown potential for the assessment of dietary intake with children. Further development is recommended. The tool would need to be expanded to cover a wider range of foods and then validated against weighed dietary intakes in a 'real-life' situation.

This work was funded by the Food Standards Agency (N08019).

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Macdiarmid JI & Blundell JE (1997) *European Journal of Clinical Nutrition* **51**, 199–200.

The need for a standard assessment of obesity as shown in a 1-year follow-up study of inner-city Dublin schoolchildren. By A.C. GRIFFIN[1], K.M. YOUNGER[1] and M.A.T. FLYNN[2], [1]*School of Biological Sciences, Faculty of Science, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland and* [2]*Nutrition and Active Living, Health Promotion and Disease Prevention, Calgary Health Region, 1509 Centre St SW, Alberta, T2T 5TI, Canada*

Positive secular trends in adolescent obesity have been documented worldwide but there is no record of an Irish trend. In addition to a lack of international consensus about assessment criteria for childhood obesity, no standard exists for assessing Irish children. In 1990, the Irish National Nutrition Survey used BMI ≥26 kg/m^2 to describe prevalence of overweight among Irish adolescents (age 12–14 years). The purposes of the present study were to examine the range in classification of Dublin schoolchildren as overweight according to four standard assessment methods and to assess changes in weight status in a 1-year follow up. In addition, to indicate trends over the past decade, the prevalence of children with a BMI ≥26 kg/m^2 was compared with that documented in the Irish National Nutrition Survey among adolescents of similar age (Hurson & Corish, 1997).

Healthy schoolchildren (*n* 199, ninety boys and 109 girls; mean age 11 years at baseline) attending seven fee-paying (six single- and one mixed-sex) and eight non-fee-paying (four single- and four mixed-sex) in Dublin city centre participated in the present 1-year follow-up study. Weight, height, waist circumference (WC) and triceps skinfold were measured and used in five definitions of overweight, including published cut-off points of BMI-for-age (CDC (National Center for Health Statistics, 2000), IOTF (Cole *et al.* 2000), UK90 (Cole *et al.* 1995)), actual relative weight (Roche, 1984) and BMI ≥26 kg/m^2 (Hurson & Corish, 1997). Actual relative weight was included to have an Irish reference by using existing growth centile charts (Hoey *et al.* 1987).

	CDC				IOTF				UK90				Actual relative weight			
	Baseline		Follow up		Baseline		Follow up		Baseline		Follow up		Baseline		Follow up	
Body weight category	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*
Underweight	2	4	1	2	–		–		1	2	1	2	11	22	8	16
Risk of underweight	5	10	5	10	–		–		6	12	4	8	19)	38	23	46
Normal weight	74	147	73	145	80	159	79	157	73	145	73	145	45	90	43	86
Risk of overweight	12	23	13	26	18	36	16	32	12	24	14	28	14	28	14	28
Overweight	8	16	9	18	2	4	5	10	9	18	9	18	11	22	13	26

The prevalence of overweight within the total group differed between the four standard definitions of weight status by 9% at baseline, and 8% at follow up. Accordingly, trends over the year ranged from increases of 0–3%. At baseline and follow-up, BMI was found to correlate more strongly than actual relative weight to triceps measurements (r−0.731 *v.* r 0.606, at mean age 11 years; r−0.662 *v.* r 0.581, at mean age 12 years). Using the criterion BMI >26 kg/m^2, 6% of Dublin schoolchildren were overweight compared with 2% of schoolchildren in 1990. According to actual relative weight, overweight children had a greater adipose tissue centrally located than their normal-weight counterparts (at baseline, WC was 712 (11) *v.* 632 (11) mm, P<0.001; and at follow-up, WC was 715 (11) *v.* 651 (11) mm, P<0.001).

The present study indicates that Irish children are getting fatter but the extent of this trend is confused by the availability of assessment methods, none of which refer to an Irish childhood population. Unfortunately, the Irish Nutrition Survey did not report waist circumference and therefore, trends in abdominal fat distribution cannot be assessed. In conclusion, a standard method for the assessment of weight status is urgently needed for the evaluation of obesity-prevention initiatives among Irish schoolchildren.

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Television viewing in adolescence and change in body mass index in the 1958 British birth cohort. By T.J. PARSONS[1], C. POWER[1] and O. MANOR[2], [1]*Paediatric Epidemiology and Biostatistics, Institute of Child Health, UCL, 30 Guilford Street, London, UK, WC1N 1EH and* [2]*School of Public Health and Community Medicine, Hebrew University, Hadassah, P.O.B. 12272, Jerusalem 91120, Israel*

A number of studies suggest that sedentary lifestyles, as indexed by higher levels of television viewing, video and computer use, relate to an increased risk of obesity, but most of these studies are cross-sectional, and therefore the direction of effect uncertain. Results from the fewer longitudinal studies in children have been mixed (Maffeis *et al.* 1998; Proctor *et al.* 2003).

We looked at the cross-sectional and longitudinal associations between television viewing and BMI in the 1958 British birth cohort, which included all children born in England, Scotland and Wales, in 1 week in March 1958. From a target population of 17 733 births, information was obtained on 98% of these births. BMI (kg/m^2) was measured at ages 16 and 33, and self-reported at 23 and 42 years. Frequency of television viewing in adolescence was collected by questionnaire at 11 and 16 years. Multi-level models, which allow for correlated data within individuals measured on several occasions, were fitted with BMI as a repeated outcome, or trajectory (four occasions, 16–42 years). Random effects were included to allow the intercept and linear slope of the BMI trajectory to vary by individual.

Mean BMI at 16 years was 20.2 (SD 2.7) kg/m^2 in males and 21.0 (SD 2.9) kg/m^2 in females. The majority of boys and girls watched television often, 86% and 84% respectively at 11y, 67% and 64% at 16y, with the remainder watching sometimes. Neither television viewing at 11 or 16 years was associated with BMI at 16 years (intercept), but in both sexes television viewing at 16y (not 11y) was related to the linear slope of the BMI trajectory. Boys who watched television often at 16 years had a faster rate of increase in BMI between 16 and 42 years, by 0.011 kg/m^2 per year than those who watched television sometimes. In girls the rate of increase was 0.016 kg/m^2 per year higher for those who watched television often. Adjusting for BMI at 11 years reduced the effect of television viewing on the BMI trajectory slope in males but not females. Physical activity at 16 years, maternal BMI, or social class, did not explain the effect of television viewing on the BMI trajectory slope in males or females.

			Intercept at 16 years (kg/m^2)		Linear change from 16 years (kg/m^2 per year)		
		Television viewing	Coefficient	95% CI	Coefficient	95% CI	*n*
11y	Males	Often[1] *v.* sometimes	0.056	−0.136, 0.248	0.006	−0.006, 0.018	6478
	Females	Often[1] *v.* sometimes	0.151	−0.053, 0.355	0.011	−0.003, 0.025	6248
16y	Males	Often[2] *v.* sometimes	−0.079	−0.226, 0.068	0.011	0.001, 0.021	5766
	Females	Often[2] *v.* sometimes	0.016	−0.143, 0.175	0.016	0.004, 0.028	5585

[1] nearly every day [2] undefined

Although television viewing may not be associated with BMI in adolescence at a given time-point, the present results suggest that it is an important factor in relation to rate of gain in BMI over time, through to 42 years. Given that children aged 4–15 years in the UK watch about 18 h of television per week (Matheson & Summerfield, 2001), reducing television viewing may be a promising strategy for reducing obesity levels.

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Matheson J & Summerfield C (2001) *Social Trends* no. 31. London: The Stationery Office.
Proctor MH, Moore LL, Gao D, Cupples LA, Bradlee ML, Hood MY & Ellison RC (2003) *International Journal of Obesity* **27**, 827–833.

An assessment of public health nutrition workforce capacity in Australia: implications for obesity prevention. By R.M. HUGHES, *Nutrition Unit, School of Health Science, Griffith University, Gold Coast, Australia*

Like many industrialised nations, Australia is in the grip of an obesity epidemic. Trend data suggest that the prevalence of overweight and obesity has increasing significantly over the last two decades, with a mean population weight gain of approximately 7 kg over this period (Australian Institute of Health and Welfare, 2004). The capacity of a nation to address a public health issue such as obesity in part depends on the capacity of the health workforce to organise societal efforts that prevent the development of obesity. The present paper interprets and reports data from a national study that investigated the determinants of public health nutrition workforce capacity in Australia to address priority issues such as obesity prevention. This assessment was conducted in order to identify workforce development and capacity-building needs at a national level, to support organised effort for obesity prevention. Data were derived from an interpretive study using multiple methods including literature review, advanced-level practitioner interviews (*n* 41), a national workforce survey (*n* 240, 87% response rate), position description analysis (*n* 46) and consensus development via a Delphi study. Interpretive analysis included the methodological triangulation of data, and a mix of quantitative and qualitative data analysis. The findings of this study suggest that five main categories of determinants currently limit the capacity of the Australian public health nutrition workforce (Table 1).

Table 1. Determinants limiting public health nutrition workforce capacity in Australia

Determinant category	Finding
Human resource infrastructure	A small designated workforce (<300 nationwide), less that 40% of the size suggested by US workforce planning benchmarks (Dodds & Kaufman, 1991). Dominated by practitioners with dietetic training backgrounds as highest qualification. Workforce instability with high turnover rates.
Organisational and policy environments	Limited collaborative and multi-disciplinary practice associated with disorganisation of the workforce (only a small proportion working within a public health unit structure).
Intelligence access and use	Perceptions of limited intelligence availability and reported use. An underdeveloped research and evaluation culture limiting intelligence dissemination.
Practice improvement and learning systems	Limited incentives for excellence in practice, an absence in consensus about the required work and competencies required for effective practice.
Workforce preparation	Inadequacies in dietetic training a major contributor to workforce unpreparedness.

(Hughes, 2003*a–d*, 2004*a*, *b*)

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Hughes R (2003*b*) *Public Health Nutrition* **6**, 599–605.
Hughes R (2003*c*) *Nutrition and Dietetics* **60**, 198–204.
Hughes R (2003*d*) *Public Health Nutrition* **6**, 839–847.
Hughes R (2004*a*) *Nutrition and Dietetics* (In the Press).
Hughes R (2004*b*) *Nutrition and Dietetics* **61**, 38–45.

Association of physical activity with body composition indices in children aged 6–8 years at varying risk of obesity. By K.L. RENNIE[1], M.B.E. LIVINGSTONE[2], J.C.K. WELLS[3], A. McGLOIN[2], W.A. COWARD[1] and S.A. JEBB[1], [1]*MRC Human Nutrition Research, Fulbourn Road, Cambridge, UK, CB1 9NL,* [2]*Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine, County Londonderry, UK, BT52 1SA and* [3]*Childhood Nutrition Research Group, Institute of Child Health, 30 Guilford Street, London, UK, WC1N 1EH*

Recent studies have reported inconsistent relationships between measures of physical activity and body composition, which may be partly due to inaccuracies in assessing both activity and body composition (Parsons *et al.* 1999). Using an objective measure of physical activity, we examined cross-sectional relationships between physical activity, activity energy expenditure (AEE), lean body mass (LBM) and fat mass (FM) in 100 healthy children recruited from the Coleraine area who are at varying risk of obesity based on parental habitus.

Sixty boys and forty girls aged 6–8 years were categorised into two risk groups; the high-risk (HR) group (*n* 50) had at least one biological parent with a BMI>29.5 kg/m^2 and the low-risk (LR) group (*n* 50) had two lean biological parents (BMI<25 kg/m^2). Total energy expenditure (TEE), LBM and FM were measured over 10 d by the doubly labelled water method (McGloin *et al.* 2002). AEE was calculated as 0.9 TEE – predicted BMR (Schofield, 1985), to include a correction for 10% thermogenesis. To adjust for body size, FM and LBM were divided by height (m) squared and expressed as FM index and LBM index respectively (Wells, 2001). Multiple linear regression was used to examine associations between FM index and BMI adjusted for sex, risk group and LBM.

Children in the HR group had significantly higher waist circumference, weight, BMI, LBM index and FM index than the LR group, but did not differ in age or height. Boys expended significantly more energy in activity than girls (mean AEE 2.66 (SD 0.76) *v.* 2.03 (SD 0.62) MJ/d; $P<0.001$); only 43% of the difference in AEE between the sexes was accounted for by LBM. There was no difference in AEE between the groups, after adjustment for LBM and sex ($P=0.25$).

	LR group (*n* 50)		HR group (*n* 50)	
	Mean	SD	Mean	SD
Age (years)	6.7	0.6	6.6	0.8
Weight (kg)	23.6	3.7	25.7*	4.7
Height (m)	1.24	0.06	1.25	0.08
Waist circumference (mm)	562	36	585*	56
BMI (kg/m^2)	15.3	1.6	16.5†	1.6
FM index (kg/m^2)	1.60	0.9	2.08*	1.3
LM index (kg/m^2)	13.7	1.2	14.3†	1.0

Mean values were significantly different from those for the LR group (one-way ANOVA): * $P<0.05$, † $P<0.01$.

AEE was significantly negatively associated with FM index after adjustment for sex, risk group and LBM ($P=0.001$), with this model explaining 17% of the variance in FM index. AEE explained 59% of this variance. Conversely, AEE was not significantly associated with BMI ($P=0.08$) after adjustment for sex, risk group and LBM.

These data suggest that lack of physical activity is associated with excess fatness in pre-pubescent children, but causality cannot be established from the present cross-sectional study. The present study also emphasises that BMI may be a misleading measure of body composition to apply when evaluating the impact of lifestyle behaviours on the risk of obesity.

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Physical activity and inactivity patterns in adolescent females living in the United Arab Emirates. By H.J. LIGHTOWLER[1], C.J.K. HENRY[1] and H.M. AL-HOURANI[2], [1]*Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford, UK, OX3 0BP and* [2]*Department of Clinical Nutrition and Dietetics, Faculty of Allied Health Sciences, The Hashemite University, PO Box 150459, Zarqa 13115, Jordan*

A high proportion of adolescent females in the United Arab Emirates (UAE) are overweight or obese (Al-Hourani *et al.* 2003), reflecting an energy intake that exceeds energy expenditure from metabolism and physical activity. Previous research has indicated that physical exercise among adults in the UAE is limited (McIlvenny *et al.* 2000). However, there is no information on physical activity and inactivity patterns in children and adolescents in the UAE. The aim of the present study was to investigate physical activity levels (PAL) and television viewing in adolescent females in the UAE.

A total of fifty-eight adolescent females, aged 11–16 years, were recruited from two female-only governmental schools in Abu-Dhabi and divided into two age groups; 11–13 years (*n* 22) and 14–16 years (*n* 36). PAL and length of time spent watching television were determined from a 3 d activity diary. Using the 85th and 95th percentiles of the sex-specific BMI-for-age growth charts as cut-off points (Kuczmarski *et al.* 2002), six subjects aged 11–13 years and seven subjects aged 14–16 years were classified as overweight or obese.

Age...	11–13 years (*n* 22)			14–16 years (*n* 36)		
	Mean	SD	Range	Mean	SD	Range
PAL						
– Schooldays	1.26	0.03	1.20–1.32	1.27	0.03	1.20–1.34
– Weekend	1.27	0.04	1.20–1.35	1.26	0.03	1.19–1.32
Television viewing (h/d)						
– Schooldays	2.0**	0.9	0.0–4.5	1.8***	0.7	0.0–4.0
– Weekend	3.1	1.0	0.0–5.0	3.1	0.8	0.0–4.5

Mean values were significantly different from those for the weekend: ** $P<0.01$, *** $P<0.001$.

Differences between BMI percentiles on PAL and on hours of television watched during schooldays and the weekend were not apparent. The poor levels of physical activity observed in the present study are of concern. Indeed, the PAL are considerably lower than those observed in females of a similar age in other countries (Torun *et al.* 1996; Henry *et al.* 1999). Participation in physical exercise has been affected by advances in technology, with many children and adolescents preferring indoor activities, such as watching television, to outdoor activities. In the present study, television watching was the predominant leisure-time pursuit.

In conclusion, the results from the present study show that the amount of physical activity undertaken by adolescent females in the UAE is very low. Cultural and weather restrictions and social change of the community in the UAE are not conducive to physical activity and play a major role in levels of physical inactivity. This may explain, in part, the rise in the incidence of overweight and obesity in this population.

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The influence of lifestyle factors on obesity in an Irish adult sample. By C.M. MURRIN[1], F. SHIELY[1], S. FRIEL[2], G. NOLAN[1] and C.C. KELLEHER[1], [1]*National Nutrition Surveillance Centre, Department of Public Health Medicine and Epidemiology, Woodview House, University College Dublin, Belfield, Dublin 4, Republic of Ireland and* [2]*Department of Health Promotion, Clinical Sciences Institute, National University of Ireland Galway, Costello Road, Shantalla, Galway City, Republic of Ireland*

The prevalence of overweight and obesity continues to increase in Ireland. The recent SLÁN survey (National Nutrition Surveillance Centre, 2003) indicated that obesity rates rose by 3% from 11% in 1998 of men to 14% in 2002 and from 9% of women in 1998 to 12% in 2002 (standardised to the 1996 census). The survey was a self-administered postal questionnaire completed by 5992 adults (aged 18 years and over) (Kelleher *et al*. 2002; J Harrington, unpublished Masters thesis). The sample, generated from the electoral register, was powerful enough to detect differences according to socio-economic status in key variables, smoking, exercise and adherence to dietary recommendations.

The aim of the present analysis was to examine the indicators most predictive of obesity in the Irish population (Hayes *et al*. 2004). A binary logistic regression was conducted with obesity (BMI $>30\,kg/m^2$) as the dependent variable. Factors included in the model were age, number of strenuous, moderate and mild exercise sessions/week, frequency of light and heavy housework, level of activity at work, number of hours sitting, number of hours watching television, smoking, alcohol consumption, compliance with food pyramid recommendations, frequency of fried-food consumption, gender, general medical services (means tested health care eligibility) status, rural or urban location, education level and marital status. Backward Wald was chosen with entry and removal set at 0.10. The total valid sample for the regression was 2789.

Results showed that those aged 35–54 years are more likely to be obese than other age groups ($P=0.089$; odds ratio (OR) 1.338). Obesity is more likely in those with none or primary ($P<0.001$; OR 2.503) and secondary education ($P<0.001$; OR 1.629) than in those with tertiary education. Those who spend more time sitting ($P=0.092$; OR 1.008), are not physically active in their job ($P<0.001$; OR 1.537), and engage in mild exercise only ($P=0.058$; OR 1.039) are more likely to be obese. Those who do light housework are less likely to be obese ($P<0.001$; OR 0.525). As the number of strenuous ($P=0.004$; OR 0.843) and moderate ($P=0.003$; OR 0.928) exercise sessions increases, respondents are less likely to be obese. Those who consume fried food more than once weekly ($P=0.004$; OR 1.433), who do not comply with the recommended servings from the bottom shelf ($P=0.049$; OR 1.293) and from the fruit and vegetable shelf ($P=0.002$; OR 1.493) of the food pyramid are more likely to be obese. Those complying with the recommendations from the dairy shelf are less likely to be obese ($P=0.012$; OR 0.694).

The present findings clearly demonstrate that obesity varies with socio-demographic patterns and thereby provide information for developing future policies tailored to the specific needs of the population.

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Social facilitation of eating behaviour: is it just distraction? By L. NEWSON[1], A.S. ANDERSON[2] and M.M. HETHERINGTON[1], [1]*School of Psychology, University of Liverpool, Liverpool, UK, L69 7ZA and* [2]*Centre for Public Health Nutrition Research, University of Dundee, Dundee, UK, DD1 9SY*

Previous research reliably demonstrates significant enhancement of food intake in the presence of familiar others (Clendenen *et al*. 1994), by as much as 70% (de Castro, 1994). It is not clear what mechanism underlies social facilitation effects; however, it is possible that distraction might play an important role. The present study was devised to examine the relative effects of distraction and the presence of familiar others on food intake.

Using a repeated-measures design, twenty-one participants visited the laboratory on four occasions to eat a meal *ad libitum*. Participants either ate alone, ate alone with a distraction (interactive TV game show), ate with two friends, or ate with two strangers. In this way, the effects of distraction and the presence of others were examined independently. A buffet-style meal was presented on trays to participants consisting of: cheese sandwiches; coleslaw; mixed salad; plain crisps; a selection of cake bars (13.92 MJ (3328 kcal) in total). This was served with chilled water and tea or coffee.

A highly significant effect of sex on food intake was found, ($F_{1,19}$ 9.736; $P=0.006$), indicating that men ate significantly more than women. Therefore, data were analysed separately by sex. A significant effect of condition ($F_{3,36}$ 3.854; $P=0.02$) on food intake (energy) was found only for men. This indicated that eating with friends enhanced food intake compared with eating alone by 28% ($P=0.025$). Men also ate 15% more when watching TV than when eating alone, although this difference failed to reach significance. These effects were not found for women who tended to eat a similar amount of food under each condition. The Table shows energy intake in each condition for males and females.

	Baseline: alone		Alone: distracted		Social: strangers		Social: friends	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Males (*n* 13)								
KJ	4146	319	4770	500	4167	412	5330*	449
Kcal	991	76.3	1140	119.4	996	98.5	1274*	107.4
Females (*n* 8)								
KJ	3046	294	3234	320	3251	546	3117	362
kcal	728	70.2	773	76.6	777	130.6	745	86.6

* Mean value was significantly different from baseline ($P<0.025$).

In conclusion, men were responsive to social facilitation of eating behaviour and this may be attributable to the effects of distraction. However females ate the same amount of food under each condition and may have restrained food intake in the presence of others.

Clendenen VI, Herman CP & Polivy J (1994) *Appetite* **23**, 1–13.
de Castro JM (1994) *Physiology and Behavior* **56**, 445–455.

Investigating enhancement of intake by variety: the role of attention. By G.N.M. NORTON[1], A.S. ANDERSON[2], R. FOSTER[1] and M.M. HETHERINGTON[1], [1]*School of Psychology, University of Liverpool, Liverpool, UK, L69 7ZA and* [2]*Centre for Public Health Nutrition Research, University of Dundee, Dundee, UK, DD1 9SY*

Enhanced consumption with increased food variety has been demonstrated in response to the presentation of multiple foods both simultaneously (Spiegel & Stellar, 1990) and sequentially (for example, Rolls *et al.* 1981). An understanding of the conditions in which, and mechanisms by which, variety can enhance food intake could provide useful dietary advice for those involved in weight-control attempts. The present study examined the effect of introducing other tastes on overall consumption of a single snack food.

Participants attended the laboratory on four occasions. They ate a snack of sweet or salted popcorn (depending on preference) under the following conditions, in counterbalanced order: baseline (no distraction); same taste (interrupted to taste and rate the pleasantness of and desire to eat the same popcorn); congruent taste (interrupted to taste and rate the pleasantness of and desire to eat a congruent food, i.e. chocolate if eating sweet popcorn, cheese if eating salted popcorn); incongruent taste (interrupted to taste and rate the pleasantness of and desire to eat an incongruent food, i.e. cheese if eating sweet popcorn, chocolate if eating salted popcorn).

Males ate significantly more in several conditions and scored significantly lower in restraint than females, therefore analyses were performed separately for males and females. Males demonstrated reduced intake in the same taste condition relative to the two novel food conditions ($F_{3,33}$ 3.238, P=0.04; see Table). This finding was not evident in females who consumed the same weight of food in each condition. However, amongst the female sample a stepwise multiple regression analysis showed external eating (measured on the Dutch eating behaviour questionnaire; van Strien *et al.* 1986) to be a significant predictor of the change in intake in the three taste conditions relative to baseline, explaining between 13 and 24% of the variance in intake. Higher external eating scores were associated with greater popcorn intake in the 'taste' conditions relative to baseline (Pearson's correlations: same taste condition r 0.458, P<0.05; congruent taste condition r 0.520, P<0.01; incongruent condition r 0.413, P<0.05). The Table shows the weight of food (g) consumed in each condition.

	Baseline		Same		Congruent		Incongruent		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P* value
Females (*n* 24)	35.5	2.9	37.4	3.5	36.6	4.4	37.6	3.2	NS
Males (*n* 12)	46.5	5.4	42.7	3.5	52.6*	4.6	59.0*	5.3	0.04

*Mean values within a column were significantly different.

In conclusion, focusing attention on food reduced intake compared with having attention drawn away from the eaten food in males. This finding suggests that the variety effect is associated with the degree to which interest in eating is maintained by the presence of other foods. This effect was not found in females, although external eating predicted susceptibility to increased intake as a result of interruption. Thus, weight-management programmes might benefit from providing advice about avoiding distraction during eating, particularly in consumers vulnerable to external cues to eat.

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Effect of the presence of fish on the amount of chips served when the thickness of chips is varied. By L.E. BURTON and A. WISE, *The Robert Gordon University, St Andrew Street, Aberdeen, UK, AB25 1HG*

Calvin & Wise (2001) showed that when individuals are asked to serve themselves a portion of chips, mean weights adjusted for sex were 187.1, 156.9 and 108.5 g for the thick, medium, and thin chips, respectively. This is an interesting finding relating to visual perception of portion size when the chips are varied, but it is more common for individuals to consume chips with other foods, and hence it is important to investigate the extent of these differences under more realistic eating situations. Three breaded haddock fillets (mean 150 g) and three varieties of McCain's chips were oven baked according to the manufacturers' instructions. Six octagonal plain white plates (230×300 mm) were placed on a table with six large bowls placed in front of them each containing one of the varieties of chips. A Latin square was used to ensure that the different thicknesses of chips, with or without fish, were presented in an unbiased order on the table. Students (twenty-seven male and thirty-five female) were individually asked to place a portion of chips that they would normally consume onto each plate as if they were having a meal and they passed down the table, doing this for each plate in turn. Whilst using the tongs, the number of dips into the bowl of chips was counted and after the student had departed, the height and weight of the chips on each plate were recorded. The data were analysed by three-way ANOVA (individual×thickness×presence or absence of fish). Students also completed a brief questionnaire.

Chip variety...	Thin		Medium		Thick	
	Mean	SD	Mean	SD	Mean	SD
Portion weight (g; with fish)	85.1	30.0	131.4	34.2	149.1	46.8
Portion weight (g; without fish)	95.4	32.2	161.5	53.5	194.7	60.7
Dips (with fish)	3.4	1.5	3.3	1.5	3.1	1.3
Dips (without fish)	3.6	1.4	3.4	1.4	3.8	1.8
Height (mm; with fish)	47	10	49	9	50	9
Height (mm; without fish)	44	9	47	8	49	8

As expected from previous studies, the weight of chips increased with their thickness (P<0.001). The mean weights of chips were lower on plates with fish, but there was a significant interaction (P<0.001) such that the proportional decrease was lowest for thin chips (to 89.2, 81.4 and 76.6% for thin, medium and thick, respectively). Similar significant conclusions were reached for both sexes when analysed separately, although the mean weights for males were 9.9% greater and the effect of having fish on the plate was generally less (to 84.3 *v.* 78.2%). Since 37% of subjects claimed to consume chips only with a meal and only 7% claimed to consume chips always without other foods, it would appear that the present study may reflect genuine food habits, particularly since when asked what they would eat with chips, 56% replied 'fish'. It is estimated that the fish reduced the area on the plate to approximately 60% (78% if the rim is included). Potential biases in the study include the fact that students were chosen, although from a wide range of courses, but diets of students may differ from those of the general public. It is possible that the timing in relation to meal consumption when individual students took part in the study may have influenced the results in that hungrier students might have loaded more chips onto their plates. They did not consume any of the food however and were simply asked how much they would normally consume. It was concluded that subjects underestimated the amount of thin chips compared with thick chips in the presence of fish in the same way as they have been found to do on empty plates. They reduced the amount of chips on plates containing fish by proportionately less for thin chips compared with thick chips.

Calvin J & Wise A (2001) *Annals of Nutrition and Metabolism* **45**, Suppl. 1, 368–369.

Mothers' educational level is positively associated with overweight among Tehrani adolescent boy students. By B. MOHAMMADPOUR-AHRANJANI[1], L. ZELLIPOUR[1], A. RASHIDI[1], N. KALANTARI[1], M. KARANDISH[2] and M.R. ESHRAGHIAN[3], [1]*National Nutrition and Food Technology Research Institute, PO Box 19395-4741, Tehran, Iran,* [2]*Ahwaz University of Medical Sciences, Ahwaz, Iran and* [3]*Tehran University of Medical Sciences, Tehran, Iran*

Overweight has been recently taken into account as a challenging health issue emerging from epidemiological and nutritional transition in developing societies (Popkin, 2001), including the Iranian population (Ghassemi *et al.* 2002). While adolescence is regarded as a critical period in determination of adulthood obesity (Dietz, 1994), very little evidence has been published on the prevalence and risk factors of overweight among Iranian adolescents. The present study was carried out in order to determine the possible correlation between overweight in adolescent male students and their mothers' educational level in Tehran city, 2000–01. Using the multistage cluster sampling method, 627 students aged 14–17 years were selected from twelve representative high schools (as clusters). Weight and height were measured and BMI was calculated for each student. Subjects were recognised as overweight if their BMI was equal or greater than the 85th percentile of age- and sex-specific NCHS/CDC2000 BMI values (NCHS/CDC, 2000). Students reported their mothers' educational level as illiterate, primary, secondary, high school diploma, and academic degrees. Statistical analysis was done using SPSS 10.0 software after excluding underweight students (BMI <5th percentile).

Weight status...	Normal		Overweight	
Mothers' educational level	*n*	%	*n*	%
Illiterate	40	8.3	6	6.5
Primary school	137*	28.5	11*	11.8
Secondary school	124	25.8	19	20.4
High-school diploma	137	28.5	36	38.7
Academic degree	43	8.9	21	22.6

* P=0.05 (logistic regression).

The χ^2 test revealed a significant difference between normal and overweight students regarding their mothers' educational level (P<0.001). The difference remained significant even after adjustment for other variables. This suggests that more years of formal education among mothers may lead to overweight in their children. A possible cause for this may be an increase in economic access to dietary energy at household and individual levels in addition to an increase in mothers' worries about their children's under-nutrition. Mothers should therefore gain information on the nutrient values of different foods, nutritional requirements, and risk of overweight among their children through nutrition education programmes, in order to provide a proper dietary pattern for the family.

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Popkin BM (2001) *Journal of Nutrition* **131**, 871S–873S.

The effects of self-recording food intake on body weight over 7 d. By S. WHYBROW[1], C. MAYER[2], T. KIRK[1] and R.J. STUBBS[3], [1]*Queen Margaret University College, Clerwood Terrace, Edinburgh, UK, EH12 8TS,* [2]*Biomathematics and Statistics Scotland, Greenburn Road, Aberdeen, UK, AB21 9SB and* [3]*Rowett Research Institute, Greenburn Road, Aberdeen, UK, AB21 9SB*

It has been suggested that an effective method of achieving a short-term weight loss is to ask subjects to weigh and record their food intake. The resultant change in feeding behaviour can be sufficient to produce a negative energy balance. This change in feeding behaviour may have consequences for the interpretation of cross-sectional dietary surveys and dietary intervention trials. The present study compared changes in body weight, as proxy measures of changes in feeding behaviour, when free-living subjects were recording food intakes, compared with when they were not, during a snacking intervention trial.

Forty-one males (age 35 (SD 7.6) years, BMI 26 (SD 4.7) kg/m^2) and forty-four females (age 34 (SD 8.4) years, BMI 26 (SD 4.4) kg/m^2) each consumed 0 MJ (control), 1.5 MJ (low-energy) and 3.0 MJ (high-energy) of snack products per d for 14 d in a counter-balanced cross-over-design study. Each intervention period comprised 7 d of equilibration where the only manipulations were measurement of body weight and consumption of the prescribed snacks, followed by 7 d when subjects self-recorded their food intakes using the weighed food record method. Body weight, corrected to nude, was investigator recorded at the beginning, middle and end of each 14 d intervention period. Suspected low-energy-reporters were not excluded from these analyses.

Changes in body weight, relative to day 1 of each intervention period, are presented in Fig. 1.

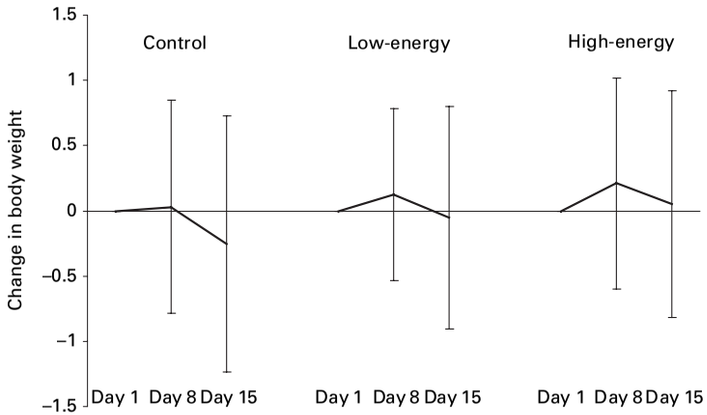

Fig. 1. Change in body weight (kg) for each level of intervention snack energy. Weight change over the equilibration period (day 1 to day 8) and over the measurement period (day 8 to day 15).

Body-weight change was more negative when subjects were self-recording their food intakes than when they were not; days 8 to 15 compared with days 1 to 8 (P=0.004). The graded effect of consuming the intervention snacks on a more positive body-weight change (P=0.02) is less clear if focusing only on weight change during the measurement period (days 8 to 15) than if the weight change over the equilibration period is also considered.

For intervention studies focusing on energy balance, measurement of body-weight change over the recording period, and over a similar time period when subjects are not recording their intakes, will be more informative than changes in self-recorded food intake alone.

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The Mediterranean Eating in Scotland Experience (MESE) project: impact of an Internet-based, tailored intervention on dietary behaviour. By A. PAPADAKI and J.A. SCOTT, *University of Glasgow, Division of Developmental Medicine, Human Nutrition Section, Glasgow Royal Infirmary, Glasgow, UK, G31 2ER*

Tailored dietary and psychosocial interventions have proved to be effective in motivating dietary change (Brug *et al.* 1999). Also, Internet-technology interventions have shown promising results in encouraging weight loss (Tate *et al.* 2001) and physical activity (Sciamanna *et al.* 2002) compared with general information. However, no tailored or Internet-technology intervention to date has encouraged the consumption of a diet consistent with the principles of the Mediterranean diet. The aim of the present study was therefore to investigate the effect of an Internet-based nutrition intervention promoting the traditional Mediterranean diet.

An intervention trial using a quasi-experimental design was carried out at two work-sites. Subjects received either tailored dietary and psychosocial feedback and Internet nutrition information (intervention group; *n* 53) or minimal dietary feedback and general healthy-eating brochures (control group; *n* 19). All subjects were recruited from the University of Glasgow (intervention) and Glasgow Caledonian University (control) and were all healthy female volunteers aged 25–55 years. Feedback provided to both groups was delivered via electronic mail. Dietary advice provided to intervention subjects via an innovative Mediterranean Eating Website focused on the increased consumption of four components of the Mediterranean diet, namely vegetables, fruit, legumes and MUFA:saturated fatty acids (SFA) ratio. Subjects completed a 7 d estimated food diary at baseline and 6 months.

Forty-one subjects in the intervention group and fourteen subjects in the control group completed both the baseline and 6-month dietary assessment. Baseline mean intakes of vegetables, fruits, nuts and seeds, legumes and MUFA:SFA ratio were similar between the two groups. At the end of the 6-month intervention, subjects in the intervention group had significantly increased their mean intake of vegetables (176.5 *v.* 226.0 g/d; P=0.002), fruits (203.9 *v.* 249.0 g/d; P=0.02) and legumes (16.8 *v.* 35.8 g/d; P=0.000), as well as the MUFA:SFA ratio in their diet (1.43 *v.* 1.84; P=0.000). Subjects in the control group significantly increased their intake of legumes (17.0 *v.* 30.9 g/d; P=0.041) and dairy products (167.9 *v.* 225.3 g/d; P=0.008) but showed no other significant differences compared with baseline.

This Internet-based, tailored intervention used to promote healthy eating in the context of the traditional Mediterranean diet holds promise in encouraging higher consumption of vegetables, fruits and legumes, as well as increasing monounsaturated fat and decreasing saturated fat in the diet. As information technology systems improve and general access to computers has increased, such interventions have the potential to promote greater consumption of plant foods in Scotland, in agreement with current dietary recommendations for health promotion and disease prevention.

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Attitudes and perceptions towards healthier eating of older adults residing in sheltered accommodation. By P.N. HINDMARCH[1], C.E. WOOD[1], C.J. SEAL[2], A.J. ADAMSON[2], J.C. MATHERS[2] and P.J. MOYNIHAN[1], [1]*School of Dental Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK, NE2 4BW and* [2]*Human Nutrition Research Centre, Wellcome Research Laboratories, RVI, Queen Victoria Road, Newcastle upon Tyne, UK, NE1*

Effective improvement of diet and health in old age requires an assessment of the attitudes and perceived barriers towards a healthier diet. The application of the theory of planned behaviour (TpB) (Ajzen, 1991) has been well documented for health education programmes and reported to be successful in the prediction of healthy eating intentions (Conner *et al.* 2002).

As part of an ongoing community nutrition intervention for older adults living in socially deprived areas of Tyne and Wear, the aim of the present analysis was to assess the attitudes towards healthier eating, the perceived behavioural control over eating more healthily, the influence of others on intention to eat a healthier diet (subjective norm) and degree of unrealistic optimism in relation to the healthiness of diet.

Older adults (*n* 288) aged 60–90 years residing in sheltered accommodation were asked to complete a self-administered, purposely designed, questionnaire based on the TpB that used a visual analogue scale. The questionnaire was constructed with items relating to attitudes towards healthier eating, perceived behavioural control over healthier eating and subjective norm, all of which were scored using a bi-polar scale (−3 to +3). Perceived need to eat more healthily and degree of unrealistic optimism concerning the healthiness of the diet were scored using a uni-polar scale (1–7). Higher scores indicate a more positive belief or influence.

Completed questionnaires were obtained from 160 subjects (77.3%) of which 16.9% were male. The mean age of respondents was 76.4 (range 61–90) years. The results are tabulated below.

Questionnaire item	Response anchors	Possible scores	Median	Range
Perceived need				
How much do you feel you need to eat a healthier diet?	Not at all – a lot	1 to 7	6	1 to 7
Attitude				
For me healthier eating would be	Extremely bad – extremely good	−3 to +3	+3	−3 to +3
For me healthier eating would be	Extremely unenjoyable – extremely enjoyable	−3 to +3	+2	−3 to +3
For me healthier eating would be	Extremely easy – extremely difficult	+3 to −3	+1	+3 to −3
Perceived behavioural control				
How much control do you believe you have over eating a healthier diet?	No control – control	−3 to +3	+3	−3 to +3
Subjective norm				
The people in my life whose opinions I value think I should try to eat a healthier diet	Strongly disagree strongly agree	3 to +3	+1	3 to +3
Optimistic bias				
Compared with persons of the same sex and age how healthy is your diet?	Much less healthy much more healthy	1 to 7	5	1 to 7

The results indicate a positive attitude towards healthier eating and a perceived need to eat more healthily. The sample population expressed a high level of control over their ability to eat more healthily with a slight influence from peers. There was a strong tendency towards unrealistic optimism concerning the healthiness of their diet, which concurrent research indicates is comparable to national data (Wood *et al.* 2004). These findings suggest that older adults from sheltered accommodation may be amenable to dietary intervention and addressing optimistic bias is an important consideration for such programmes.

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Mean intakes of fifteen classes of dietary (poly)phenols in subjects following two commercial weight-loss programmes. By R.K. AYLEN[1], M.N. CLIFFORD[1], D.J. MILLWARD[1], H. TRUBY[1], A. DELOOY[2], K. FOX[3], P.J. ROBSON[4], I. MACDONALD[5] and L.M. MORGAN[1], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH,* [2]*Queen Margaret's University College, Edinburgh, UK, EH12 8TS,* [3]*University of Bristol, Bristol, UK, BS8 1TH,* [4]*University of Ulster, BT52 1SA, UK and* [5]*University of Nottingham, NG7 2RD, UK*

Epidemiological studies suggest that increased consumption of fruit and vegetables reduces the risk of chronic diseases, but which components are responsible is less certain. Such diets are richer in 'phytochemicals' and there is evidence that (poly)phenols may be important protective agents.

Excess weight is an independent risk factor for premature death and is associated with CVD, type 2 diabetes, and some types of cancer. Two popular commercial weight-loss programmes are the Weight Watchers diet and Dr Atkins Diet Revolution, the former encouraging increased consumption of fruit and vegetables, whereas the latter exploits a low-carbohydrate regimen resulting in a reduced consumption of fruit and vegetables, and associated (poly)phenols. While both diets can be effective in reducing body weight, if (poly)phenols are health protecting, a reduced intake might paradoxically compromise the benefit of a reduced body weight.

To define the effect of these weight-loss regimens on (poly)phenols intake and profiles, the University of Surrey (poly)phenol content database (Gosnay *et al.* 2002; Woods *et al.* 2003) has been applied to 7 d diet diaries shown to be reliable by the Goldberg method. Analyses were made at baseline and 2 months for ten subjects for each diet, and ten controls. The consumptions were estimated for fifteen classes of (poly)phenols, i.e. anthocyanins, cinnamates (including chlorogenic acids), derived polyphenols, dihydrochalcones, ellagitannins, flavanols, flavanones, flavones, flavonols, hydroxybenzoic acid derivatives, hydroxybenzyl alcohols, isoflavones, lignans, proanthocyanidins and stilbenes. Data were tested for normality of distribution and for changes in (poly)phenols intake over the three time points for each diet group. There were no statistically significant differences in baseline data for the three groups, and no effects of season or other variables for the controls. Results are expressed as mean$\pm$SEM.

The overall estimated mean total (poly)phenols intakes at 2 months were 654$\pm$72 mg/d (Atkins) and 863$\pm$125 mg/d (Weight Watchers) and did not differ significantly. However, at 2 months the intake of (poly)phenols from food commodities alone (primarily fruit and vegetables) was significantly greater on the Weight Watchers diet (365$\pm$77 mg/d) compared with the Atkins diet (128$\pm$26 mg/d) (P=0.001). The (poly)phenol subgroups most affected were the hydroxybenzoic acid derivatives (P=0.0007), the cinnamates (P=0.0019), and the flavanols (P=0.0039). The Atkins dieters, but not the Weight Watchers dieters, demonstrated a significant decline over time in their intakes of total (poly)phenols (P=0.04) and (poly)phenol intake from alcoholic beverages (P=0.01), especially of ellagitannins (P=0.01).

Non-alcoholic beverages (tea and coffee) were the major contributor to (poly)phenols intakes for both the Atkins (75$\pm$5%) and the Weight Watchers (54$\pm$6%) groups at 2 months. At 2 months, the cinnamates dominated the (poly)phenols intakes from the total diet (Atkins, 337$\pm$62 mg/d; Weight Watchers, 420$\pm$103 mg/d). This dominance remained (Atkins, 53$\pm$15 mg/d; Weight Watchers, 186$\pm$31 mg/d) when considering the intake only from food (i.e. predominately fruit and vegetables).

The present study has demonstrated that although these two diet regimens *per se* do not greatly affect total (poly)phenols intakes, this is due largely to the dominant role of tea and coffee, and intakes of those (poly)phenols derived from foods were significantly lower on the Atkins diet. The relative importance to health of derived (poly)phenols (from tea) and cinnamates (from coffee) relative to those (poly)phenols subgroups provided only by foods is unclear, but it must be noted that tea and coffee do not supply significant fibre, ascorbate, or glucosinolates, whereas fruits and brassica vegetables do.

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Diet Trials: weight and fat loss in a randomised controlled trial of commercially available slimming diets. By D.J. MILLWARD[1], P. TOTHILL[2], A. DELOOY[2], K. FOX[3], C.M. LOGAN[4], I. MACDONALD[5], L. MORGAN[1] and H. TRUBY[1], [1]*Centre for Nutrition & Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH,* [2]*Queen Margaret's University College, Edinburgh,* [3]*University of Bristol,* [4]*University of Ulster and* [5]*University of Nottingham*

Diet Trials was a randomised, 6 months, controlled trial of weight and fat loss on four commercially available slimming diets, conducted at five participating regional centres in the UK. A total of 293 subjects, seventy-nine men and 214 women, selected on the basis of age (>18 and <65 years), BMI (>27 and <40 kg/m^2), living within a 30-mile radius of a test centre, and lack of specific exclusion factors, were randomised to one of five groups. These were: Atkins, Weight Watchers, Rosemary Conley and Slimfast, or a non-dieting control group. Subjects received no specific instructions about any of the diets, other than the Atkins book *Dr Atkins New Diet Revolution* (Atkins, 1999), the Slimfast information pack, or the standard information received at Weight Watchers or Rosemary Conley slimming clubs meetings. The major outcome was fat loss assessed by dual-energy X-ray absorptiometry (DXA) measurements at baseline, 2 months and 6 months when measurements were also made of blood biochemistry, fitness, physical activity and food intake by a 7 d diary. In addition, weight, blood pressure and waist circumference was measured at monthly intervals at the local investigation centre. Here we report weight and fat loss.

Of the subjects, 28% withdrew (n 83); 25% men, 29% women, within the 'normal range' of longitudinal weight-loss studies with free-living subjects. Weight loss varied over a wide range, with individuals achieving >20 kg weight loss on each diet. Those on the Atkins diet lost weight faster than average during month 1, with a higher fat loss amongst men but not women at 2 months, but their weight loss was slower than average after 4 months. Whilst there were differences between centres in terms of the total time on the diets, there were no diet–centre interactions for weight loss or differences between centres in weight loss or in outcomes for weight loss or weight loss adjusted for time on diets. Weight loss (% initial), adjusted for initial fatness, indicated no gender effects (women: 6.1 (95% CI 5.1, 7.1) %; men: 8.7 (95% CI 7.0, 10.3) %), with no differences between diets apart from control.

Fat loss by DXA was adjusted by centre because of variable machine characteristics indicated by variable slopes of the body fat (% weight) BMI regression between the Hologic fan beam (at two centres) and the Prodigy fan beam (two centres) and a Pencil beam instrument. An algorithm was developed to correct for errors which resulted in very small changes in mean values for body fat for any diet group (i.e. 0.1–1.5%), but which did reduce overall CV and with no effect on outcome.

At 6 months completers lost on average 18% of initial fat, more in men (23%) than women (16%), with a wide range of loss on each diet (see Table). Fat loss and fat loss as a percentage of initial were related to initial size (height or lean tissue mass) and initial fatness (% fat) indicating that bigger and fatter subjects lost proportionately more fat. For men there were no differences between the diets but for women fat loss as percentage initial fat was highest in the two assisted programmes, Weight Watchers and Rosemary Conley. However, inclusion of initial fatness in an ANCOVA removed the gender effect, and these adjusted values are shown in the Table together with actual minimum and maximum values.

Fat loss (% initial fat at 6 months)	n	Mean	95% CI	Minimum	Maximum
Atkins	40	18.4	15.2, 21.5	−6.0	41.9
Weight Watchers	48	18.1	15.2, 21.0	−5.1	47.6
Slimfast	40	13.8	10.7, 17.0	−4.7	46.3
Rosemary Conley	38	20.8	17.5, 24.1	0.4	55.4
Control	39	−1.6	−4.8, 1.7	−11.0	9.2

After trimming for outliers (>+3 SD), ANCOVA indicated no gender differences, or diet–gender interactions with the significant dietary effect reflecting mainly the differences between dieters and non-dieting controls (P=0.07, Slimfast *v.* Rosemary Conley; Kruskal–Wallis test).

Clearly, effective weight and fat loss can be achieved by a variety of approaches so that individuals need to decide what is likely to work best for them.

We thank the BBC for financial support.

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Diet Trials: composition of weight loss in relation to exercise and fitness in a randomised controlled trial of commercially available slimming diets. By D.J. MILLWARD[1], A. DELOOY[2], K. FOX[3], M. DAVIS[3], M.B.E. LIVINGSTONE[4], I. MACDONALD[5], L. MORGAN[1] and H. TRUBY[1], [1]*Centre for Nutrition & Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH,* [2]*Queen Margarets University College, Edinburgh,* [3]*University of Bristol,* [4]*University of Ulster and* [5]*University of Nottingham*

Diet Trials was a randomised, 6 months, controlled trial of weight and fat loss on four commercially available slimming diets, conducted at five participating regional centres in the UK. A total of 293 subjects (seventy-nine men and 214 women, aged >18 and <65 years, BMI >27 and <40 kg/m^2), who lacked specific exclusion factors were randomised to one of five groups: Atkins, Weight Watchers, Rosemary Conley and Slimfast, or a non-dieting control group (for details, see Millward *et al.* 2004). Here we report on how physical activity, assessed at baseline and 24 weeks in terms of 7 d pedometer recordings, and fitness, as assessed by change in sub-maximal (70%) work capacity (Aerobic Adaptation Test; Church *et al.* 2001), related to weight loss and its composition in terms of fat and fat-free mass (FFM), assessed by dual-energy X-ray absorptiometry (DXA) measurements at baseline and 6 months.

For completers (*n* 210), after trimming for weight loss <5 kg and outliers (>3 SD), lean amounted to 24.3 and 14.4% of weight loss for men and women respectively but initial leanness in the model removed the gender effect (21.3 and 18.3% men and women respectively) and there were no dietary effects with an average loss of 3.0% initial lean.

Gain in fitness occurred in all dietary groups and was higher in men than in women, but after adjusting for initial fitness, dietary effects were only marginal (P=0.066).

Interactions between fitness and activity and the composition of weight loss were examined in subjects losing >5 kg. Fitness and activity were related to weight loss and its composition. Thus fat loss (% initial fat) correlated with both initial activity and 6-month fitness, and with improvement in fitness per subject (r 0.32; P=0.002) or per kg FFM (r 0.31; P=0.003). Absolute lean loss (% initial lean) was unrelated to activity or fitness but lean loss as a percentage of weight loss was inversely correlated with increased fitness/kg FFM (r −0.22; P=0.037). Within individual diet groups, fat loss correlated with increased fitness for Atkins and Slimfast while lean loss (% weight loss) correlated with increased fitness for Rosemary Conley. To better identify any benefit of increased fitness for weight loss and its composition, subjects losing >5 kg were stratified according to fitness gain/FFM and differences in outcome were identified between the higher and lower fitness quartile.

Outcome variables by fitness	Weight loss (kg)	Fat loss (% initial)	Lean loss (% weight loss)
Top quartile (*n* 29)	12.3	26.8	14.2
Bottom quartile (*n* 34)	9.2	19.6	24.2
Difference (top–bottom)			
Atkins (*n* 14)	3.2	6.0	−6.3
Weight Watchers (*n* 17)	−0.8	−5.2	3.3
Slimfast (*n* 15)	6.7	13.4	−16.0
Rosemary Conley (*n* 17)	2.7	7.9	−11.7
All groups (*n* 63)	3.1	7.2	−10.0

An increase in fitness was particularly effective for subjects following Atkins, Slimfast and Rosemary Conley diets, for whom getting fitter meant extra loss of weight (3–7 kg), higher percentage fat loss (6–13%) and lower proportion of weight loss accounted for by lean tissue (6–16% lower).

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Diet Trials: influences of weight loss on lipid-based cardiovascular risk factors in a randomised controlled trial of commercially available slimming diets. By L. MORGAN[1], I. DAVIES[1], B. GRIFFIN[1], A. DELOOY[2], K. FOX[3], M.B.E. LIVINGSTONE[4], P. ROBSON[4], I. MACDONALD[5], H. TRUBY[1] and D.J. MILLWARD[1], [1]*Centre for Nutrition & Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH,* [2]*Queen Margarets University College, Edinburgh,* [3]*University of Bristol,* [4]*University of Ulster and* [5]*University of Nottingham*

Weight loss is known to have favourable influences on blood lipids but to date there is no consensus on the relative efficacy of low-fat, compared with low-carbohydrate diets, especially their relative impact on the structural properties of LDL which, together with changes in triacylglycerol (TAG), are considered most important in terms of atherogenicity (Griffin & Zampelas, 1995). We investigated this during 'Diet Trials', a randomised, 6 months, controlled trial of weight and fat loss on four slimming diets: Atkins (low-carbohydrate), Weight Watchers, Rosemary Conley and Slimfast (all generally low-fat), and a non-dieting control. This was conducted at five participating regional centres in the UK with 293 subjects, seventy-nine men and 214 women, selected on the basis of age (>18 and <65 years), BMI (>27 and <40 kg/m^2), and lack of specific exclusion factors. Blood biochemistry was measured at baseline and 24 weeks, fitness assessed as sub-maximal (75%) work capacity, and fat loss assessed by dual-energy X-ray absorptiometry measurements (for details, see Millward *et al.* 2004). The distribution and density of LDL sub-fractions was examined by iodixanol density centrifugation.

After 6 months LDL-cholesterol had fallen in all four diet groups, on average by 0.29 mmol/l, but not in the control group. ANOVA showed significant dietary effects, especially after adjusting for baseline LDL ($P \leq 0.0001$), which remained after adding fat loss to the model (P=0.04) with Rosemary Conley and Weight Watchers but not Atkins and Slimfast different from control. Analysis of LDL sub-fractions showed that at baseline only fourteen of fifty-seven men and three of 147 women exhibited an atherogenic lipoprotein phenotype in terms of >50% little dense LDL. This number fell to four men and two women after 6 months. In fact, LDL peak density fell in all groups, to a greater extent in men. However, ANCOVA corrected for baseline LDL peak density removed the gender effect and showed the fall was greatest for the Atkins, Weight Watchers and Rosemary Conley groups, but only Atkins differed from control. HDL-cholesterol fell in all groups including control by on average 0.16 mmol/l, and changes were unrelated to weight or fat loss. The fall was least in the male Atkins group but ANCOVA with baseline HDL-cholesterol indicated only marginal gender (P=0.08) and dietary effects (P=0.036), with no between-group differences indicated by Bonferroni *post hoc* testing. The LDL:HDL ratio was higher in men (3.5) than women (2.9), but did not vary between diets at any time, increasing slightly at 6 months.

TAG fell on average by 0.28 mmol/l, the fall strongly correlated with initial LDL density (r^2 29%), and fat loss (r^2 9.5%). Adjustment for baseline TAG removed any gender effect, showing a very similar pattern of changes to those observed for LDL density, i.e. the fall was greatest for the Atkins diet and Weight Watchers and least for Slimfast and Rosemary Conley who did not differ from control.

Insulin levels varied markedly, with mean levels falling at 6 months in the four diet groups but not in controls. The falls were related to fat loss ($P \leq 0.005$), increase in fitness ($P \leq 0.05$), fall in TAG ($P < 0.001$), and decrease in LDL density ($P < 0.05$). After adjusting for baseline insulin, ANCOVA indicated no differences between the four diets but a significantly greater fall with the Atkins diet compared with control.

Overall these results indicate the profound beneficial impact of negative energy balance and weight loss on plasma lipids, especially those factors most strongly related to atherogenic risk. The results also tend to confirm that during weight loss low-carbohydrate high-fat diets have no detrimental effects on lipid-based cardiovascular risk factors.

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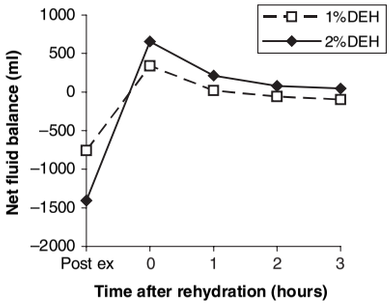
Effect of two levels of exercise-induced hypohydration on post-exercise rehydration in man. By D.T. ARCHER, D. BALL, N.A. LEWIS and V. MAGUIRE, *School of Medical Sciences, College of Life Sciences, University of Aberdeen, Foresterhill, Aberdeen, UK, AB25 2ZD*

The current recommendations for fluid replacement after exercise are to ingest a volume of fluid equivalent to 150% of body-mass loss during exercise (American College of Sports Medicine, 2000). These recommendations are primarily based on data obtained following 2% body-mass loss (Shirreffs *et al.* 1996). The aim of the present study was to examine if these recommendations were equally applicable to hypohydration at both 1% and 2% body-mass loss.

With local ethics committee approval, seven healthy subjects (four males and three females) gave informed consent to participate in the present study. Subject characteristics were as follows: age 24 (SD 4) years; height 1.73 (SD 0.11) m; body mass 69.27 (SD 9.28) kg. Trials took place at the same time of day 1 week apart in randomised order. Subjects were dehydrated by 1.1% (1% DEH) and 2.0% body mass (2% DEH) using low-intensity intermittent cycle exercise performed in a warm (34 °C), humid (76% relative humidity) environment. Beginning 30 min after exercise and over a 60 min period, subjects ingested a fluid containing Na (54 mmol/l) and carbohydrate (20 g/l) in a volume equivalent to 150% of their body-mass loss. Urine samples were obtained before exercise, 30 min after exercise and every 1 h until 4 h post-exercise (4 h). Data were analysed by repeated measures ANOVA, paired *t* tests and one-way ANOVA and *post hoc* Tukey tests.

Drink volume was greater on 2% DEH than 1% DEH due to the greater loss of body mass on 2% DEH (Table; $P<0.001$). Total urine production was greater on 2% DEH than 1% DEH (Table; $P<0.05$). A higher percentage of the drink was retained on 2% DEH in comparison with 1% DEH (Table; $P<0.05$). Net fluid balance, which was calculated as fluid gains (drinking) minus fluid losses (dehydrating exercise and urination) at each time point, was not significantly different between trials (Figure; $P>0.05$) despite significant differences in drink retention.



	1% DEH		2% DEH	
	Mean	SD	Mean	SD
Drink volume (ml)	1200	321	2134†	294
Urine volume (ml)	510	189	655*	274
Drink retention (%)	56	16	69*	14

* $P<0.05$ *v* 1% DEH; † $P<0.001$ *v.* 1% DEH.

In summary, there was no significant difference in whole-body net fluid balance between trials, indicating that subjects returned to a similar state of euhydration following fluid intake equivalent to 150% body-mass loss despite two different degrees of hypohydration. However, drink retention was increased following 2% DEH compared with 1% DEH.

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Relationship between attitudes towards perceived physical activity level and estimated physical activity levels in a representative adult Irish population. By A.P. HEARTY, S.N. McCARTHY and M.J. GIBNEY, *Department of Clinical Medicine, Trinity College Dublin, Republic of Ireland*

Theoretical and empirical research suggests that physical activity is a complex phenomenon related to personal and social variables (Biddle & Mutrie, 1991; de Almeida *et al.* 1999). Despite extensive research in the area of physical activity, little is known about the association between attitudes towards self-perceived physical activity levels and actual measures of habitual physical activity. The majority of studies that have addressed physical activity have focused only on certain aspects of activity, such as leisure-time activity. To gain a holistic understanding of physical activity behaviour, it is essential to examine all components of physical activity.

Information on habitual physical activity and on attitudes towards self-perceived physical activity level was collected via self-administered questionnaires as part of the North/South Ireland Food Consumption Survey (NSIFCS; Irish Universities Nutrition Alliance, 2001). An estimated physical activity level (ePAL) value, which represents total physical activity over 24 h periods, was calculated for 1331 respondents in the NSIFCS (McGowan, 2003). The present paper describes the relationship between self-perceived attitudes towards physical activity and ePAL of Irish adults. Mean ePAL and time spent in recreational, home and work activities (h/week) were examined across attitudes towards physical activity.

	ePAL			TV or video (h/week)			Recreation (h/week)			Home activities (h/week)			Work activities* (h/week)		
Attitude	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
'Thinking about exercise, do you feel you need?'															
A lot more	1.69[a]	0.3	531	20.4[a]	10.1	527	6.4[a]	5.5	506	19.9[a]	15.9	517	36.7[NS]	14.2	401
A little more	1.74[b]	0.3	436	18.7[b]	10.0	427	7.4[b]	5.9	428	17.4[b]	15.4	428	37.7	13.8	337
Same or less	1.84[c]	0.4	213	17.1[b]	9.3	210	7.7[b]	6.6	208	14.3[c]	12.8	198	39.5	17.5	172
'Would you say you enjoy exercise?'															
Always	1.79[NS]	0.4	275	17.2[a]	9.5	270	7.8[a]	6.5	267	17.9[ab]	13.8	259	38.5[NS]	15.6	208
Most of the time	1.72	0.3	482	18.3[a]	9.5	475	7.6[a]	6.2	470	16.5[a]	14.2	470	37.0	13.5	382
Sometimes	1.73	0.4	326	21.4[b]	10.8	323	6.1[b]	5.0	311	18.7[ab]	16.1	319	37.9	15.9	251
Hardly ever or never	1.68	0.3	108	22.3[b]	10.5	106	4.5[c]	3.7	103	23.5[b]	20.2	106	37.3	14.8	75

[a,b,c] Mean values within a column within each attitudinal statement with unlike superscript letters were significantly different ($P<0.05$). *Only those who spent >1 h/d in work activities are included in this analysis.

Subjects with the highest ePAL values were most satisfied with their perceived level of physical activity and were most likely to enjoy exercise ($P<0.05$). As time spent in recreational activities ($P<0.05$) increased, subjects were more likely to be satisfied with their exercise levels and to enjoy exercise. Increased time spent watching TV or video was associated with perceiving the need for 'a lot more' exercise ($P<0.05$), and with a decreased enjoyment of exercise ($P<0.05$). Similarly, increased time spent in home activities was associated with decreased satisfaction with perceived exercise levels ($P<0.05$), which indicates that subjects may not necessarily consider home activities to constitute physical activity. Energy expenditure for self-reported physical activity was expressed in terms of metabolic equivalents (MET; Ainsworth *et al.* 1993). MET for recreation, home and work activities were examined across attitudes towards exercise, and similar trends to those described were observed (data not shown).

Overall, the present study has shown that Irish individuals are aware of and are relatively able to self-rate their physical activity level. These results also suggest that it may be necessary to increase awareness of the contribution of home activities in relation to total physical activity behaviour.

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Household activity is a significant component of physical activity estimation in the Irish population. By F. SHIELY[1], C.M. MURRIN[1], S. FRIEL[2], G. NOLAN[1] and C.C. KELLEHER[1], [1]*National Nutrition Surveillance Centre, Department of Public Health Medicine and Epidemiology, Woodview House, University College Dublin, Belfield, Dublin 4, Republic of Ireland and* [2]*Department of Health Promotion, Clinical Sciences Institute, National University of Ireland Galway, Costello Road, Shantalla, Galway City, Republic of Ireland*

As occupations demanding heavy physical work continue to decline, leisure-time physical activity, including housework, has emerged as an important preventive action against chronic diseases, especially taking into account the high prevalence of sedentary professional occupations (Martinez-Gonzales *et al*. 2000). The SLÁN surveys sample a representative cross-section of the Irish adult population (*n* 5992; 53% response rate). The data provide opportunities for the exploration of lifestyle trends and health behaviours in relation to adult physical activity patterns. The recommendations for moderate physical activity are 20 min or more three times per week (Department of Health and Children, 2000). An additional 20 min of strenuous activity is recommended by the World Health Organization (2002) for younger individuals.

The SLÁN surveys indicate that those participating in regular moderate or strenuous exercise are less likely to be overweight or obese ($P<0.001$). However, as indicated in the Table, there are extremely high percentages of non-participants both in moderate and strenuous terms. Males are more likely to meet the strenuous physical activity recommendations ($P<0.001$). Females are more likely to meet the moderate recommendations ($P<0.001$) except those aged 55 years and over where there is no significant sex difference.

Exercise	BMI (kg/m^2)				Exercise	BMI (kg/m^2)			
Strenuous (times/week)	Normal (%)	Overweight (%)	Obese (%)	Total (%)	Moderate (times/week)	Normal (%)	Overweight (%)	Obese (%)	Total (%)
0	79.3	82.4	89.1	81.7	0	47.7	53.1	63.8	51.8
1–3	15.8	13.2	8.2	13.9	1–4	33.4	32.1	24.5	31.7
≥4	4.9	4.4	2.8	4.4	5–6	11.5	9.3	7.0	10.1
					≥7	7.4	5.6	4.7	6.4

Females of all ages are more likely to engage in regular (≥3–4 times per week) light and heavy housework ($P<0.001$). Between 81.2 and 92.4% of females engage in regular light housework compared with 44.0 to 65.8% of males. Between 45.6 and 62.8% of females engage in regular heavy housework compared with 24.4.0 to 28.7% of males.

Occupational physical activity differed significantly for males and females in the 18–34 age group and the 35–54 age group ($P\leq0.001$). The proportion of males reporting very physically active occupations did not vary between age groups. However, females who reported they were very physically active in their job increased with age.

Further analysis using categorical principal components analysis, encompassing all exercise variables from SLÁN, indicated that for both males and females, under and over 55 years, housework is the most important explanatory physical activity variable (between 15.6 and 16.3% explained variance for males and between 18.6 and 18.9% explained variance for females). These results indicate that domestic activity should be incorporated in future physical activity policy development.

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What determines a child's intake of fruit and vegetables? By L.R. GLYNN, C.D. STEER, I.S. ROGERS, P.M. EMMETT and the ALSPAC STUDY TEAM, *Unit of Paediatric and Perinatal Epidemiology, Division of Community Medicine, University of Bristol, Bristol, UK, BS8 1TQ*

Despite several campaigns promoting the importance of fruit and vegetables, UK studies have shown that children are not eating enough of these foods. The Health Survey for England recorded that 11% of boys and 6% of girls aged 7–9 years ate fruit less than once per week and 37% of boys and 32% of girls ate vegetables less than once per d (Prescott-Clarke & Primatesta, 1998). One of the most influential determinants of children's intake of fruit and vegetables is likely to be parental role-modelling. Parents make the decision about the types of foods that are offered to their child based on their own attitudes and beliefs about food. Consequently a child's food choices are mostly limited to the range of foods provided by their parents. The mother's educational status may be a determinant; mothers who are better educated might encourage their children to eat more fruit and vegetables.

The aim of the present study was to establish any relationship between mother's intake of fruit and vegetables and the child's intake. To assess if having parental rules about providing fruit and vegetables daily increases the child's intake, and if the mother's educational status determined intake.

The subjects were 814 children aged 7 years, a sub-cohort of the Avon Longitudinal Study of Parents and Children, a cohort of children resident in the South-west of England. Diet was assessed using three 1 d unweighed food diaries. The parents were asked to record everything the child ate and drank for 3 d, preferably 2 weekdays and 1 weekend day using household measures. The child's daily fruit and vegetable intake was calculated by summing the weight of each type of vegetable and fruit consumed. The children's intakes were adjusted for energy and categorised into tertiles. Maternal diet was assessed using the food-frequency method 4 years after the birth of the study child. The mother's intake was categorised into tertiles. Parents were asked if they tried to ensure that the child has 'fresh fruit every day' and/or 'vegetables/salad every day'. The mothers were assigned to one of three education categories according to their highest attainment; low (none, CSE and vocational), medium (O level) and high (A level or degree). These outcomes were analysed by ordinal logistic regression.

Initially univariable analyses adjusted for sex were performed. These showed that maternal intake of both fruit ($P<0.001$) and vegetables ($P<0.005$) was positively related to their child's intake, and having parental rules about fruit ($P<0.001$) and vegetables ($P<0.001$) also resulted in increased consumption; maternal education influenced fruit ($P<0.001$) but not vegetable intake ($P=0.723$). Then specific multivariable hypotheses were tested related to maternal education, diet and rules concerning vegetable and fruit consumption. The findings showed that education was still a determinant of fruit intake but its effect was weakened by the other variables ($p=0.001$). Maternal diet remained a strong influence for fruit ($P<0.001$), but was no longer a determinant for vegetables ($P=0.0.104$), the effect of having parental rules about fruit ($P<0.001$) and vegetables ($P<0.001$) remained.

Maternal fruit consumption was associated with increased fruit consumption in the children. Parents should lead by example and eat fruit themselves. They should try to make sure fruit and vegetables or salad are part of the meals they provide daily since having these rules can overcome educational bias.

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Dietary differences and similarities amongst 5–9-year-old children from schools classified by the new Scottish indices of multiple deprivation. By W.L. WRIEDEN[1], J.E. CECIL[2], I. MURIE[2], C. BOLTON-SMITH[3], P. WATT[4], C.N.A. PALMER[5] and M.M. HETHERINGTON[6], [1]*Centre for Public Health Nutrition Research,* [2]*Department of Psychology,* [3]*MRC Human Nutrition Research, Cambridge, UK, CB1 9NL,* [4]*University of Brighton, Brighton, UK, BN20 7SP,* [5]*Biomedical Research Centre, University of Dundee, Dundee, UK, DD1 9SY and* [6]*School of Psychology, University of Liverpool, Liverpool, UK, L69 7ZA*

The Scottish Health Survey of 1998 (Scottish Executive, 2001) and the National Diet and Nutrition Survey of young people aged 4–18 years (Gregory & Lowe, 2000) showed higher intakes of fruit, and lower intakes of soft drinks, and fried foods amongst children from families classified as non-manual as opposed to manual social class. The new Scottish Indices of Multiple Deprivation (SIMD; Scottish Executive, 2003) provide an area (ward)-based combined measure of deprivation using five measures; employment, health, education, income and geographical access to services. This is a useful measure to apply where data have been collected from groups within a community including schools.

As part of a study investigating the interactions between eating style, diet, activity and genotype as determinants of energy balance in pre-pubertal children a food-frequency questionnaire (adapted from Hammond *et al.* 1993) was used to collect dietary information from 100 children selected from twenty-four schools across Tayside and Fife, Scotland. The ward and hence the SIMD of each school was determined and the range of scores was 3.65 to 74.29. The most deprived, with a score of 74.29 represented the sixteenth most deprived ward in Scotland and the least deprived with a score of 3.65 ranked 1156 out of a total of 1222 wards). Median (Med; and interquartile ranges; IQR) weekly intakes of the foods, calculated from fifteen frequency categories ranging from never to five times per d were compared for girls and boys from schools with an SMID less than 20 (less deprived) and those with SIMD of 20 (more deprived) or more. Most food frequencies showed similarities across deprivation indices. The Mann–Whitney *U* test was used to compare differences between children from the two deprivation groups.

	Less deprived (SIMD<20)				More deprived (SIMD>20)				
	Boys (*n* 34)		Girls (*n* 20)		Boys (*n* 17)		Girls (*n* 29)		
Food frequency (times per week)	Med	IQR	Med	IQR	Med	IQR	Med	IQR	*P**
Cooked green vegetables	2	0.0–7.0	2	1.0–4.8	0.5	0.0–1.8	2.0	0.2–4.0	NS
Cooked other vegetables	3	0.5–3.2	2	0.5–3.0	1	0.5–1.0	1.0	0.4–3.0	<0.05 B
Salads	0	0.0–1.3	2	0.6–3.8	0	0.0–3.0	0.5	0.0–3.0	<0.05 G
Fresh fruit	7	3.0–14	5.5	3.3–7.0	7	3.2–14	7.0	5.0–14	NS
Crisps and packet snacks	4	3.0–7.0	7	5.0–7.0	5	3.0–7.0	7.0	5.0–7.0	NS
Fried, chipped and roast potatoes	1	0.5–2.0	2	1.0–3.0	1.5	1.0–3.8	1.0	0.5–3.0	NS
Breakfast cereals, high fibre	2	0.9–5.2	2	0.6–4.0	0.5	0.3–3.0	0.5	0.0–4.5	0.058 B
Sweets and chocolates	4	2.0–5.8	4	3.0–7.0	7	4.3–7.0	7.0	4.0–7.0	<0.05
Non-diet squashes and carbonated drinks	2	0.5–7.0	5.5	0.1–21	1.3	0.1–12	1.0	0.2–14	NS
Diet squashes and carbonated drinks	0.5	0.0–14	4.5	0.6–14	7	0.6–26	21	3.8–28	<0.05 B 0.056 G

B, boys; G, girls.
* *P* for difference between less and more deprived.

The results show that the differences in diet across deprivation groups in this sample of children are not as pronounced as seen in previous studies. In general the data suggest a poor diet across the range of children regardless of deprivation status. The higher intake of sweets and chocolates in the more deprived group may reflect parental restriction in the less deprived group.

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A city-wide survey of the diet of three cohorts of 9 and 10 year old children in Liverpool in 2000, 2001 and 2002. By A.F. HACKETT[1], M. GIBBON[1], S. TAYLOR[1], G. STRATTON[1] and E. LAMB[2], [1]*Liverpool John Moores University, Faculty of Education, Community and Leisure, Barkhill Road, Liverpool L17 6BD and* [2]*Leisure Services Directorate, Liverpool City Council, Millennium House, Victoria Street, Liverpool L1 6JH*

We have previously reported the dietary intake of children collected as part of the city-wide *Sports***Linx** project (Hackett *et al.* 2001) which provides an annual supply of dietary and nutritional information on Liverpool children. This is a report of the dietary data collected from three successive cohorts of children (school years beginning 2000, 2001 and 2002).

All primary schools in Liverpool were invited to take part (about 117), approximately ninety did so each year and 9776 year 5 children (3890 in 2000, 3007 in 2001 and 2879 in 2002); 5070 boys and 4706 girls completed the survey. Diet was recorded using a questionnaire based on recalling intake of fifty-one foods the previous day (only the fifteen foods mentioned by most children are shown). The Table shows the proportion (%) of children who claimed to have eaten each food and whether there were differences across the year groups. Differences between years were evaluated with the χ^2 test.

	Cohort			
Food	2000–2001	2001–2002	2002–2003	*P*
Fruit	71.1	71.6	72.9	0.28
White bread	70.4	70.5	72.0	0.34
Fizzy drink (sugared)	64.2	62.8	62.5	0.32
Low fat milks	62.2	62.4	57.3	0.00
Cordial drinks (sugared)	61.5	61.6	55.5	0.00
Chocolate biscuits	60.8	60.3	57.8	0.04
Crisps	58.8	59.5	61.5	0.12
Chocolate	54.2	54.0	56.2	0.19
Sweets	54.2	53.2	50.8	0.03
Butter	50.5	50.5	52.7	0.15
Chips	50.1	49.5	53.4	0.01
Sugar added to a drink	47.0	46.3	43.4	0.02
Plain biscuits	44.3	44.8	42.0	0.08
Meat (not in pies etc)	43.1	43.8	47.5	0.00
Diet fizzy drink	42.3	41.5	38.9	0.03

After-school clubs to try to encourage healthier eating, which are informed by these data, began in some of these schools in 2002. The food claimed to have been eaten by most children was fruit in every year, but most foods in the top fifteen were less desirable items. Some changes in food choice apparently occurred but there is no strong pattern suggesting either general improvement or deterioration in choices being made.

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Children's opinions on food: a culturally appropriate tool for healthier eating in Sandwell. By R.E. KYLE, *Rowley Regis and Tipton PCT, 438 High Street, West Bromwich, West Midlands, UK, B70 9LD*

Health promotion strategies designed to alter the population's eating habits do not appear to have brought about the changes identified in the nutrition policies from which they were generated. Developing food policy for schools in Sandwell, a Black Country borough in the West Midlands, has led to the generation of data, which are being used to underpin a health promotion tool for primary school children. Sandwell has high levels of disadvantage and the inevitable health inequalities associated with this. Health promotion designed to modify food choice is often based on education about 'healthy eating', using universal information, for example The Balance of Good Health plate (Department of Health, 1991). But food choice is not free from restrictions. Practical matters such as cost or availability influence choice, but so do taste, social attitudes and cultural conventions.

Teachers and school health nurses had drawn attention to a perceived increase in the numbers of overweight children in primary schools, and the social and physical problems associated with this. A need to develop appropriate health education was identified. Height and weight measurements for 8367 primary school children, previously recorded by school health nurses, were used to calculate BMI using BMI reference curves for the UK (Cole *et al.* 1995).

Class and year	*n*	Overweight (%)	Obese (%)
Reception boys 1997–98	1328	10	4
Reception girls 1997–98	1254	14	5
Reception boys 2000–01	1400	11	5
Reception girls 2000–01	1333	16	6
Year 5 boys 2000–01	1567	16	8
Year 5 girls 2000–01	1485	23	8

Qualitative semi-structured interviews were undertaken with eight groups of four children (aged 7–11 years), seven groups of eight children (aged 4–11 years) and two groups of parents. In addition, 209 children (aged 7–11 years) kept 1 d food diaries. The data suggest that many children eat alone during the week. Vegetables were often only eaten at the weekend as part of a 'proper dinner', frequently prepared by a grandmother. Children and parents had a well-developed sense of the appropriateness and symbolic significance of food and meals (Murcott, 1982). Although children have a very clear idea of what is meant by 'healthy eating', this knowledge does not impinge on their eating habits.

An 'Interactive Food Diary' has now been developed. This not only provides information and allows children to record elements of their daily food, but suggests small changes within cultural norms. Physical activity is also recorded. The diary is currently being piloted in Sandwell's three Primary Care Trusts, for use in health promotion and in nurse-led obesity clinics. Preliminary feedback from parents, nurses and teachers has been encouraging.

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Is there a link between the increasing use of inhaled corticosteroids to treat asthma and increasing obesity prevalence in children? By J. GANDY[1], V. TUFFREY[2] and S. MUKHOPADHYAY[3], [1]*Research Centre for Health Studies, Buckinghamshire Chilterns University College, Chalfont St Giles HP8 4AD,* [2]*School of Integrated Health, University of Westminster, London W1W 6UW and* [3]*Division of Maternal and Child Health Sciences, Ninewells Hospital and Medical School, Dundee DD1 9SY*

Over the last 10 years the prevalence of obesity has doubled to 8.5% in 6-year-olds and trebled in 15-year-olds to 15%. The prevalence of childhood asthma diagnosis and symptoms has also increased especially in pre-school children (Kuehni *et al.* 2001). This has resulted in the increased use of inhaled corticosteroids and the introduction of higher potency inhaled steroids for general use. Concerns are being increasingly expressed about possible links between these two phenomena. While the increased risk of developing asthma in obese children has received much attention, the effect of steroid inhalation on body weight has not been investigated. Hedberg & Rössner (2000) used self-reported asthma, medication (use but not type of medication was recorded), height and weight of over 8000 adults in the Sweden Living Condition Surveys. They concluded that there was no strong evidence to suggest that asthma medication contributes significantly to the development of obesity. No similar information is available for children.

Data from the 2001 Health Survey for England (Data Archive, University of Essex) were analysed, which included complete information on prevalence of asthma, asthma medication, and anthropometry for 3222 children aged 2 to 16 years of age. The characteristics of the three groups of children; non-asthmatics, asthmatics receiving inhaled corticosteroids and asthmatics not receiving corticosteroid medication are shown in the Table. The data were normalised for sex and age using the international cut-off points in BMI for overweight and obesity (Cole *et al.* 2000). A new variable, the percentage of the age- and sex-specific value of BMI equivalent to a BMI of 25 at age 18, was derived from the Health Survey data.

The data were transformed by taking reciprocals, to adjust for their severe positive skewness. A significant difference was found between the asthmatics (n 712; mean 94.2% of BMI cut-off) and non-asthmatics (n 2510; mean 92.8% of BMI cut-off) in the means of the reciprocal of percentage BMI cut-off (F 5.0; P=0.026) by two-way ANOVA with sex as the other independent variable (F 10.0; P=0.002) and age as covariate (F 35.3; P<0.001). However, there was no significant difference between the means of the transformed percentage of BMI cut-off in asthmatics using inhaled corticosteroids (n 233) and those not using these drugs (n 479) by two-way ANOVA (F 0.6, P=0.45, with F 3.8, P=0.05 for sex and F 9.1, P=0.003 for age as covariate).

		Age (years)			BMI (kg/m^2)		Percent of BMI cut-off		
Group	*n*	Mean	SD	Male:female	Mean	SD	Mean	SD	Median
Non-asthmatic children	2510	9.01	4.24	1196:1314	18.60	3.67	92.77	14.11	90.63
Asthmatic children not receiving inhaled corticosteroids	479	10.19	3.96	265:214	19.51	4.11	94.43	16.57	91.13
Asthmatic children receiving inhaled corticosteroids	233	9.12	3.99	129:104	18.74	3.85	93.70	15.74	91.14

The findings of the present study are in agreement with other studies in that asthmatic children tend to have higher BMI than non-asthmatic children. However, in this sample of English children the use of inhaled corticosteroids for the treatment of asthma does not appear to be associated with overweight or obesity. Further studies using longitudinal data are required to provide a more definitive answer to this question.

Data from the Health Survey for England were used with the permission of the Data Archive, University of Essex. The Health Survey was carried out by the Joint Health Survey Unit, Social and Community Policy Research, University College London. The survey was funded by the Department of Health.

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Fat-free mass composition in obese and non-obese children: matched case–control analyses. By D. HAROUN, J.C.K. WELLS, J.E. WILLIAMS, N.J. FULLER, M.S. FEWTRELL and M.S. LAWSON, *MRC Childhood Nutrition Research Centre, Institute of Child Health, 30 Guilford Street, London, UK, WC1N 1EH*

Childhood obesity is increasing at an alarming rate. There is insufficient information on the body-composition characteristic of obese children; hence measuring body composition in children is vital in researching the aetiology of childhood obesity and evaluating the relative success of various treatment programmes. Most body-composition techniques assume constant properties of the fat-free mass (FFM) such as hydration (H_{ffm}) and density (D_{ffm}). However, according to previous research, FFM composition in obesity has been shown to be altered, with one study reporting a 3% difference in H_{ffm} between obese and lean children (Bray *et al.* 2001).

Matched case–control analyses of obese and non-obese children aged 7–14 years were conducted. Obesity was defined as BMI centile >95. Twenty-two matched pairs of children (ten boys, twelve girls) were compared using the three-compartment (3C) model, and eleven matched pairs (six boys, five girls) were compared using the four-compartment (4C) model. Measurements were made of weight, height, total body water (TBW), body volume (BV) and bone mineral content.

	3C difference*(*n* 22)			4C difference (*n* 11)		
	Mean	SD	*P* value	Mean	SD	*P* value
TBW (litres)	5.2	5.1	<0.001			
BV (litres)	22.3	12.6	<0.001			
Fat mass (kg)	14.7	8.5	<0.001	18.8	7.8	<0.001
FFM (kg)	6.2	6.7	<0.001	10.4	9.8	0.006
% Fat	16.7	10.3	<0.001	17.5	8.1	<0.001
H_{ffm} (%)	1.3	2.8	<0.05	3.1	2.7	0.003
D_{ffm} (kg/l)	−0.005	0.012	0.057	−0.009	0.007	0.002
Protein:mineral ratio				−0.4	0.9	0.17

* Difference calculated as obese minus control value.

Obese children had significantly higher TBW and BV than controls ($P<0.001$). According to both the 3C and 4C models, obese children had significantly greater fat mass, FFM and percentage fat. H_{ffm} of the obese children was significantly higher by both models; the difference being larger in the 4C model (4C, 3.1%; 3C, 1.3%). D_{ffm} was lower in obese children, only reaching statistical significance in the 4C model (4C, $P<0.002$; 3C, $P=0.057$). The protein:mineral ratio was also reduced in obese children, however not significant ($P=0.17$). This analysis indicates that the 3C model underestimates the differences in H_{ffm} and D_{ffm} between obese and lean children. Compared with the 4C model, the 3C model assumes a constant protein:mineral ratio and the difference in this ratio shown by the 4C model accounts for this underestimation.

Since previous studies used dual-energy X-ray absorptiometry, which assumes constant properties of FFM (Wong *et al.* 2002), the present study provides improved evidence that H_{ffm} is increased in obese children. Both the 3C and 4C models detected differences in body composition between obese and lean children; however the 4C model was more accurate as it also took into account the increased mineralisation of FFM observed in obese children. This in turn is relevant to which methods should be chosen to measure body composition in future studies. Multi-compartment models are preferable to two-component methods, such as isotope dilution or densitometry, as these approaches cannot take the FFM alterations characteristic of obesity into account.

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Wong WW, Hergenroeder AC, Suff JE, Butte NF, Smith EO & Ellis KJ (2002) *American Journal of Clinical Nutrition* **76**, 384–389.

The effect of breakfast type on lunch intake in primary school children. By C.M. STRIK, J.M. WARREN and C.J.K. HENRY, *Nutrition and Food Science Group, School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane Campus, Headington, Oxford, UK, OX3 0BP*

Currently there is much interest in low-glycaemic index (GI) foods and their potential to enhance satiety and reduce subsequent food intake. Recently a low-GI breakfast was shown to produce increased satiety and reduced *ad libitum* lunch intake when compared with a high-GI breakfast (Warren *et al.* 2003). In the present study different breakfasts were offered within the low-GI and high-GI breakfast groups. The present study examines the impact of individual low-GI breakfast type on subsequent food intake at lunch. It represents preliminary observations from a larger ongoing cross-over trial comparing the effects of a low- *v.* a high-GI breakfast on *ad libitum* food intake at a buffet-style lunch.

Thirty-six children, aged between 8 and 11 years, were matched for age, sex, weight and height and randomly allocated to three groups. Each group was allocated to one of the following breakfasts; traditional porridge (Quaker Oats, Southall, UK), soya and linseed bread (Burgen, Allied Bakeries, Maidenhead, UK), or All Bran (Kelloggs, Manchester, UK). The respective GI values of these foods, as estimated from the international GI tables, are 58, 49, and 42 (Foster-Powell *et al.* 2002). Each of the breakfasts provided approximately 1273 kJ (300 kcal) and were matched as closely as possible for macronutrient and NSP content. After breakfast the children were instructed not to eat or drink anything other than water and a small serving of fruit, which was provided. Their subsequent intake at an *ad libitum* lunch sitting was observed unobtrusively and recorded. Breakfast palatability, satiation after breakfast and satiety before lunch were measured by rating scales used in the previous breakfast study (Warren *et al.* 2003).

			Lunch intake (kJ)		Breakfast palatability		Post-breakfast satiety		Pre-lunch satiety	
Breakfast	*n*	GI	Mean	SD	Mean	SD	Mean	SD	Mean	SD
All Bran	12	42	2874	1264	5.7	1.5	4.6	2.0	1.2	0.4
Soya and linseed bread	12	49	3059	753	5.9	1.2	5.0	1.5	1.2	0.4
Porridge	12	51	3623	1377	6.0	1.3	5.8	1.6	1.3	0.5

The results show that *ad libitum* lunch intake increased progressively as breakfast GI increased (2874 kJ (687 kcal) compared with 3623 kJ (866 kcal)). This trend suggests that even small differences in GI may influence appetite and food intake at subsequent meals. However, possibly because of the limited number of subjects involved and the large inter-subject variability in lunch intake, analysis by one-way ANOVA showed no significant differences in lunch intake between breakfasts ($P=0.330$). There were no differences between the breakfast palatability, post-breakfast satiation or pre-lunch satiety scores. However these scores are subjective and it may be difficult to see differences in a small sample of subjects.

In conclusion, small differences in the GI of individual breakfast types may have a significant effect on subsequent food intake at lunch. Further research with a larger number of subjects and breakfasts with various GI values are needed to confirm our preliminary results.

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Effect of parity on maternal plasma leptin and adipose tissue growth in the resulting offspring.
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There is now extensive epidemiological and experimental evidence that the risk of obesity can be programmed *in utero*, which may be mediated in part through changes in maternal leptin secretion (for example, Bispham *et al.* 2003). Exposure of the fetus to a suboptimal nutritional environment early in gestation results in greater fat mass at birth but it is not known if maternal parity alone promotes later adiposity. The present study aimed to determine the effect of parity on maternal plasma leptin and the extent to which adipose tissue growth and leptin secretory capacity may also be reset.

Fourteen twin-bearing sheep of similar body weight were entered into the study of which seven were primiparous (P) and seven multiparous (M). Each animal was fed 100% of total metabolisable energy requirements throughout gestation and all ewes delivered normally at term. Hourly blood samples were taken over about 8 h from all ewes at 140 d gestation. Plasma concentration of leptin was then determined by radioimmunoassay. One randomly selected lamb was then humanely euthanased at 1 d of age and the other at 1 month of age. Tissue sampling was performed and peri-renal adipose tissue immediately snap-frozen in liquid N_2 and stored at −80 °C until analysis. Total RNA was extracted and reverse-transcriptase PCR analysis was performed using oligonucleotide primers specific to ovine leptin, insulin-like growth factor (IGF)-I and II receptors (Bispham *et al.* 2003). Results are expressed in arbitrary units (a.u.) as a ratio of an 18S rRNA internal control. Statistical differences were analysed using Kruskal–Wallis and Mann–Whitney *U* tests.

			Postnatal 1 d				Postnatal 30 d			
	Maternal plasma leptin (ng/ml)		IGF-I receptor mRNA (a.u.)		IGF-II receptor mRNA (a.u.)		Leptin mRNA (a.u.)		Fat mass (g)	
Parity	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
P	1.2**	0.5	197*	14	121	10	75*	18	153**	10
M	5.3	1.1	123	10	74	7	31	7	108	14

Mean values were significantly different from M: * $P<0.05$, ** $P<0.01$.

Maternal weight gains were similar between the two groups and birth weight, in the present study, was not affected by parity. However, the maternal plasma concentration of leptin during late gestation was substantially lower in primiparous compared with multiparous mothers despite similar adiposity. In the offspring, although growth rate over the first month of life was unaffected by parity, lambs born to primiparous mothers had a much greater fat mass (both total and relative to body weight) at 1 month of age. At birth, fat from primiparous offspring exhibited a significantly raised mRNA abundance for both IGF-I and -II receptors. These adaptations did not persist up to 1 month of age, but leptin mRNA abundance was greater at both 1 and 30 d of life.

In conclusion, maternal parity has a major effect on maternal endocrine status for which an increase in maternal leptin following exposure to one successful pregnancy may dramatically change the relationship between fat mass and leptin synthetic capacity, potentially re-setting appetite regulation. One consequence of this adaptation would be to improve the partition of nutrients between maternal and fetal tissues via the placenta, so as to have the capacity to increase fetal growth in subsequent pregnancies. Maternal parity also has a pronounced effect on later fat growth, which is greatly increased in offspring of primiparous ewes. Enhanced adiposity in the absence of any change in total body weight suggests an explanation for this adaptation resides within the adipocyte itself rather than centrally mediated mechanisms such as increased food intake or differences in maternal milk composition. Support for this proposal is the greater IGF receptor mRNA abundance and leptin synthetic capacity.

Bispham J, Gopalakrishnan GS, Dandrea J, Wilson V, Budge H, Keisler DH, Broughton Pipkin F, Stephenson T & Symonds ME (2003) *Endocrinology* **144**, 3575–3585.

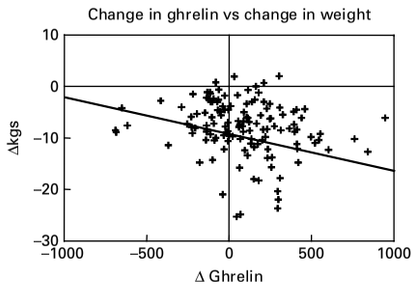
Fasting ghrelin concentrations in a large cohort of overweight and obese volunteers following four commercial weight-loss programmes.
By A.E. BRYNES[1], G.S. FROST[1], M. GHATEI[1], S.R. BLOOM[1], A. DELOOY[2], K. FOX[3], P.J. ROBSON[4], I. MACDONALD[5], D.J. MILLWARD[6], H. TRUBY[6] and L. MORGAN[6], [1]*Nutrition and Dietetics Research Group, ICSM, Hammersmith Hospital, London, UK, W12 0HS,* [2]*Queen Margaret's University College, Edinburgh, UK, EH12 8TS,* [3]*University of Bristol, Bristol, UK, BS8 1TH,* [4]*University of Ulster, UK, BT52 1SA,* [5]*University of Nottingham, Nottingham, UK, NG7 2RD and* [6]*School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH*

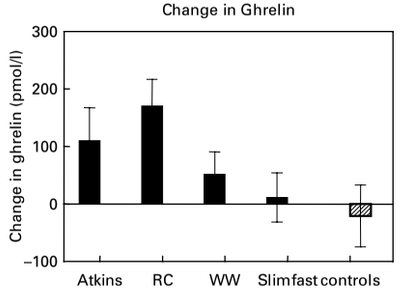
Ghrelin is a twenty-eight-amino acid peptide secreted mainly in the stomach, which may signal meal initiation (Cummings *et al.* 2001) and satiety (Tschop *et al.* 2001). Plasma ghrelin levels increase before meals and decrease rapidly postprandially. They also increase with decreasing BMI (Tschop *et al.* 2001; *n* 30) and weight loss in human subjects (Ravussin *et al.* 2001; *n* 7). Here we report on a large cohort of 170 overweight and obese healthy adults at the beginning and end of 6 months of dieting. This represents 77% of the original 'Diet Trials' population (a randomised controlled trial of four commercially available weight-loss programmes) of which, in this subset 71% were women (baseline weight 84.1 (SD 10) kg, BMI 31.4 (SD 2.7) kg/m^2 and 29% were men (104.4 (SD 12.3) kg, BMI 32.7 (SD 2.6) kg/m^2).

Baseline ghrelin was significantly different by sex (male 473 (SEM 32) *v.* female 643 (SEM 32) pmol/l; $P<0.001$). This remains when adjusted for weight ($P<0.001$), BMI ($P<0.001$), percentage body fat ($P<0.001$) or waist ($P<0.001$) but not homeostasis model assessment (HOMA) %S ($P=0.13$). There was a significant negative correlation between baseline fasting ghrelin and weight (r −0.2; $P<0.01$), BMI (r −0.18; $P=0.02$), waist (r −0.21; $P<0.01$) and insulin (r −0.36; $P<0.0001$).

Mean weight loss at the end of 6 months was −9.6±1.2% in the group following the Atkins diet (*n* 25), −8.4±0.8% on the Rosemary Conlley (RC) diet (*n* 38), −7.9±0.8% on Weight Watchers (WW) (*n* 38) and −6.6±0.9% on Slimfast (*n* 37). The control group did not lose weight (+0.99±0.5%; *n* 37).

No relationship was found between baseline plasma ghrelin and magnitude of body-weight change in the dieting groups. There was a significant negative correlation between change in ghrelin and change in weight (r −0.25; $P<0.01$), change in waist (r −0.2; $P<0.01$) and change in insulin (r −0.36; $P<0.0001$). Change in ghrelin *v.* change in HOMA (%S) was positively correlated (r +0.2; $P=0.04$). There was a small but significant increase in ghrelin over time on the RC diet *v.* control (171±46 *v.* −21±54 pmol/l; $P=0.04$) using ANOVA with *post hoc* differences. This was not related to change in waist, change in percentage body fat or change in insulin sensitivity by HOMA.



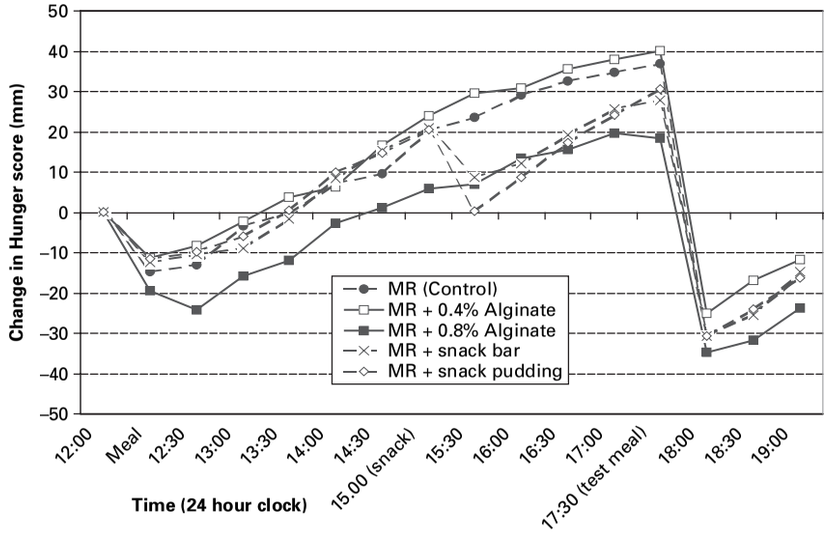


In conclusion the observed increase in ghrelin with diet-induced weight loss is consistent with the hypothesis that ghrelin has a role in the long-term regulation of body weight. In this population women had higher fasting plasma ghrelin than men. The possibility that ghrelin is affected by different types of diets and/or macronutrients and exercise is exciting and warrants further investigation.

Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. (2001) *Diabetes* **50** 8: 1714–9.
Ravussin E, Tschop M, Morales S, Bouchard C & Heiman ML (2001) *Journal of Clinical Endocrinology and Metabolism* **86**, 4547–4551.
Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E & Heiman ML (2001) *Diabetes* **50**, 707–709.

Addition of alginate fibre to a liquid meal replacement: effects on satiety and food intake. By K.M. APPLETON[1], J. HILL[1], E. HADDEMAN[2], P. RAYMENT[3], P.J. ROGERS[1] and D.J. MELA[2], [1]*Department of Experimental Psychology, University of Bristol, 8 Woodland Road, Bristol, UK, BS8 1TN,* [2]*Unilever Health Institute, Unilever R&D Vlaardingen, The Netherlands and* [3]*Unilever R&D Colworth, Sharnbrook, UK, MK44 1LQ*

Meal replacers (MR) have been shown to be effective for weight loss and maintenance in a number of clinical trials; however, feelings of hunger are a problem which can reduce compliance with MR and other reduced-energy diet plans. Fibres such as alginates may be used to enhance feelings of satiety through their physical effects within the gastrointestinal tract. We assessed hunger, mood and food intake in thirty normal and overweight subjects (mean BMI 25.9 kg/m^2), following each of five conditions in a within-subject repeated-measures design: (1) A liquid MR with no added fibres; (2) MR+0.4% alginate; (3) MR+0.8% alginate; (4) MR followed by a commercial MR plan snack bar; or (5) MR followed by a snack pudding containing 0.8% alginate. MR were always eaten at 12.00 hours. In the two snack conditions, the snacks (snack bar or pudding) were equal in energy content, and eaten at 15.00 hours. Hunger measures and mood were measured at 30 min intervals across the afternoon, and food intake measured in an *ad libitum* meal at 17.30 hours.



Compared with the control MR, addition of 0.8% alginate fibre significantly reduced the magnitude of the post-MR hunger profile. Both snacks also produced significant reductions in hunger measures. However, there was no added effect of 0.4% alginate fibre, or difference between the two snacks. Despite the effects of the 0.8% alginate and the snacks on reducing hunger scores, there were no treatment-related effects on test meal intake. There were also no consistent treatment effects on mood.

We conclude that the addition of a gelling fibre such as alginate to a liquid MR can significantly reduce feelings of hunger for several hours, although this did not influence intakes at a later *ad libitum* test meal. Further research will establish the preferred types and levels of fibres to optimise the effects in different food product formats.

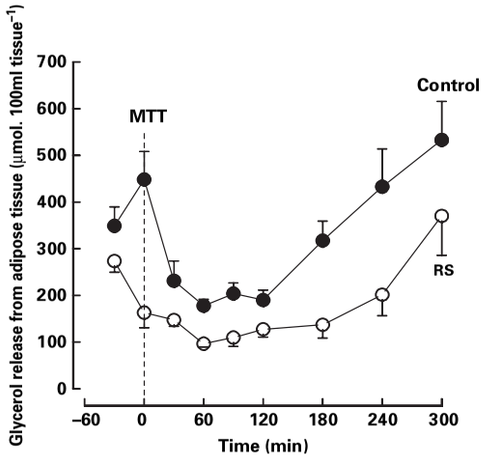
Change in adipose tissue function following a high-fibre diet. By M.D. ROBERTSON, A.L. DENNIS, A.S.T. BICKERTON, J.M. CURRIE and K.N. FRAYN, *Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK, OX3 7LJ*

Despite having a negligible effect on fasting plasma lipids or postprandial glycaemia, diets rich in insoluble fibre such as resistant starch (RS) have been linked to a decreased incidence in both type 2 diabetes and heart disease (Jenkins *et al.* 2000). The biological mechanisms underlying this remain unclear.

Ten healthy subjects (six female; age range 24–61 years; BMI 18.4–32.3 kg/m^2) consumed one of two dietary supplements for 4 weeks in a randomised, single-blind cross-over design. The supplements consisted of 50 g Novelose 260$^{(R)}$/d (30 g RS) or 20 g Amioca$^{®}$/d (0 g RS). At the end of week 3, a hyperinsulinaemic–euglycaemic clamp (35 mU insulin/m2 per min) was performed in the fasting state. At the end of week 4, a meal tolerance test (MTT; 60 g glucose, 21 g fat) was undertaken in which metabolic flux across both skeletal muscle and adipose tissue was calculated using arterio-venous sampling combined with measurements of blood flow.

During the clamp, the insulin sensitivity was increased from 8.5 to 9.7×10^{-2} mg glucose/kg per min×mU insulin during RS supplementation (P=0.027). Following the MTT, there was a significant increase in the oral insulin sensitivity (P=0.05), an elevation in fasting and postprandial plasma ghrelin concentrations (P=0.027) and an increase in the C-peptide:insulin molar ratio (P=0.034) following RS supplementation compared with control.

The metabolic function of adipose tissue was also significantly changed following RS intake. There was a significant decrease in fatty acid and glycerol release from adipose tissue despite a lower prevailing insulin concentration which may be an indicator of decreased adipose tissue lipolysis (see Figure).



There was also an increase in the postprandial glucose uptake (P=0.007) and an increased uptake of the SCFA, acetate (P=0.034) and propionate (P=0.048), by adipose tissue following RS supplementation.

The change in insulin sensitivity observed with diets high in insoluble fibre may be mediated primarily through changes in adipose tissue metabolism.

We acknowledge the BBSRC, UK for financial support and National Starch and Chemical (Manchester) for the provision of test starches.

Jenkins DJ, Kendall CW, Axelsen M, Augustin LS & Vuksan V (2000) *Current Opinion in Lipidology* **11**, 49–56.

Glucocorticoid-independent programming of angiotensin receptor expression in hypertensive rats exposed to a low-protein diet *in utero*. By S. McMULLEN and S.C. LANGLEY-EVANS, *Centre for Reproduction and Early Life, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, UK, LE12 5RD*

Human epidemiological studies and supporting animal models have demonstrated that undernutrition in pregnancy is related to hypertension and renal disease in the subsequent offspring. Hypertensive rats exposed to a low-protein (LP) diet *in utero* exhibit abnormalities of the renin–angiotensin system, including elevated angiotensin-converting enzyme (ACE) activity in plasma and lung (Langley-Evans & Jackson, 1995) and down regulation of type 2 angiotensin receptor (AT_2) expression and increased pressor responses to angiotensin II (McMullen *et al.* 2004). Overexposure of fetal tissues to glucocorticoids (GC) of maternal origin has been implicated as a prerequisite for the programming of later CVD. The present study examined the relationship between the renin–angiotensin system and GC in the LP rat model of programmed hypertension.

Wistar rats were fed isoenergetic diets containing 180 g casein/kg (control; *n* 6) or 90 g casein/kg (LP) throughout gestation. On days 0–14, LP rats were injected with saline (LP; *n* 6), an inhibitor of maternal corticosteroids synthesis (metyrapone; LP_m, *n* 6), or metyrapone with corticosterone replacement (LP_{mc}; *n* 5). At birth all litters were culled to a maximum of eight. Blood pressure was assessed at 4 weeks using the tail-cuff method. Plasma and tissues were collected from 4-week-old offspring. The relative expression of renal angiotensin receptor expression was assessed by real-time RT-PCR and normalised to β-actin. Pulmonary and renal ACE was assessed using the FAPGG assay.

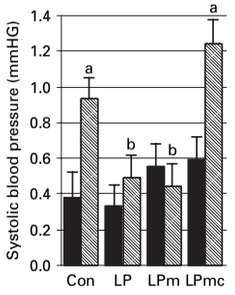


Fig. 1. Systolic BP in males (solid) and females (hash).

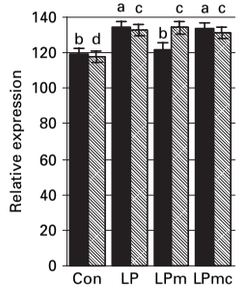


Fig. 2. Renal AT_2 expression in males (solid) and females (hash).

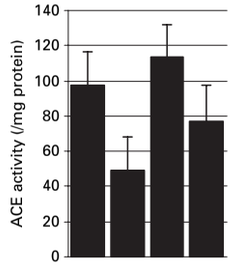


Fig. 3. Renal ACE activity in males and females.

Data are shown as mean ± SEM for *n* 20 to 24 (BP and AT_2) or *n* 40 to 48 (ACE) observations. Different superscripts indicate significant difference between groups (within each sex, $P<0.05$).

The significant increase in blood pressure in LP offspring (Fig. 1; $P<0.01$) was GC-dependent in males only. At both time points, AT_1a and b expression was unaltered by *in utero* dietary experience, although the expression of AT_1a did appear to be GC-regulated. AT_2 expression was down regulated in LP rats at 4 weeks in females only (Fig. 2; $P<0.05$). Although not influenced by metyrapone, AT_2 expression was up regulated in response to corticosterone ($P<0.01$). There appeared to be a glucocorticoid-dependent down regulation of renal ACE activity, although this failed to reach statistical significance.

Down regulation of AT_2 receptor expression may contribute to programmed hypertension in females through its role in opposing the AT_1 receptor-mediated vasoconstrictive actions of angiotensin II. Although it appears that AT_2 expression is regulated by GC, the LP-induced down regulation of expression does not appear to be GC-dependent. This is in contrast to the GC-dependent programming of nephron number and blood pressure in males, indicating an additional and alternative pathway of blood pressure programming. The present study suggests that the factors contributing to LP-programmed hypertension are sex-specific. Examination of the differential regulation of the renin-angiotensin system in males and females may partly explain such sex differences.

S.McM. is supported by the British Heart Foundation.

Langley-Evans SC & Jackson AA (1995) *Comparative Biochemistry and Physiology* **110**A, 223–228.
McMullen S, Gardner DS & Langley-Evans SC (2004) *British Journal of Nutrition* **91**, 133–140.

The effect of body composition on reproductive performance in C57BL/6J mice. By S.L. JOHNSTON[1], L.M. BELL[1], S.J. MURRAY[1], B.J. TOLKAMP[2], J. YEARSLEY[3], I. KYRIAZAKIS[2], A.W. ILLIUS[4] and J.R. SPEAKMAN[1,5], [1]*Aberdeen Centre for Energy Regulation and Obesity (ACERO), Rowett Research Institute, Aberdeen, UK, AB21 9SB,* [2]*SAC, Bush Estate, Penicuik, UK, EH26 OQE,* [3]*Macaulay Institute, Aberdeen, UK, AB15 8QH,* [4]*University of Edinburgh, Edinburgh, UK and* [5]*ACERO, University of Aberdeen, Aberdeen, UK, AB24 2TZ*

Energy restriction has been associated with benefits such as a delay in the onset of age-related disorders whilst excessive energy intake and obesity has been associated with costs such as problems with female fertility. If body fatness is associated with reproductive failure then perhaps leanness increases reproductive capability. In the present study we investigated the effect of different body compositions on the reproduction of female C57BL/6J mice. We designed the experiment so that the treatment would alter body composition of the mice without directly affecting the offspring. Mice were initially mated at 7 weeks of age and pups were weaned at 21 d, to ensure only reproductively capable animals entered the study. Fertile mice (*n* 115) received either an *ad libitum* supply of a control (10% energy as fat; *n* 25), medium-fat (45% energy as fat; *n* 30) or high-fat (60% energy as fat; *n* 30) diet, or a restricted diet (daily ration of 75% of *ad libitum* control diet; *n* 30) for 9 weeks. Body composition was measured by dual-energy X-ray absorptiometry at 6 months of age. Body mass (BM), fat mass (FM) and lean tissue mass (LTM) were strongly influenced by diet (BM general linear model (GLM): $F_{3,1128}$ 638, $P<0.001$; FM GLM: $F_{3,111}$ 52.6, $P<0.001$; LTM GLM: $F_{3,111}$ 33.4, $P<0.001$). Weight loss in restricted-diet animals averaged –1.5 (SEM 0.46) g and weight gain in control-, medium-fat- and high-fat-diet-fed animals averaged 0.7 (SEM 0.24) g (2.8% of baseline), 5.0 (SEM 0.59) g (20.5% of baseline) and 8.3 (SEM 0.61) g (33.9% of baseline), respectively. Restricted-diet animals were different from controls in LTM but not FM (control FM 3.0 (SEM 0.25) g, LTM 19.2 (SEM 0.17) g; restricted FM 3.2 (SEM 0.61) g, LTM 17.1 (SEM 0.22) g). Medium-fat- and high-fat-diet animals had more FM but not LTM than controls (medium-fat FM 7.1 (SEM 0.61) g, LTM 19.8 (SEM 0.26) g; high-fat FM 11.3 (SEM 0.76) g, LTM 19.3 (SEM 0.17) g). To minimise the effect of diet directly on pregnancy and lactation all mice were removed from treatment and placed on standard laboratory chow 10 d before being paired with a proven breeder male for 6 d. The dam and litter were weighed at birth and weaning 21 d later. Mice on the restricted diet gained weight before being paired for mating but the medium- and high-fat-diet-fed animals still had greater BM than controls at birth and the BM of high-fat-diet animals was higher than controls at weaning (birth GLM: $F_{3,69}$ 8.11, $P<0.05$; weaning GLM: $F_{3,64}$ 4.59, $P<0.05$).

	Birth					Weaning			
	Litter		Pup			Litter		Pup	
Diet	Number	Mass (g)	Number	Mass (g)*		Number	Mass (g)	Number	Mass (g)*
Restricted	27	13.2^{c}	6.98^{b}	1.97^{a}		24	66.3^{c}	6.83^{c}	10.1^{ab}
Control	18	11.3^{bc}	4.89^{a}	2.71^{b}		18	47.2^{b}	4.83^{b}	10.7^{b}
Medium-fat	16	10.3^{ab}	4.38^{a}	3.00^{b}		16	42.6^{ab}	4.38^{ab}	10.0^{ab}
High-fat	12	9.0^{a}	3.67^{a}	2.81^{b}		10	35.0^{a}	3.41^{a}	9.5^{a}
LSD		1.892	1.293	0.575	LSD		10.56	1.321	1.108
$F_{3,69}$		8.15	11.58	5.89	$F_{3,64}$		15.38	11.39	1.55
P		<0.001	<0.001	0.001	*P*		<0.001	<0.001	0.211

[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0.05$).
* Pup mass is derived from the average mass of pups per litter for each treatment.

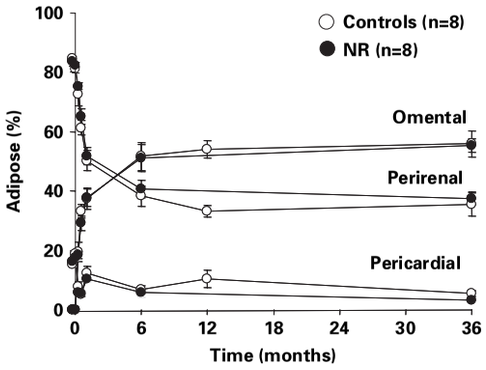
Animals on the restricted diet had the highest birth rate (90%) whereas 72% of the controls, 53% of medium-fat- and 40% of high-fat-diet animals gave birth. Of the animals that gave birth those fed the high-fat diet weaned significantly fewer pups, and these were also significantly smaller than the offspring born to control mice. Mice on the restricted diet had a greater number of pups than the controls. Because the mice were removed from dietary treatment before reproductive performance was tested, the effects on litter and pup sizes can be linked to differences in body composition. These results indicate that mice on a low-fat diet produce more and bigger offspring than mice on high-fat diets because they have less body fat. Reproduction is severely compromised by increased FM resulting from the intake of a high-fat diet.

Maternal diet and development of regional adiposity in sheep. By D.S. GARDNER, S. PEARCE, T. STEPHENSON and M.E. SYMONDS, *Centre for Reproduction and Early Life, Institute of Clinical Research, University Hospital, Nottingham, UK*

Obesity is becoming the major health burden facing Westernised societies. Excess adipose tissue mass, specifically around the abdomen, increases the risk of a range of adverse health outcomes. Adult adiposity is influenced by the prenatal diet both in man and in experimental animal models. The present study describes a retrospective analysis of the ontogeny of regional adipose tissue deposition in sheep, which tend to lay down the majority of their fat in four regions; omental, peri-renal, cardiac and subcutaneous. In addition to a control group, regional adiposity has also been examined in the offspring of ewes nutrient-restricted (NR) during early gestation.

The present study involves several groups of ewes that were fed at or above nutrient requirements throughout gestation and lactation (controls, fed 1–1.5 maintenance ration (M); AFRC 1993) or NR (fed at 0.5×M) during early gestation (either 0–30 d or 30–80 or 95 d). Offspring were delivered by Caesarean section at 140 d gestation or naturally at term and immediately euthansed (sodium pentobarbitone) for tissue collection. Offspring from separate groups of control and NR ewes were subsequently euthanased at 1 d, 30 d, 6 months, 12 months or 3 years of age after birth for detailed measurements and analysis of fat from each major depot. Lambs were weighed at birth, ewe-reared as singletons to weaning and grass-fed thereafter until tissue collection. Data are means and SEM and were analysed by two-way ANOVA (for time for example, 1 d *v.* 30 d and treatment, i.e. control *v.* NR) using SPSS.

In fetuses and term neonates, adipose tissue constituted 4.9 (SEM 0.25) g/kg body weight. The majority (about 80%) was peri-renal, with cardiac comprising the remaining 20%. Over the next 30 d, adipose tissue increased by an order of magnitude (from about 25 g total fat to 250 g) and began to be preferentially deposited abdominally. For example, the percentage of total fat had declined in the peri-renal and cardiac regions to 50 (SEM 3) % and 12 (SEM 2) %, respectively; whereas the proportion in the omental region now comprised 37 (SEM 3) % of total fat. By 6 months of age, adipose tissue constituted 18 (SEM 2) g/kg, and increased in proportion to the increase in body weight thereafter. In addition, at this age, the regional depots of adipose constituted 50 (SEM 3), 38 (SEM 2) and 4 (SEM 1) % total fat in omental, peri-renal and cardiac areas, respectively and changed little from these values over the next 2.5 years (Fig. 1). Prenatal nutrient restriction tended to increase the propensity to store energy as fat (for example, total fat at 3 years of age was 1283 (SEM 242) *v.* 1451 (SEM 284) g in control and NR animals, respectively), but regional adiposity was unaffected at each age studied (Fig. 1).



To conclude, the data first highlight the remarkable rate of deposition of abdominal fat in neonatal sheep over the periods of lactation and early independent life. Second, in the sheep, the proportion of body fat and regional deposition appears fixed from as early as 6 months of age. Prenatal nutrient restriction appears to facilitate greater overall fat deposition in offspring, but the extra adipose appears to be deposited evenly throughout the body stores. The early postnatal period in sheep appears crucial for the programming of later regional fat deposition offering huge potential for possible modification of this distribution through nutritional intervention at this time.

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Chronic umbilical cord compression promotes maturation of fetal brown adipose tissue. By M.G. GNANALINGHAM[1], A. MOSTYN[1], M.E. SYMONDS[1], D.A. GIUSSANI[2], T. STEPHENSON[1] and D.S. GARDNER[1], [1]*Centre for Reproduction and Early Life, Institute of Clinical Research, University of Nottingham, Nottingham, UK, NG7 2UH and* [2]*Department of Physiology, University of Cambridge, Cambridge, UK, CB2 3EG*

Chronic umbilical cord compression in fetal sheep sufficient to restrict fetal blood supply by 30% mimics the degrees of cord compression noted in about 40% of human pregnancies, and results in a range of fetal endocrine adaptations, including increased fetal plasma cortisol (Gardner *et al.* 2001). Fetal plasma cortisol promotes brown adipose tissue (BAT) maturation by increasing the thermogenic capacity of BAT-specific uncoupling protein-1 (UCP1) (Mostyn *et al.* 2003*a*). Voltage-dependent anion channel (VDAC) and cytochrome c are also important in cellular energy metabolism in BAT (Mostyn *et al.* 2003*b*). The present study was designed to determine the effect of chronic umbilical cord compression on the abundance of UCP1 mRNA, mitochondrial proteins UCP1, VDAC and cytochrome c, guanosine diphosphate (GDP) binding (estimate of the potential thermogenic activity) and glucocorticoid receptor (GCR).

Nine ewes were entered into the study and were chronically instrumented with fetal vascular catheters at 118±2 d (term is at about 147 d). Five fetuses were then subjected to 3 d umbilical cord compression beginning at 125 d gestation by automated compression of the umbilical cord, with the remaining four acting as controls. At 137±2 d gestation, all fetuses were humanely euthanased (100 mg sodium pentobarbitone/kg), BAT dissected and frozen in liquid N_2. RT-PCR utilising specific ovine oligonucleotide primers determined UCP1 and GCR mRNA abundance. Mitochondrial fractions were prepared and analysed using immunoblotting (Mostyn *et al.* 2003*a*) with antibodies specific for cytochrome c, VDAC and UCP1 that produced single bands at 16, 35 and 32 kDa respectively. GDP binding was determined by a competitive radioactive assay (Symonds *et al.* 1992). Results are given as means and SEM. Statistical differences between groups were analysed by an unpaired Mann–Whitney *U* test.

	UCP1 mRNA (% 18S rRNA)		GCR mRNA (% 18S rRNA)		UCP1 protein (% reference sample)		VDAC (% reference sample)		Cytochrome c (% reference sample)		GDP binding (pmol/mg protein)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Controls	70.0	2.4	52.1	2.3	57.4	1.3	100.6	2.6	80.0	0.7	19.3	1.3
Umbilical compression	121.8	0.2*	69.0	4.2*	108.5	4.7*	132.6	1.9*	92.4	1.2*	36.3	2.8*

* Significantly different from controls ($P<0.05$).

There were no significant differences in body or BAT weights between the groups. Umbilical cord compression resulted in an enhanced abundance of UCP1 and GCR mRNA, mitochondrial UCP1, VDAC and cytochrome c proteins and GDP binding.

In conclusion, chronic umbilical cord compression results in precocious maturation of BAT, an effect that may be mediated by increased fetal plasma cortisol and/or glucocorticoid receptor abundance. The improved thermogenic capacity of BAT may subsequently act to prevent hypothermia in the potentially growth-restricted fetus at birth following cold exposure to the extra-uterine environment.

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The effect of calcium supplementation during pregnancy on birth weight and length. By A. DJAZAYERY[1], M. KARANDISH[2], F. MOREMAZI[2], A. BEHROOZ[2], E. KAZEROUNINEJAD[2] and M. MAHMOUDI[1], [1]*School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran and* [2]*Paramedical School, Ahvaz University of Medical Sciences, Ahvaz, Iran*

Studies on the effect of Ca intake during pregnancy on pregnancy outcome have not had consistent results (Merialdi *et al*. 2003). The aim of the present double-blind, placebo-controlled randomised trial was to determine the effect of Ca supplementation (two 500-mg doses/d as calcium carbonate capsules) during the last trimester of pregnancy on birth weight and length.

The participants were sixty-eight healthy 18–35-year-old pregnant women selected randomly from among those consulting the Ahvaz University of Medical Sciences main teaching hospital in Ahvaz, south-east of Iran, allocated randomly, as they came in, into 'Ca supplement' (A) or 'placebo' (B) groups, matched for confounding factors-age, weight, height, parity, and pre-pregnancy BMI. Inclusion criteria were: parity<three, singleton pregnancy, intention to breastfeed infant, no history of bone or metabolic diseases, and intake of no supplements except Fe and folic acid. Daily dietary Ca intakes were estimated once per month by using a locally validated food-frequency questionnaire. The length of the experimental period was from week 26–28 to delivery (9–11 weeks). The numbers of boys and girls born were, respectively, 17 and 16 in the experimental group, and 17 and 18 in the placebo group.

	Ca supplement (*n* 33)*		Placebo (*n* 35)*	
Variable	Mean	SD	Mean	SD
Pre-pregnancy BMI (kg/m^2)	23.1	4.2	24.0	4.1
Maternal dietary Ca intake (mg)	539.0	172.0	579.0	176.0
Pregnancy weight gain (kg)	12.8	3.8	11.7	3.1
Birth weight (g)	3241.0	486.0	3011.0†	434.0
Birth length (mm)	498.0	18.0	496.0	25.0

*Compliance >80%. †Student's t test (P<0.05; SPSS version 9).

The results are shown in the Table. Birth weight was significantly increased in the supplementation group although birth length was not different, confirming the results reported by Purwar *et al*. (1996) and Niromanesh *et al.* (2001). No such an effect, however, was observed in the largest trial reported so far (Levine *et al*. 1997). One reason could be different baseline dietary Ca intakes, which are low in many developing countries. Although it has been suggested that the effect of Ca supplementation during pregnancy on birth weight could be due to better intra-uterine growth and increased length of pregnancy (prevention of prematurity), in the present study the difference in birth weight still existed even after removing the four low-birthweigth cases (1 in the experimental and 3 in the placebo group).

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Differences in nutrient intake in Pakistani- and European-origin women in pregnancy. By A. VYAS[1,2], J. OLDROYD[1,2], L.A. JAMES[1,2], A.J. KHAN[1,2], P. McELDUFF[2] and J.K. CRUICKSHANK[1,2], [1]*Clinical Epidemiology Group, University Department of Medicine, Manchester Royal Infirmary, Manchester, UK and* [2]*Unit of Chronic Disease Epidemiology, University of Manchester, Manchester, UK, M13 9PT*

Maternal nutritional status has an impact on fetal outcomes and hence on disease risk in later life. Systematic comparative data by ethnic group are scarce. The present report compared nutrient intake during pregnancy in samples of antenatal women by ethnic group, self- and grandparentally-defined. Women were included if they were over 18 years, had been scanned before 24 weeks, and were on no medication. Those on *in vitro* fertilisation treatment, with multiple pregnancies or who had or developed diabetes were excluded. Food-frequency questionnaires (FFQ), specifically designed for each ethnic group, were administered around 28 weeks gestation by one of four trained interviewers.

The FFQ were calibrated by 4 d food diaries in subsamples. Of 675 women with FFQ data, 101 were over- or under-reporting nutrient intake, defined as <1.2×estimated BMR or >17.8 MJ/d. Misreporting was 16% in the White European (WE), and 31% in the Pakistani origin (PO) women; these are not included in the Table, which shows the nutrient intakes in the two groups of women, adjusted for age and to 4.2 MJ. PO women were younger and had higher BMI than WE but total energy intakes were similar (11.8 *v.* 11.9 MJ). Polyunsaturated : saturated fatty acid ratios and percentage energy from polyunsaturated fats were higher in the PO group compared with the WE group (see Table).

		PO		WE		% <RNI		
	RNI	Mean	95% CI	Mean	95% CI	PO	WE	*P* value
n		122		392				
Age (years)		28.5	27.7, 29.3	30.8	30.0, 31.0			0.009
BMI (kg/m^2)		28.6	21.2, 30.0	27.4	26.7, 28.1			0.05
Total energy (MJ)		11.8	11.4, 12.3	11.9	11.7, 12.1			NS
Protein (g)		32.4	31.4, 33.4	37.1	36.4, 37.7			0.000
% Energy from protein		13.0	12.6, 13.4	14.8	14.6, 15.1			0.000
Fat (g)		43.3	42.3, 44.3	39.6	38.9, 40.3			0.000
% Energy from fat		39.0	38.1, 39.9	35.6	35.0, 36.2			0.000
% Energy from polyunsaturated fat		9.2	8.7, 9.7	6.5	6.2, 6.7			0.000
Polyunsaturated : saturated fatty acid ratio		0.88	0.81, 0.94	0.56	0.53, 0.55			0.000
Carbohydrate (g)		129.4	127.1, 131.7	131.9	130.2, 133.6			0.011
% Energy from carbohydrate		48.5	47.7, 49.7	49.5	48.8, 50.1			0.014
Sugar (g)		60.6	57.3, 62.6	65.6	63.8, 67.8			0.006
Ca (mg)	700 mg	428.9	411.0, 446.8	494.2	480.1, 508.2	16	7	0.03
Fe (mg)	8.7 mg	5.8	5.6, 6.0	5.7	5.5, 5.8	17	11	0.14
Folate (μg)	300 μg	121.8	116.3, 127.2	137.9	134.1, 141.8	18	8	0.002
Vitamin B_6 (mg)	1.5 μg	1.0	0.8, 1.1	1.5	1.3, 1.7	4	1	0.01
Vitamin B_{12} (μg)	1.2 μg	1.8	1.6, 2.0	2.1	2.0, 2.2	6	2	0.05
Vitamin C (mg)	50 mg	72.1	65.8, 78.4	80.4	76.5, 84.4	2	2	0.06

RNI, reference nutrient intake per d.

Micronutrient and protein intakes are lower in these PO women, despite their marginally higher total energy and fat consumption. A further analysis examines relationships to fetal outcomes (Oldroyd *et al.* 2004) and plasma value validation is in process.

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Faecal microflora and short-chain fatty acid concentrations in patients receiving enteral tube feeding. By K. WHELAN[1], P.A. JUDD[2], K.M. TUOHY[3], G.R. GIBSON[3], V.R. PREEDY[1] and M.A. TAYLOR[4], [1]*Department of Nutrition and Dietetics, King's College London, London, UK, SE1 9NN,* [2]*Lancashire School of Health and Postgraduate Medicine, University of Central Lancashire, Preston, UK, PR1 2HE,* [3]*Food Microbial Sciences Unit, University of Reading, Reading, UK, RG6 6AP and* [4]*School of Biomedical Sciences, University of Nottingham, Nottingham, UK, NG7 2UH*

Diarrhoea is a common complication of enteral tube feeding (ETF) with a pathogenesis that includes an increased risk of *Clostridium difficile*-associated diarrhoea (CDAD; Bliss *et al.* 1998) and an abnormal secretion of water into the colonic lumen. The colonic microflora may protect against enteropathogens, such as *C. difficile*, whilst the SCFA they produce stimulate colonic water absorption (Bowling *et al.* 1993). However, standard, fibre-free enteral formula, consumed by healthy subjects, causes a reduction in total faecal bacteria and SCFA concentrations (Whelan *et al.* 2003). In order to further investigate the interaction between the microflora and diarrhoea during ETF, we analysed the faecal microflora and SCFA concentrations in patients during 14 d of ETF, comparing those who did, and did not, experience diarrhoea or CDAD.

Twenty patients (five males, fifteen females) starting fibre-free ETF as a sole source of nutrition for 14 d were recruited from St George's Hospital, London. Faecal output was characterised daily by nursing staff using a validated chart (Whelan *et al.* 2004). Fresh faecal samples were collected at the start (days 1–4), middle (days 6–9) and end (days 11–14) of the 14 d period. Samples were analysed for major bacterial groups by fluorescent *in situ* hybridisation, using the probes EUB338 (total bacteria), Bif164 (bifidobacteria) and EREC482, Chis150 and Clit135 (major clostridia). Faecal SCFA concentrations were measured by GLC and *C. difficile* enterotoxins A and B were detected by ELISA.

There were no significant within-subject time effects in the concentration of faecal microflora or SCFA during the 14 d period. However, some patients had large alterations in microflora during the 14 d period, which may have been, in part, due to the majority (80 %) being prescribed as many as four different antibiotics. Mean faecal microflora and SCFA concentrations were compared between patients who did (*n* 10) and did not (*n* 10) experience diarrhoea, and those who did (*n* 4) and did not (*n* 16) experience CDAD.

	No diarrhoea (*n* 10)		Diarrhoea (*n* 10)			No CDAD (*n* 16)		CDAD (*n* 4)		
	Mean	SD	Mean	SD	*P* value	Mean	SD	Mean	SD	*P* value
Total bacteria (log_{10}/g dry faeces)	10.59	0.37	10.37	0.36	0.208	10.44	0.40	10.63	0.14	0.137
Bifidobacteria	9.4	1.16	8.64	0.42	0.078	9.21	0.94	8.25	0.35	0.005
Clostridia	9.73	0.43	10.12	0.26	0.029	9.91	0.44	10.00	0.25	0.595
Total SCFA (μmol/g dry faeces)	265	162	599	304	0.009	323	180	867	254	0.018
Acetate	159	97	399	191	0.003	210	126	557	160	0.016
Propionate	43	33	100	82	0.060	47	31	169	90	0.071
Butyrate	32	22	54	33	0.103	34	24	79	25	0.027

Patients who experienced diarrhoea had higher concentrations of faecal clostridia and a trend towards lower bifidobacteria, whilst those with CDAD had lower bifidobacteria. The higher concentrations of total SCFA in patients with diarrhoea or CDAD are probably due to a reduction in absorption rather than an increase in production.

Whether the differences in microflora are directly involved in the pathogenesis of diarrhoea and CDAD during ETF, or merely indicative of antibiotic prescription, is unclear. Studies investigating whether correcting alterations in the microflora will reduce the incidence of these complications are justified.

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Changes in dietary patterns over a 5-year period in perimenopausal and early postmenopausal Scottish women. By H.M. MACDONALD[1,2], S.A. NEW[3] and D.M. REID[1,2], [1]*Osteoporosis Research Unit, University of Aberdeen, Woolmanhill Hospital, Aberdeen, UK, AB25 1LD,* [2]*Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, UK, AB25 2ZD and* [3]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH*

The National Diet and Nutritional Survey (NDNS) provides valuable information on trends in dietary habits of the UK population from its series of cross-sectional surveys (Henderson *et al.* 2003). However, there are few truly longitudinal studies that have examined dietary change in specific population groups. The aim of the present study was to examine the change in both types and quantities of key foods consumed over a 5-year period in a cohort of perimenopausal and early postmenopausal women from North-East Scotland.

The subjects were a subset of 1064 women from the Aberdeen Prospective Osteoporosis Screening Study who completed a validated food-frequency questionnaire (FFQ) following the baseline visit (v1) between 1990 and 1993, of whom 898 women (84.4 %) completed a second FFQ at the follow-up visit (v2) between 1998 and 1999. The mean age of the women was 47.6 (SD 1.5, range 45–53) years at v1 and 53.9 (SD 1.5, range 50–59) years at v2. The amount of foods, representative of the plate model, that were eaten on each occasion was calculated from the FFQ and intakes compared by paired *t* test of the log-transformed variables. Since FFQ are particularly important for ranking individuals in epidemiological studies, the percentage of women who had changed ranking by more than one quartile (Q) was assessed and a measure of agreement between the ranking on both occasions was given by weighted kappa (K_w). K_w values between 0.61 and 0.80 indicate good agreement, 0.41–0.60 moderate agreement, 0.21–0.40 fair agreement, and <0.21 poor agreement (Landis & Koch, 1977).

The Table shows that mean intakes of red meat, processed meat, bread and potatoes had decreased whereas intakes of poultry, cereals, rice and pasta, and fruit had increased. The number of portions of fruit had increased from 2.5 to 2.8/d. Vegetable intake had not changed significantly (1.9 portions/d at each visit). In terms of ranking of individuals, there was moderate agreement between the visits for most foods with the exception of bread and potatoes for which there was fair agreement. Poor agreement was observed for poultry, with 31 % of women ranked differently by more than 1 Q at visit 2 compared with visit 1.

					Difference between visits*					Ranking	
	v1 (g/week)		v2 (g/week)		v1–v2 (g/week)*					% Moved	
Food	Mean	SD	Mean	SD	Mean	SD	95% CI	Correlation	% Change	>1 Q	K_w
n	898		898								
Red meat	361	248	295	202	−67†	190	−80, −54	0.53†	−20.8	15.5	0.42
Processed meat	116	126	97	127	−24†	86	−31, −17	0.50†	−19.1	13.5	0.44
Poultry	169	161	261	165	+82†	169	+70, +94	0.02	+55.3	30.8	0.03
Cereals	164	137	192	137	+31†	137	+21, +41	0.48†	+20.0	16.3	0.41
Bread	586	421	520	357	−49†	350	−72, −25	0.44†	−10.4	20.5	0.32
Rice and pasta	362	293	415	299	+4†	230	+26, +58	0.50†	+12.8	15.7	0.41
Potatoes	604	307	547	237	−49†	283	−68, −30	0.46†	−9.1	17.6	0.36
Vegetables	1326	638	1339	602	+16	533	−19, +51	0.58†	+1.4	14.1	0.44
Fruit	1391	903	1579	928	+200†	859	+143, +257	0.52†	+17.5	14.5	0.42

* Paired *t* test after natural log-transformation. Anti-logged results are shown.
† $P<0.001$.

The increases in poultry, fruit and cereal consumption were consistent with the findings of NDNS, although overall fruit and vegetable intake (4.4 portions/d) was higher than the 2.9 portions consumed by women in the NDNS. In contrast to NDNS we found that red meat consumption had decreased in this age group of women. Further work is required to establish how these changes affect overall nutrient intake.

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Health-related characteristics and behaviours of dietary supplement users at age 53 years in the 1946 British birth cohort. By S.J. DOHERTY[1], S.A. McNAUGHTON[1], A.A. PAUL[1] and M.E.J. WADSWORTH[2], [1]*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL and* [2]*MRC National Survey of Health and Development, University College and Royal Free Medical School, 1–19 Torrington Place, London, UK, WC1E 6BT*

Use of dietary supplements may be associated with a range of healthy behaviours and those taking supplements may be the least likely to need them (Kirk *et al.* 1999). The objective of the present study was to investigate the health-related characteristics and behaviours of individuals using dietary supplements. The MRC National Survey of Health and Development (1946 birth cohort) is a longitudinal study of a social class-stratified sample of 5362 singleton births occurring in England, Scotland or Wales during the first week of March 1946. At age 53 years, 1776 subjects completed 5 d food diaries, which included questions on daily supplement use including the type, brand and dose of the supplement (Tumilty *et al.* 2001). Food diaries were coded using an in-house dietary coding program, combined with a newly created database of over 800 dietary supplements. Subjects provided information on alcohol consumption, exercise and smoking behaviours, a physical examination was conducted including anthropometric and blood pressure measurements and a blood sample was collected (Wadsworth *et al.* 2003). Statistical analysis was conducted using SPSS version 11.

		Men (*n* 827)					Women (*n* 949)				
		Users* (*n* 211)		Non-users (*n* 616)			Users* (*n* 433)		Non-users (*n* 516)		
		Mean	SD	Mean	SD	*P* value†	Mean	SD	Mean	SD	*P* value†
Smoking status (%)	Never	38		38		0.05	49		54		0.007
	Past	50		42			35		26		
	Current	13		20			16		20		
Participates in vigorous leisure activities (%)	Yes	64		52		0.001	60		47		<0.001
	No	36		49			40		53		
Alcohol consumption in the past year (%)	None	5		5		0.3	7		8		0.08
	Special occasions	10		14			23		29		
	More frequently	85		81			70		63		
Waist circumference (mm)		964	114	970	103	0.6	825	110	858	133	<0.001
BMI (kg/m^2)		27.0	4.1	27.1	3.7	0.9	26.2	4.7	27.5	5.7	<0.001
Total cholesterol (mmol/l)		6.0	1.0	6.0	1.1	0.7	6.0	1.1	6.2	1.2	0.04
Blood pressure (mmHg)	Systolic	139.9	19.8	140.0	19.7	0.9	132.3	18.2	133.1	19.3	0.5
	Diastolic	86.2	11.0	87.5	12.0	0.2	81.7	10.9	82.4	10.6	0.3

*Supplement users were defined as those subjects who reported taking at least one supplement during the 5 d food diary.
†Comparing supplement users and non-users; χ^2 for proportions and independent samples *t* test for means.

More women than men reported supplement use (45.6 *v.* 25.5%; $P<0.001$). Supplement use was significantly associated with smoking and exercise behaviour. Among women, non-supplement users had higher BMI and waist circumference than users and a significantly greater proportion were obese (25.4 *v.* 17.8%; $P<0.023$). Women supplement users had lower mean total cholesterol levels than non-users, but there were no differences in men. There were no significant differences between supplement users and non-supplement users for any of the health status indicators in men. These results confirm that supplement users, particularly women, tend to be different with respect to a number of health-related behaviours and characteristics and support the notion of a clustering of healthy behaviours (Schuit *et al.* 2002). An understanding of the characteristics of supplement users may help to inform public health programmes.

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Patterns of vegetable and fruit consumption in compliers and non-compliers with dietary recommendations. By M.M. O' BRIEN, M.A. GALVIN, M. KIELY and A.F. FLYNN, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Intakes of vegetables and fruit in the Irish adult population are low (mean intake 276 g/d in 18–64-year-old adults; O'Brien *et al.* 2003). The present study investigated patterns of vegetable and fruit intake in order to identify possible strategies to increase compliance with dietary recommendations.

Dietary intake data from the North/South Ireland Food Consumption Survey (Irish Universities Nutrition Alliance, 2001) was used to identify patterns of vegetable and fruit consumption in compliers and non-compliers with the population goal for vegetable and fruit intake of ≥400 g/d (World Health Organization/Food and Agriculture Organization, 2003). Food intake was estimated, using a 7 d food diary, in a randomly selected representative sample of 18–64-year-old adults (*n* 1379; 662 men and 717 women). In order to identify a subgroup of the population who were compliers with the population goal of ≥400 g/d an approach described by Wearne & Day (1999) was used. Mean daily intake of vegetables and fruit (g) in all individuals was ranked from highest to lowest. Starting from the highest intake, successive individuals were added until the addition of one more individual caused the average intake of the group to fall below 400 g. The subgroup (52%, *n* 718; 334 men, 374 women) was referred to as 'compliers' and the remaining individuals were 'non-compliers'. Analysis was carried out separately for men and women in the compliers and non-compliers group.

	Men (*n* 662)						Women (*n* 717)					
	Compliers (*n* 318)			Non-compliers (*n* 344)			Compliers (*n* 343)			Non-compliers (*n* 374)		
Vegetable or fruit group	% Con	Freq/ week	Mean per freq (g)	% Con	Freq/ week	Mean per freq (g)	% Con	Freq/ week	Mean per freq (g)	% Con	Freq/ week	Mean per freq (g)
Fruit juice	60	2.5	214	21	0.4	148	64	2.5	188	30	0.6	179
Bananas	64	2.2	189	22	0.4	161	72	2.1	160	36	0.6	142
Citrus fruit	51	1.6	210	15	0.2	130	63	1.8	189	17	0.2	117
Apples	80	2.8	139	42	0.8	97	82	2.9	136	48	1.0	97
Other fruit	99	2.3	119	96	0.6	94	100	2.7	103	98	0.8	66
Total fruit	99	9.1	232	75	2.1	144	100	10.0	195	83	3.0	129
Peas, beans and lentils	87	2.4	73	83	2.1	80	80	1.9	53	76	1.8	56
Carrots	88	2.5	89	73	1.6	67	88	2.4	70	80	1.7	62
Salad vegetables	64	1.7	28	41	0.8	22	77	2.2	30	57	1.3	21
Mushrooms	56	1.1	52	40	0.6	42	57	1.1	41	44	0.7	35
Onions	91	3.4	42	84	2.3	40	92	3.2	36	85	2.3	32
Peppers	44	0.9	49	26	0.4	38	54	1.1	41	32	0.5	33
Green vegetables	77	1.8	96	57	1.1	77	80	1.8	80	63	1.1	69
Tomatoes	89	3.4	83	71	2.0	68	96	4.2	76	82	2.2	62
Other vegetables	99	6.2	55	96	4.2	50	100	6.3	53	98	4.6	46
Total vegetables	100	10.3	181	99	7.8	131	100	10.3	152	100	8.0	112

% Con, percentage consumers of each group.

There were large differences between compliers and non-compliers in patterns of vegetable and fruit consumption and differences were more marked for fruit than for vegetables. Compliance with the population goal of ≥400 g/d is low in Irish adults. Strategies for increasing compliance with this goal should include increasing the number of consumers, frequency of consumption and amount per eating occasion for all fruit categories and selected vegetable categories.

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Fruit intake of hospital staff: how does it compare with national data? By V. REID[1], N. MAGOCHE[2], P. FITZPATRIC[2], C. MURRIN[2], D. COMERFORD[1] and C. KELLEHER[1,2], [1]*Department of Preventive Medicine and Health Promotion, St Vincent's University Hospital, Dublin 4, Republic of Ireland and* [2]*Department of Public Health Medicine and Epidemiology, University College, Dublin 2, Republic of Ireland*

Current Irish guidelines recommend an intake of four or more portions of fruit and vegetables per day. In other countries such as the UK and US the recommended intake ranges from five to nine portions. The health promotion unit at the Department of Health and Children runs an annual public awareness-raising campaign on healthy eating. In 2003 this involved a Focus on Fruit week in June and a Focus on Vegetables week in September. During Focus on Fruit week we conducted a quiz among hospital staff, the aim of which was to raise awareness in the hospital of the Focus on Fruit campaign and to encourage hospital staff to eat more fruit. Ease of completion facilitated participation.

The questionnaire included general questions on fruit, frequency of eating fruit and types of fruit eaten, in addition to basic demographic information derived from the SLAN instrument. Data from the National Health and Lifestyles Survey (SLAN, 2003) were used for comparison with the general population.

Approximately 900 copies of the quiz were distributed to staff over lunchtime on 1 d during Focus on Fruit week via a stand in the main concourse of the hospital and in the restaurant. Collection boxes were placed at the stand, in the restaurant and at the porter's desk. Two fruit hampers were offered as prizes. Literature from the health promotion unit ('Fast fruit: the tasty way to snack') was available at the stand and in the restaurant. Catering staff provided a daily fruit display in the restaurant and additional fruit choices were highlighted on service counters.

There was a high level of interest in the quiz and 547 (60%) entries were returned. The majority (82%) of respondents were females and 86% were in the younger age groups (<55 years), representative of the hospital staff profile. Of individuals, 90% knew that they should eat fruit every day and 98% ate one or more portions of fruit per d. The average fruit intake was two to three portions per d, with women eating more than men. Most individuals ate more than one type of fruit; whole fruit and fruit juice were the most popular. Younger individuals had more whole fruit and juice whereas the >55 years age group had more dried and cooked fruit. Compared with national data, age- and sex-matched respondents in hospital staff ate twice as much fruit as the general population. The most popular fruits were the same for both groups; banana, apple and orange, in that order. Strawberries were in season when the quiz was undertaken and were popular with females.

Types of fruit eaten		Portions eaten per d	Hospital staff	SLAN
Whole fruit	88%	<2	22%	61%
Fruit juice	64%	2 to 3	52%	26%
Fruit salad	30%	>3	26%	13%
Dried fruit	20%			
Cooked fruit	16%			
Fruit smoothie	12%			

Since whole fruit and fruit juice were the most popular types of fruit eaten by hospital staff, these should be promoted in future public health campaigns. The increasing availability of fruit salads and smoothies in food outlets provides a convenient and easy-to-eat form of fruit and fruit juice, which should appeal particularly to younger individuals.

The quiz proved to be popular with staff and should help raise awareness of fruit as a contributor to health. A follow-up article was published in the hospital newsletter, Healthwise, which gave the results of the quiz; the health benefits of an increased fruit intake were emphasised. There is research evidence that worksite food services have potential to increase fruit and vegetable consumption (Lassen *et al*. 2004). This type of activity at local level can support a national health promotion campaign.

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Knowledge of food safety practices amongst older adults. By P.J. MOYNIHAN[1], C.E. WOOD[1], P.N. HINDMARCH[1], A.J. ADAMSON[2], C. SEAL[2] and J.C. MATHERS[2], [1]*School of Dental Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK, NE2 4BW and* [2]*Human Nutrition Research Centre, Wellcome Research Laboratories, RVI, Queen Victoria Road, Newcastle upon Tyne, UK, NE1 4LP*

Individuals aged 65 years and over are more susceptible to morbidity and mortality from food-borne-induced gastroenteritis than younger individuals (Smith, 1998). Whilst this group of the population is concerned about food poisoning, individuals may be putting themselves at greater risk due to poor food storage practices (Johnson *et al*. 1998; Lilley, 2000).

As part of a larger nutrition intervention study, the aim of the present research was to assess knowledge and attitudes towards food safety of 288 older adults living in sheltered accommodation from socially deprived areas of north-east England. Subjects were asked to complete a questionnaire which included questions similar to those used in previous surveys (for example, Lader, 1999; Food Standards Agency, 2001) and modified to the study group. The questionnaire included items relating to knowledge of correct food storage, knowledge of food safety practices and attitudes towards manufacturers' storage and cooking instructions.

The final analysis included 163 completed questionnaires (57% completion rate), collected from twenty-seven males (16.6%) and 136 females, with a mean age of 76.48 (range 61–90) years. For each question, the percentage of the sample group answering correctly is shown in the Table.

Question	Correct answer	Percentage correct
What temperature should your fridge be?	Between 1–5 °C	22
Which shelf in the fridge should pork chops be put on?	The bottom shelf	47
If the 'use by' date on a food product passed yesterday what do you think you should do?	The food product could be unsafe to eat and should be thrown away	33
If the 'best before' date on a food product passed yesterday what do you think you should do?	The food product is past its best but not necessarily unsafe to eat	25
How long is it safe to keep a sandwich out of the fridge?	2 h	40
How should pork chops be defrosted?	In a container in the fridge	49
How should a leftover meat pie be stored?	In a container in the fridge	93

One-fifth of the total sample (23%) stated they owned a fridge thermometer. Despite overall poor knowledge of food safety, a positive attitude towards the importance of correct storage and cooking was expressed by the majority of respondents. Of the respondents, 90% stated it was very important to follow food manufacturers' storage instructions; 87% stated it was very important to follow manufacturers' cooking instructions and 91% stated it was very important not to reheat food more than once.

The present findings confirm earlier findings of Lilley (2000) and suggest that independent-living older adults from socially deprived areas have poor knowledge of food safety and are therefore a group to whom food safety advice should be targeted.

The present study was funded by the Food Standards Agency contract N09015. The views expressed are the authors' own.

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Dietary practices of a Somali population in Liverpool. By S.M. SALAH[1], J.E.G. BUNN[2] and S.M. MAXWELL[3], [1]*Faculty of Health and Applied Social Sciences, Liverpool John Moores University, Tithebarn Street, Liverpool, UK, L2 3ER,* [2]*Department of Tropical Child Health, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, UK, L3 5QA and* [3]*Faculty of Education, Community and Leisure, Liverpool John Moores University, Barkhill Road, Liverpool, UK, L17 6BD*

Rickets and osteomalacia are caused by a deficiency of vitamin D or Ca in the diet, or by a lack of sunlight. Reports indicate that this disease is common in ethnic minority groups from Asia and Africa living in the UK (Swan & Cook, 1971; Ford *et al.* 1972; Datta *et al.* 2002). There are over 2000 Somalis living in Liverpool and substantial populations of other ethnic minority groups. Strict purdah in young women from this community may put them at risk through reduced exposure to sunlight. In 2003 we surveyed 307 Somalis in Liverpool and found vitamin D deficiency in 82% and high blood parathyroid hormone levels in 34.1%, indicating osteomalacia or rickets, and low Ca excretion in 73%. Diet in ethnic minority groups is resistant to change and remains culturally identifiable for many generations. The aim of the present study was to identify the frequency of consumption of food items, especially those containing substantial quantities of Ca and vitamin D. The dietary intake of seventy Somali volunteers (eighteen males, fifty-two females), aged 6–81 years, was assessed using a food-frequency questionnaire.

Food items considered to be good sources of Ca and vitamin D were consumed infrequently. Of all the food items under consideration, 46% of respondents or more consumed these items less than once per week with the exception of bread, rice, ghee and milk. Of those who drank fresh milk, 92% stated that they consumed this item only in tea, less than half a pint a day.

	Proportion of respondents (%)										
Food item	Every day	3–6 times per week	1–2 times per week	Less than once per week	Never	Food item	Every day	3–6 times per week	1–2 times per week	Less than once per week	Never
Milk	42.9	22.9	8.6	10.0	15.7	Oranges	21.4	7.2	11.4	4.3	55.7
Cheese	7.1	14.3	15.7	12.9	35.5	Beans	0	23.0	27.1	18.6	31.4
Yoghurt	5.7	24.4	14.3	17.1	37.1	Bread	58.6	28.6	8.6	1.4	2.8
Cream	0	8.6	5.8	12.9	72.8	Chocolate	5.7	8.6	8.6	11.4	58.6
Eggs	1.4	14.3	18.6	20.0	44.3	Ice cream	3.0	12.9	5.8	22.9	52.9
Margarine	40.0	10.1	2.9	14.3	35.7	Rice	5.8	80.0	7.1	2.9	4.3
Butter	35.7	14.3	1.5	15.7	30.0	Green leafy vegetables	8.6	7.3	5.7	4.3	74.1
Ghee	12.1	24.2	21.2	14.3	18.2						

A significant number of subjects stated that they did not like certain foods containing Ca such as milk (11.4%), cheese (35.7%), green vegetables (8.6%), and yoghurt (8.6%). Bony fish was not eaten by any subject. Only one subject was taking Ca and three multivitamin supplements. Subjects aged under 30 years more frequently consumed cheese, yoghurt, cream, eggs, chocolate and ice cream than those aged 30 years and over ($P<0.05$). No differences were found in the frequency of consumption of any food item between men and women, although only a few men were studied.

The dietary practices of these individuals with regard to bone health is a cause for concern and indicates Ca and vitamin D intake to be low, contributed in part by a low intake of fortified foods or use of supplements. In women lack of sunlight may also contribute. Community Ca and vitamin D supplementation has been initiated to address this problem in the Somali community, and its effect will be assessed.

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Dietary intake and sources of phylloquinone (vitamin K_1) according to social class of British adults surveyed in 1986–7 and 2000–1. By C.W. THANE and W.A. COWARD, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL*

National diet and nutrition surveys of British adults have reported clear differences, by social class, in the intake of several nutrients (Gregory *et al.* 1990; Finch *et al.* 1998). Similar differences have not been reported for phylloquinone (vitamin K_1) owing to a previous lack of reliable content values for a comprehensive range of foods. Using newly available data, such a comparison can now be made in tandem with a scrutiny of changes in the intake and food sources of vitamin K_1 over a 14-year period.

Dietary vitamin K_1 intake was estimated in two nationally representative samples of British adults aged (a) 16–64 years from the 1986–7 Dietary and Nutritional Survey of British Adults (Gregory *et al.* 1990) and (b) 19–64 years from the 2000–1 National Diet and Nutrition Survey (Henderson *et al.* 2002). After excluding those who reported being unwell with eating habits affected, 7 d weighed dietary records were used to estimate dietary vitamin K_1 intake, and the relative contributions of different food groups. Vitamin K_1 content values (Food Standards Agency, 2002; C Bolton-Smith and M Shearer, unpublished results) were assigned for all foods consumed. Intake and food sources were examined by age, sex, region, season, occupational social class and cigarette smoking habit. Differences between the two surveys are reported here by occupational social class, with adjustment for the other factors.

Whether expressed in terms of μg/d or μg/kg body weight per d, vitamin K_1 intakes were significantly lower in participants with manual *v.* non-manual occupational backgrounds at both time points, with the greatest reduction in intake from 1986–7 to 2000–1 observed in those of manual social class. Food sources also changed from 1986–7 to 2000–1, when there were latterly lower contributions from vegetables (particularly cooked leafy green vegetables; LGV) accompanied by higher contributions from potatoes and potato products, especially among those of manual social class.

Occupational social class …	Non-manual				Manual			
Year of national survey …	1986–7 (*n* 1043–1052)		2000–1 (*n* 877–921)		1986–7 (*n* 876–884)		2000–1 (*n* 552–583)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
K_1 intake (μg/d)†	76	73, 78	73	70, 75	68*	65, 71	58	55, 61
K_1 intake from vegetables (%)‡	64*	63, 65	62	61, 63	62*	61, 63	56	55, 58
of which: cooked LGV (%)‡	25*	24, 27	20	19, 22	25*	24, 27	17	15, 19
K_1 intake (μg/kg body weight per d)	1.11*	1.07, 1.15	0.98	0.95, 1.02	0.96*	0.92, 1.00	0.78	0.74, 0.82
K_1 intake <1 μg/kg body weight per d (%)	43*		51		53*		71	

Mean values were significantly different between surveys for non-manual and manual occupational social class (*$P<0.001$, otherwise $P>0.01$; ANOVA for continuous variables, multiple logistic regression for discontinuous proportions, with each model adjusted for other socio-demographic and lifestyle factors).
† Geometric means obtained from antilog of $\log_e$-transformed data; ‡ arithmetic means.

The greater differences in vitamin K_1 intake and food sources (expressed as percentage change from 1986–7 values) with manual occupation applied to both men and women, and were not altered significantly after excluding likely under-reporters (energy intake:estimated BMR <1.1).

In contrast with recommendations to consume more fruit and vegetables for various health benefits, and with emerging evidence suggesting that low vitamin K intake may lead to poor vitamin K status and contribute to osteoporosis and increased risk of fracture (Shearer, 2000), it is concerning that from 1986–7 to 2000–1 these data indicate a decline in vitamin K_1 intake and contribution from vegetables, particularly among those of manual social class.

This work was funded by the Food Standards Agency. We also thank Drs Caroline Bolton-Smith and Martin Shearer for allowing us to use the unpublished database of food vitamin K_1 contents.

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The effect of cook–hot-hold and cook-chill processing on ascorbic acid levels in potatoes. By R. HALKYARD, E. COWIE and S. CUNNINGHAM, *The Robert Gordon University, St Andrew Street, Aberdeen, UK, AB25 1HG*

There is currently concern about the amount of water-soluble vitamins lost in hospital food due to industrial processing methods. It is estimated that 25% of ascorbic acid is lost during the cooking of potatoes by boiling, and this loss is likely to be compounded still further in a hospital catering system, due to the need for bulk preparation and distribution of meals over considerable distances (Williams, 1996). The two most common meal service and distribution systems used in hospitals are the cook–hot-hold and the cook–chill systems. Ascorbic acid losses are known to occur in both systems, but surprisingly little research has compared the potential nutritional consequences of applying these systems. So, a comparative investigation was undertaken to examine the effects of these processing techniques on the ascorbic acid content of potatoes.

Unpeeled Maris Piper potatoes (sample weights 540–660 g) were washed and cooked by simmering in water for 20 min. After cooking, a 50 g sample of potato, minus the skin, was removed for ascorbic acid determination. In the hot-hold experiments, the remaining portion of the potato was placed in a pre-heated ovenproof dish and stored in the oven at 100 °C. Samples of potato (50 g) were then removed at 60 and 180 min intervals. In the cook–chill experiments, the remaining portion of potato was divided into 150 g portions and placed in a chiller. Once chilled to below 5 °C, the potato samples were stored in a refrigerator for 48 and 96 h, respectively. On removal from refrigeration, the samples were re-heated in an oven at 200 °C for 20 min, before a 50 g sample was removed for ascorbic acid analysis. Ascorbic acid in potato samples was preserved by mixing samples with meta-phosphoric acid and *n*-octanol, which were then stored at –18 °C until required for further analysis. Before analysis, samples were allowed to defrost at room temperature in the absence of light. Ascorbic acid was determined using a commercial enzymic kit (R-Biopharm GmbH, Germany) and colorimetry. All assays were performed in duplicate (four in each sample group) and a Student's *t* test was used to compare test samples with the respective freshly cooked samples. The significance level was taken as $P<0.05$.

	Ascorbic acid (mg/100 g)		
	Mean	SD	*P* value
Freshly cooked (hot-hold)	7.86	2.34	–
1 h hot-hold	4.46	1.00	0.043
3 h hot-hold	2.85	1.04	0.013
Freshly cooked (cook–chill)	7.74	0.31	–
48 h chill	2.54	0.99	0.002
96 h chill	0.41	0.40	0.001

Potato samples that had been hot-held for 1 and 3 h contained 43 and 64% less ascorbic acid than freshly boiled samples, respectively. In the chilled storage experiments, potato samples that had been stored below 5 °C for 48 and 96 h contained 67 and 94% less ascorbic acid, respectively, than samples of freshly boiled potatoes. Thus, chilled storage over a period of 3 d, followed by re-heating of potato samples resulted in an approximately 30% greater loss of ascorbic acid than hot-holding potato samples for 3 h.

In conclusion, these results indicate that hot-holding is preferable to cook–chill methods for the preservation of ascorbic acid levels in potatoes. It remains to be elucidated whether these results are indicative of a general trend in the preservation of water-soluble vitamins in both hospital food and the ready-meal industry.

The technical assistance of Ms Heather Scott is gratefully acknowledged.

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Turmeric extract may reduce symptoms of irritable bowel syndrome and improve quality of life in otherwise healthy adults: a pilot study. By R. BUNDY[1], A.F. WALKER[1], R.W. MIDDLETON[2] and J. BOOTH[3], [1]*Hugh Sinclair Unit of Human Nutrition, The University of Reading, Reading, UK, RG6 6AP,* [2]*Lichtwer Pharma (UK) Ltd, Dedmere Road, Marlow, UK, SL7 1FJ and* [3]*Gastroenterology Department, Royal Berkshire and Battle Hospitals NHS Trust, London Road, Reading, UK, RG1 5AN*

Irritable bowel syndrome (IBS) is a very common functional disorder characterised by symptoms including abdominal pain, altered bowel habit and changes in stool frequency (Camilleri, 2001). Evidence also exists that health-related quality of life (QoL) is significantly reduced in IBS patients compared with healthy controls (El-Serag *et al.* 2002). Hypotheses promulgated for the aetiology of the condition include infection, leading to changes in colonic microflora, food sensitivities, antibiotic use, and genetic factors (Maxwell *et al.* 1997). It has also been suggested that many of these factors may promote and perpetuate an underlying low-grade inflammation of the gut mucosa (Barbara *et al.* 2002).

Although few nutrient deficiencies have been linked to the condition, phytochemicals in the form of herbal remedies may be more promising in terms of improving symptomology, for example peppermint oil (Pittler & Ernst, 1998). Turmeric, a strongly anti-inflammatory herb with anti-microbial and cholerectic properties, was investigated in the present study as a further means to reduce symptoms.

Five hundred otherwise healthy adults with self-reported IBS were screened using the ROME II criteria (in the simplified modular format) for IBS, and 207 suitable volunteers were randomised to receive either one or two tablets of standardised turmeric extract (equivalent to 1800 mg turmeric root per tablet) daily for 8 weeks in a partially blinded postal study. Volunteers were 72 and 71% female, with 35% over 60 years of age, and with an average BMI of 25.5 and 25.8 in the one- and two-tablet groups respectively. IBS symptomology and health-related QoL were assessed at baseline and after treatment using validated questionnaires (the ROME II criteria in the more comprehensive integrative format plus the IBS QoL questionnaire).

Unexpectedly, IBS prevalence fell significantly in both groups between screening and baseline (41 and 57% in the one- and two-tablet groups respectively; $P<0.001$), possibly due to differences in the format of the ROME II criteria questionnaires used, and because screening was completed just before Christmas, an often stressful time which may have exacerbated symptoms. There were significant improvements in IBS QoL scales of between 5 and 35% in both groups ($P=0.015$ to $P<0.001$) and approximately two-thirds of all subjects self-reported a definite or some improvement in IBS symptoms after treatment. There were no significant differences between the groups. Due to the unexpected decrease in statistical power to detect differences between groups, changes in abdominal pain and discomfort frequency were measured *post hoc* in the complete randomised data set. This showed reductions of 22% in the one-tablet group and 25% in the two-tablet group, the difference tending towards significance ($P=0.071$).

The present pilot study hints that turmeric extract shows promise for reducing IBS symptomology and improving health-related QoL in otherwise healthy adults. More rigorous trials are now needed to confirm these findings.

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The effect of physiologically relevant concentrations of dietary anthocyanins on their ability to prevent low-density lipoprotein oxidation in the presence and absence of serum albumin. By J.E. BROWN, *Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Stag Hill, Guildford, Surrey, UK, GU2 7XH*

The distinctive cardioprotective properties of red wine and red-coloured fruits, observed in epidemiological (Frankel *et al.* 1993) and experimental studies, are considered in part to reside in the phenolic and anthocyanin content of these foods. Anthocyanins have been shown to possess a variety of biological effects including their ability to act as antioxidants and to protect against peroxynitrite damage (Serraino *et al.* 2003). Recent evidence has shown that anthocyanins are absorbed intact (as glucosides) and are present in the systemic circulation after the consumption of anthocyanin-rich foods (Cao & Prior, 1999; Mazza *et al.* 2002). Typical concentrations are generally low (about 100 nM) but their role in the vasculature may still be of importance. The present study examined the efficacy with which low concentrations (25–500 nM) of cyanidin-3-O-glucoside and malvidin-3-O-glucoside delayed the oxidation of LDL. The present study also investigated the effectiveness of the two anthocyanins in terms of their ability to delay LDL oxidation in the presence of serum albumin.

	Lag phase (min)							
	Control		25 nM		100 nM		500 nM	
Anthocyanin	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cyanidin-3-O-glucoside	92.0	15.3	92.8	15.1	97.2*	15.5	131.3*	19.6
Normalised to control (100)	100		101		105.7		142.7	
Cyanidin-3-O-glucoside with albumin	101.4	11.1	106.9	6.3	ND		129.7	16.5
Normalised to control (100)	100		105.4				127.9	
Malvidin-3-O-glucoside	83.5	18.8	81.0	15.6	80.5*	17.1	85.1	17.8
Normalised to control (100)	100		97.0		96.4		101.9	
Malvidin-3-O-glucoside with albumin	100.3	5.6	126.3	15.3	ND		141.9†	14.7
Normalised to control (100)	100		125.9				141.5	

ND, not determined.
*Significant *v.* control ($P<0.05$).
†Trend observed ($P<0.07$).

A clear dose-response relationship was observed in terms of the lag-phase extension with increasing concentrations of cyanidin-3-O-glucoside. This pattern was not demonstrated for malvidin-3-O-glucoside which appeared to be pro-oxidant at 100 nM but was not different from control at 500 nM. Albumin (0.1 mg/ml) increased the lag phase to oxidation as expected ($P<0.08$; data not shown). The interaction between cyanidin-3-O-glucoside and albumin resulted in a pattern not dissimilar to that observed in the absence of albumin. However, for malvidin-3-O-glucoside the addition of albumin enhanced this anthocyanin's ability to extend the lag phase to oxidation ($P<0.07$). These properties and the differential interaction between anthocyanins and serum albumin may be important in terms of their beneficial effects *in vivo*.

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Immunomodulatory effects of β-sitosterol and β-sitosterol oxides. By Y.C. O'CALLAGHAN and N.M. O'BRIEN, *Department of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*

Phytosterols are plant components with a structure similar to cholesterol. Due to the unsaturated nature of the phytosterol molecule, it is subject to oxidation. Unlike cholesterol oxides, the biological effects of phytosterol oxides have not as yet been fully determined. Preliminary studies have indicated that phytosterols may enhance the immune response but also possess anti-inflammatory properties as they promote a balance between the various cells and cytokines of the immune system (Bouic, 2001). Cholesterol oxides have previously been shown to possess potent immunosuppressive effects. The aim of the present study was to investigate the immunomodulatory effects of phytosterols, stigmasterol and β-sitosterol, and a number of β-sitosterol oxides.

Jurkat cells, a human T-cell line, are a useful model for the determination of immunological effects and were employed for the present investigation. Cells were seeded at a density of 2×10^5 cells/ml in RPMI medium, supplemented with fetal bovine serum (100 ml/l). Control cells were treated with phorbol 12-myristate (PMA, 25 ng/ml) and ionomycin (100 ng/ml) to stimulate the production of IL-2. Cells were treated with stigmastanol (60 μM) or β-sitosterol (60 μM) in the presence of PMA and ionomycin. The cells were also treated with a range of β-sitosterol oxides including an uncharacterised mix of oxides (20 μM) or α-epoxide (20 μM) or an α,β-epoxide mix (20 μM). Cells were incubated (37 °C, 5% CO_2) for a period of 6 ds which was found to be optimal for the production of IL-2. IL-2 production was assessed by ELISA, cell viability was assessed by fluorescein diacetate–ethidium bromide staining and cell proliferation was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Blaszczyk *et al.* 2004).

	Control		Stigmastanol		β-Sitosterol		β-Sitosterol oxides		α-Epoxide		α,β-Epoxide	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
IL-2 (% control)	100	0	117*	4	109	3	76	8	88	8	49*	10
Viability (% control)	100	10	103	7	99	6	105	4	88	9	67*	13
MTT (% control)	100	2	102	9	77	8	89	8	103	5	54*	6

Values are means of three independent experiments, *$P<0.05$.

IL-2 promotes the proliferation, differentiation and cytolytic activity of cells involved in the immune response and is not involved in the inflammatory response. IL-2 production was enhanced in the presence of PMA and ionomycin (results not shown). β-Sitosterol, the uncharacterised β-sitosterol ooxides and the α-epoxide derivative of β-sitosterol did not alter IL-2 levels significantly. The production of IL-2 was enhanced following exposure to stigmastanol but was inhibited by the α,β-epoxide. Cell viability was not affected under the conditions of the present study except following exposure to α,β-epoxide, which significantly reduced cell viability relative to the control value. Cell proliferation was decreased in the presence of the α,β-epoxide derivative of β-sitosterol but was not altered following incubation with stigmastanol, β-sitosterol, the uncharacterised β-sitosterol oxide mix or the α-epoxide. The findings of the present study indicate that stigmastanol may enhance the immune response while certain products of β-sitosterol oxidation may have immunosuppressive effects.

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Genetic taste sensitivity to *n*-propylthiouracil (PROP) is associated with dietary fat intake but not energy density. By D. MISKIN[1] and I. DAVIDSON[2], [1]*Food Industry Foundation and* [2]*Dietetics, Nutrition and Biological Sciences, Queen Margaret University College, Edinburgh, UK, EH12 8TS*

An inverse relationship between an individual's taste sensitivity to the bitter tasting compound *n*-6 propylthiouracil (PROP) and BMI has previously been demonstrated (Tepper & Ullrich, 2002). In addition, it has been reported that PROP-sensitive individuals have significantly lower preference ratings for high-fat foods than their PROP-insensitive counterparts (Davidson & Miskin, 2003) and which may impact on their food selection (Yackinous & Guinard, 2001). We have also previously shown PROP-sensitive individuals' dietary intakes to have a significantly higher percentage energy from carbohydrate (Savage & Davidson, 2000). The present study aimed to assess whether the genetically determined perceived intensity to PROP could predict change in preference ratings for foods with different fat contents and whether this was associated with the energy density of the diet.

Subjects aged between 18 and 60 years were recruited through email from a University College environment. All sensory procedures adopted standard protocols and were carried out in a sensory analysis laboratory. Three yoghurt mixtures containing no flavourings and with 0.2, 5.6 and 11% fat respectively were used. The subjects were asked to rate on a continuous nine-point hedonic scale their preference for the specific attributes: aroma, sweetness, creaminess, mouth feel, flavour and overall liking of the mixtures. Additionally, the subjects' perceived intensity of the bitter taste of a 3.2×10^{4} M-PROP solution was determined (as previously described Tepper *et al.* 2001) quantitatively using the magnitude of estimation scale. Dietary intakes were assessed using the 3 d non-weighed diary method and nutritionally analysed using Compeat-Pro for determination of macronutrient content. Energy density of the diet based on these diaries was also estimated.

Fifty-five subjects were recruited to the study (nine males and forty-six females; age range 18–59 years). Preference ratings for all three yoghurts were similar for aroma, sweetness and flavour. Significant differences were evident for preference ratings of the three yoghurts ($P<0.05$; ANOVA) for the attributes creaminess (lowest fat, 5.29±1.25; medium fat, 6.27±0.8; highest fat, 6.54±0.8; medians±interquartile ranges) and mouth feel (lowest fat, 5.39±1.2; medium fat, 6.27±0.8; highest fat, 6.21±1.25; medians±interquartile ranges). The difference in preference ratings between the highest-fat and lowest-fat yoghurts and also between the lowest-fat and medium-fat yoghurts predicted the PROP perceived intensity score ($P<0.05$; regression analysis). In addition, the perceived intensity of PROP was found to be significantly correlated with energy intake (r 0.33; $P<0.05$) and weight of fat ingested (r 0.30; $P<0.05$). No significant correlation was evident for energy density.

These findings suggest that increased PROP sensitivity is associated with an increased ability to discriminate between low- and higher-fat foods in terms of their rheological properties. In addition the data suggest that this increased discrimination favours preference for higher-fat foods. However, despite this, PROP-sensitive individuals tend to have a lower BMI (Tepper *et al.* 2001). An explanation for this may lie with a greater sensitivity of oro-sensory properties in these individuals which would initiate satiety mechanisms contributing to physiological regulation of energy intake.

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Effects of the dietary ratio of *n*-6:*n*-3 polyunsaturated fatty acids on clotting factor VII in the fasting and postprandial state: the 'OPTILIP' study. By T.A.B. SANDERS[1], F.J. LEWIS[1], S. SLAUGHTER[1], M.D. GRIFFIN[2], D.J. MILLWARD[2], J.A. COOPER[3] and G.J. MILLER[3], [1]*Nutrition, Food and Health Research Centre, King's College London, London, UK, SE1 9NN,* [2]*Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK, GU2 7XH and* [3]*Wolfson Institute of Preventive Medicine, Queen Mary College, London, UK, EC1M 6BQ*

Prospective epidemiological studies suggest that elevated factor VII coagulant (FVIIc) activity is associated with an increased risk of fatal IHD. FVIIc is associated with elevated plasma triacylglycerol (TAG) concentrations and it is known that *n*-3 fatty acids decrease plasma TAG concentrations and are associated with a decreased risk of fatal IHD. We investigated the hypothesis that a decreased dietary *n*-6:*n*-3 ratio as can be attained through modification of the typical UK diet would favourably alter FVII. Male and female subjects (aged 45–70 years) were recruited through general practices participating in the MRC General Practice Research Framework and through advertisement in King's College and its associated hospitals. The mean age of the subjects completing the subjects was 60 years. A parallel design was employed to test the effects of four diets with *n*-6:*n*-3 ratios of between 5:1 and 3:1 with a control diet with an *n*-6:*n*-3 ratio of 10:1. FVII zymogen (FVIIag) concentration, FVIIc activity and FVII activated (FVIIa) concentration were determined at the beginning and after 6 months of dietary intervention. FVIIa concentrations were also determined in response to a standardised test meal containing 50 g fat. Table 1 shows the results for fasting measurements of FVII. Changing the *n*-6:*n*-3 fatty acids ratio in the diet did not influence fasting FVIIag and FVIIa concentrations.

		Treatment effect (% change in geometric mean)					
		FVIIag		FVIIc		FVIIa	
Diet	*n*-6:*n*-3	Mean	95% CI	Mean	95% CI	Mean	95% CI
Control (*n* 40)	10:1	0.9	−5.7, 7.9	3.5	−2.5, 9.7	6.3	−7.5, 22.1
n-3 LCP (*n* 57)	3:1	−1.2	−6.6, 4.6	3.2	−1.2, 7.8	14.3	1.6, 28.8
Linolenate (*n* 46)	3:1	−2.9	−8.0, 2.4	0.3	−3.2, 3.9	5.4	−4.7, 16.6
n-3 LCP+linolenate (*n* 50)	3:1	3.0	−2.0, 8.2	4.6	−0.5, 9.8	4.6	−7.5, 18.4
Moderate linolenate (*n* 44)	5:1	0.1	−5.1, 5.7	4	−1.6, 9.9	−0.02	−12.2, 13.9
P (ANOVA)		0.65		0.77		0.60	

LCP, long-chain PUFA.

FVIIa 6 h following the test meal was substantially increased compared with the fasting value on all diets ($P<0.001$). Table 2 shows the increments at baseline and at follow-up. The increment at follow-up was not significant for any diet or overall ($P=0.32$) compared with that at baseline.

		Change in FVIIa from fasting 6 h following a 50 g fat test meal (%)					
		Baseline		Follow-up		% Change	
Diet	*n*-6:*n*-3	Mean	95%CI	Mean	95%CI	Mean	95%CI
Control (*n* 40)	10:1	40.2	26.3, 55.6	45.4	32.2, 59.8	3.5	−7.7, 14.6
n-3 LCP (*n* 57)	3:1	41.5	32.7, 50.9	48.0	37.3, 59.6	3.3	−5.6, 12.2
Linolenate (*n* 46)	3:1	51.0	38.5, 64.6	57.3	42.2, 74.2	2.5	−8.7, 13.7
n-3 LCP+linolenate (*n* 50)	3:1	50.4	40.0, 61.4	46.7	33.5, 61.3	−2.0	−11.4, 7.5
Moderate linolenate (*n* 44)	5:1	47.7	34.3, 62.4	51.1	38.4, 64.9	1.4	−10.0, 12.9

Fasting serum TAG concentrations were significantly lower in subjects who received the diets containing *n*-3 LCP compared with the subjects receiving the other diet (mean difference 0.118 (95% CI 0.032, 0.204) mmol/l; $P=0.007$). However, there was no evidence of any decrease in either fasting measures of FVII or in the postprandial increase in FVIIa. We conclude that changing the *n*-6:*n*-3 fatty acids ratio in the diet by using linolenic acid-rich vegetable oil and consuming oily fish does not influence FVII.

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Conjugated linoleic acid suppresses interleukin-12 and enhances interleukin-10 production in murine bone marrow-derived dendritic cells. By C.E. LOSCHER[1], K.H. MILLS[2] and H.M. ROCHE[1], [1]*Nutrigenomics Group, Department of Clinical Medicine, Institute of Molecular Medicine and* [2]*Department of Biochemistry, Trinity College Dublin, Dublin, Republic of Ireland*

Dietary fatty acids, in particular PUFA, have been shown to modulate cytokine production in a number of cell types. Specifically, linoleic acid decreases interferon γ production by IL-12- and IL18-stimulated intra-epithelial lymphocytes and conjugated linoleic acid (CLA) suppresses TNFα production in lipopolysaccharide (LPS)-stimulated RAW macrophages. However, to date no study has examined the effect of PUFA on dendritic cells (DC). Activation of DC by microbial stimuli, such as LPS, induces maturation and the subsequent production of cytokines such as IL-12, IL-4 and IL-10, which selectively differentiate precursor T helper cells into Th1, Th2 or Tr1 cells respectively. The pivotal role of IL-12 in Th1-driven autoimmune diseases suggests that this cytokine is a key therapeutic target. The present study examines the potential of CLA for modulation of DC function.

Using bone marrow-derived DC, the present study demonstrates that both the *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA isomers suppressed IL-12p70 and enhanced IL-10 production in response to LPS (see Table). Furthermore this modulation was evident at the level of transcription with a significant decrease in IL-12p70 mRNA and increase in IL-10 mRNA in these cells. The suppression of IL-12p70 mRNA and protein production by *cis*-9, *trans*-11 CLA but not *trans*-10, *cis*-12 CLA was partially mediated by IL-10 as the addition of an anti-IL-10 antibody to the culture resulted in recovery of IL-12p70 production. The Table shows cytokine production by bone marrow-derived DC 24 h after LPS stimulation.

	Dimethyl sulfoxide control		*cis*-9, *trans*-11 CLA		*cis*-9, *trans*-11 CLA+αIL-10		*trans*-10, *cis*-12 CLA		*trans*-10, *cis*-12 CLA+αIL-10	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
IL-12p70 (pg/ml)	80.81	5.64	33.72	2.47	59.25	1.81	10.88	1.28	3.37	0.72
IL-10 (pg/ml)	37.16	2.65	73.15	0.7	66.67	2.82	72.34	0.4	39.58	1.07

Given the importance of DC in directing T helper cell responses, it is possible that CLA may influence T cell responses, through their effects on DC function. Given the pivotal role of IL-12 in Th1-driven autoimmune diseases, such nutritional interventions that attenuate an excessive Th1-cell response by down regulating IL-12 and up regulating IL-10 production may play a role in the treatment of such diseases.

α-Tocopherol protects against D-galactosamine-induced hepatic injury. By M.C.Y. WONG[1], R. RAJENDRAM[1], R. DUFFY[1], B. PORTMANN[2], H. WISEMAN[1] and V.R. PREEDY[1], [1]*Nutritional Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9NN and* [2]*Institute of Liver Studies, King's College Hospital, Denmark Hill, London, UK, SE5 9RS*

D-Galactosamine (GALN) administration has previously been shown to induce oxidative stress and necrosis in rat models of hepatic injury (Sun *et al.* 2003). We hypothesised that the liver injury seen in GALN dosage can be ameliorated with α-tocopherol (ATC), a well-known antioxidant.

To test this hypothesis, male Wistar rats (0.1 kg body weight; BW) were ranked and assigned to four groups (six rats per group) as follows: group A, carrier+saline; group B, carrier+GALN; group C, ATC+saline; group D, ATC+GALN. The protocol involved a pre-treatment for 1 h followed by treatment for 23 h. The carrier was Intralipid (20% fat emulsion). ATC was dissolved in the carrier and injected at a dose of 30 mg/kg BW. GALN was dissolved in saline (0.9 mol NaCl/l) and injected at a dose of 1 g/kg BW. All rats were injected intraperitoneally. Rats were killed 24 h after the first injections and livers were dissected for subsequent assessment of hepatic abnormalities.

Paraffin sections (4 μm thick) were stained with haematoxylin and eosin and assessed using a histological scoring system. For micro-steatosis and lobular inflammation, samples were graded 0 (rarely seen) to 3 (widespread). For necrosis (acidophilic bodies), samples were graded 0 (absent) to 5 (confluent necrosis). The scores were ranked and analysed using 2×2-way ANOVA, followed by a *post hoc* least significant differences (LSD) test, where a P value <0.05 was considered to be significant (see Table).

	Histopathology scores*					
	Necrosis (acidophilic bodies)		Micro-steatosis		Lobular inflammation	
Treatment	Mean	SEM	Mean	SEM	Mean	SEM
Group A: carrier+saline (*n* 6)	0.00	0.00	0.00	0.00	0.00	0.00
Group B: carrier+GALN (*n* 6)	2.67	0.71	2.33	0.49	1.83	0.48
Group C: ATC+saline (*n* 6)	0.00	0.00	0.00	0.00	0.00	0.00
Group D: ATC+GALN (*n* 6)	0.83†	0.31	1.83	0.60	0.83†	0.31

* There was a significant effect ($P<0.001$) due to GALN for all three histopathological indices (2×2-way ANOVA).
† Mean value was significantly different from that for group B (LSD analysis; $P<0.05$).

The results of the analysis showed that GALN administration induced marked necrosis, steatosis and inflammation. However, the degree of necrosis and reactive inflammation were significantly less after ATC pre-treatment. This suggests that ATC administration may be beneficial in preventing liver injury.

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Absence of milk in the diet is associated with higher bone resorption in postmenopausal Saudi Arabian women. By S.O. KHOJA[1], J.A. KHAN[1], A-R.A. MAIMANI[2] and S.A. NEW[3], [1]*Biochemistry Department and* [2]*Medical School, King Abdul Aziz University PO Box 1540, Jeddah 21441, Kingdom of Saudi Arabia and* [3]*School of Biomedical and Life Sciences, University of Surrey, Guildford, UK, GU2 7XH*

Few data exist on the effect of dietary and lifestyle factors on indices of bone health in women living in Middle Eastern countries. The aim of the present study was to assess the effect of milk consumption on markers of bone turnover in Saudi Arabian women.

As part of our ongoing bone health study in 212 Saudi Arabian women (Khoja *et al.* 2002), a total of 100 premenopausal and 112 postmenopausal women living in the city of Jeddah were studied. They were aged 20–30 years and 45–60 years respectively, had not suffered from any known condition, and were not taking any medication likely to affect bone metabolism. Measurements were made on weight and height as well as spine, hip and calcaneal bone mass. Bone resorption was assessed by ELISA measurements of pyridinium crosslinks (pyridinoline; PYD) and deo-oxypyridinoline (DPD) using a second morning urine sample. Bone formation was assessed by radioimmunoassay of bone-specific alkaline phosphatase (BSAP) and osteocalcin (OC) using a fasted blood (serum) sample. All subjects were interviewed concerning their habitual dietary intake, physical activity levels and general lifestyle. Women were divided into three groups according to their level of milk consumption: non-milk group (NMG); low-milk group (LMG; <1 pint/d); high-milk group (HMG; >1 pint/d).

	Premenopausal women						Postmenopausal women					
	NMG (*n* 20)		LMG (*n* 70)		HMG (*n* 4)		NMG (*n* 29)		LMG (*n* 79)		HMG (*n* 4)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DPD (nmol/l)	10.4	4.1	10.6	4.4	9.2	5.5	12.0[a]	4.7	9.6[b]	3.1	9.4[b]	2.9
PYD (nmol/l)	37.8	8.2	39.2	8.1	29.8	7.1	40.8	15.6	36.9	12.2	39.4	17.3
BSAP (U/l)	18.9	5.8	17.5	5.5	17.7	5.6	21.5	8.1	20.0	7.4	24.6	8.7
OC (ng/ml)	13.5	5.3	12.5	3.6	11.4	3.0	9.5	3.7	10.1	3.6	8.9	1.0

[a,b]Mean values within a row are significantly different (ANOVA; $P<0.05$).

As shown in the Table, postmenopausal women in the NMG were found to have a significantly higher DPD excretion, indicative of increased bone resorption. No significant changes were seen in markers of bone formation (OC or BSAP). No effects of milk consumption were seen in the premenopausal group. These intriguing results indicate that an exclusion of milk in the diet may be an important risk factor for poor bone health in Saudi Arabian postmenopausal women. Further analysis of vitamin D status and markers of Ca metabolism are currently underway and may provide an insight into the mechanisms underlying this increased bone turnover with absence of milk in the diet.

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Khoja SO, Khan JA, Maimani AR & New SA (2002) *Proceedings of the Nutrition Society* **79**, 108A.

Moderate alcohol ingestion acutely inhibits bone resorption in a parathyroid hormone- and calcitonin-independent fashion. By S. SRIPANYAKORN[1,2], R. JUGDAOHSINGH[1], S. DAVIDSON[2], R.P.H. THOMPSON[1] and J.J. POWELL[1,3], [1]*Gastrointestinal Laboratory, The Rayne Institute, St Thomas' Hospital, London, UK, SE1 7EH,* [2]*Department of Nutrition and Dietetics, King's College London, 150 Stamford Street, London, UK, SE1 9NN and* [3]*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge, UK, CB1 9NL*

Although abusive alcohol consumption is associated with increased morbidity and reduced life expectancy, a moderate intake of alcohol appears beneficial for overall mortality and for a number of organs including bone (De Lorimier, 2000). Mechanisms are not clear although oestrogenic effects of alcohol have been described. Here we investigated the acute effects of ethanol ingestion on bone resorption by assessing changes in serum C-terminal telopeptide of type I collagen (CTx), intact parathyroid hormone (iPTH), and calcitonin in sixteen healthy volunteers. Ca was a positive control and ultra high purity (UHP) water was a negative control. Sixteen healthy, fasted volunteers (mean age, 27 years) were studied in two separate groups (4 females, four males/group). The first group ingested either 0.6 litres of 4.6% (v/v) ethanol (about three units) or an equivolume of UHP water on alternate weeks. The second group ingested 182 mg Ca in 0.6 litres UHP water. Blood samples were collected over a 6 h period and sera analysed for iPTH, CTx, and calcitonin using ELISA.

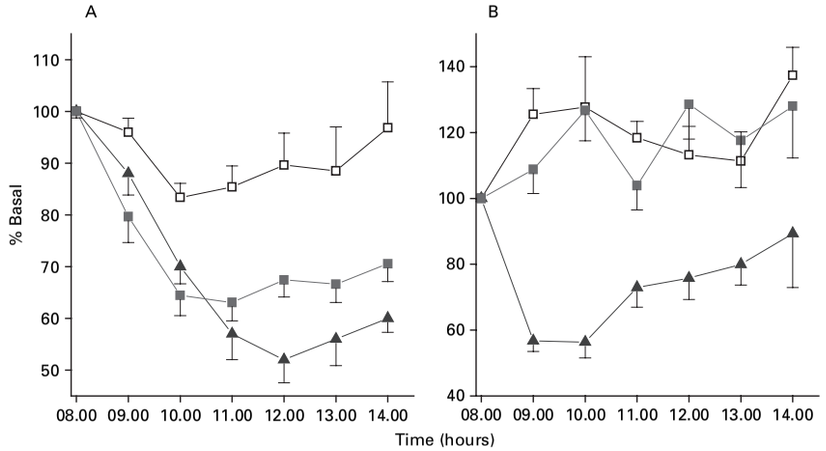

Fig. 1. Percentage basal value (*n* 8) of (A) serum CTx and (B) iPTH following ingestion of water (fasting period; □), 4·6% (v/v) ethanol (■) and a solution of Ca (▲).

As expected, the ingestion of Ca induced a marked reduction in serum CTx ($P<0.001$; Fig. 1 (A)). Serum CTx was similarly reduced following the ingestion of ethanol ($P=0.004$) but not water (Fig. 1 (A)). Serum iPTH levels also fell following Ca ingestion ($P<0.005$; Fig. 1 (B)) but not following ethanol or water ingestion (Fig. 1 (B)). In addition, serum calcitonin levels did not differ following the ingestion of ethanol compared with water (data not shown).

In conclusion, the acute ingestion of three units of alcohol induced a marked reduction in the bone resorption marker, CTx. The effect is independent of PTH and calcitonin, suggesting suppression of bone resorption through a potentially novel pathway.

De Lorimier A (2000) *American Journal of Surgery* **180**, 357–361.

Risk factors for breast cancer: anthropometric and physical activity results from the UK Women's Cohort Study. By J.E. CADE, V.J. BURLEY and D.C. GREENWOOD, *Nutritional Epidemiology Group, University of Leeds, 71–75 Clarendon Road, Leeds, UK, LS2 9PL*

The relationship between body size and breast cancer risk has been the subject of numerous investigations (van den Brandt *et al.* 2000). The links between physical activity and breast cancer have been less well explored. The present study aims to explore the impact of different measures of body size and physical activity in relation to incident risk of breast cancer in the UK Women's Cohort Study (UKWCS). This is a national cohort of 35 372 women, aged 35–69 years at baseline, who have all completed a 217-item food-frequency questionnaire and detailed lifestyle and activity questionnaire.

	Premenopausal women				Postmenopausal women			
	Breast cancer (*n* 149)	No breast cancer (*n* 13 779)	Unadjusted OR	Adjusted OR*	Breast cancer (*n* 158)	No breast cancer (*n* 13 326)	Unadjusted OR	Adjusted OR*
	Mean SD	Mean SD	OR 95% CI	OR 95% CI	Mean SD	Mean SD	OR 95% CI	OR 95% CI
Vigorous physical activity (h/d)	0.18 0.24	0.27 0.47	0.47 0.26, 0.88	0.36 0.15, 0.87	0.15 0.32	0.24 0.53	0.53 0.29, 0.95	0.19 0.06, 0.65
BMI (kg/m^2)	23.7 4.0	23.9 4.3	0.99 0.95, 1.03	0.98 0.92, 1.03	25.7 4.7	25.0 4.6	1.03 1.0, 1.05	1.02 0.98, 1.05
Weight change since age 20 years (kg)	7.3 9.2	6.6 9.3	1.01 0.99, 1.03	1.02 0.99, 1.06	11.2 11.4	9.2 10.7	1.02 1.00, 1.03	1.03 1.0, 1.07

*Adjusted for age, social class, smoking, education, parity, total energy intake, vegetarian or fish eating, alcohol, BMI, vigorous activity.

As of March 2004, with around 4 years of follow-up, there were 355 women with incident breast cancer and, of these, 158 were postmenopausal and 149 premenopausal. Univariate analysis showed a statistically significant relationship between a number of variables and breast cancer incidence. Women with breast cancer reported less previous vigorous physical activity and were taller. Postmenopausal breast cancer incidence was associated with higher values for BMI, current weight, waist and hip sizes and weight change since age 20 years compared with women without breast cancer.

Logistic regression models were created to study risk of breast cancer associated with these anthropometric and activity-related variables. The models were adjusted for a number of potential confounders listed in the table footnotes. In the models, vigorous physical activity (h/d) was statistically significantly inversely associated with both pre- and postmenopausal breast cancer incidence. Although the odds ratio (OR) for BMI was higher in postmenopausal women, it was not statistically significant. Weight change since age 20 years, however, was positively associated with the incidence of postmenopausal but not premenopausal breast cancer.

In the present analysis, women who were undertaking more vigorous physical activity were less likely to develop breast cancer. In addition, for postmenopausal women those who had gained more weight from the age of 20 years were more likely to develop breast cancer. Further exploration of the UKWCS will allow us to explore interactions between body size and physical activity independently of other risk factors.

The UK Women's Cohort Study is funded by the World Cancer Research Fund. J.E.C. is in receipt of a National Public Health Career Scientist Award. Thanks to James Thomas for database management and the data entry team, Claire Calvert and Alyson Greenhalgh for baseline data collection support, and the UKWCS steering group for continued advice.

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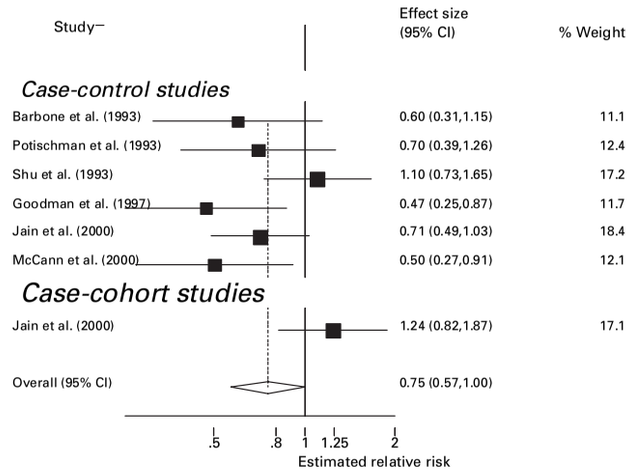
A systematic literature review and meta-analysis of the effects of dietary fibre consumption on risk of endometrial cancer. By V.J. BURLEY[1], D.C. GREENWOOD[2], E.V. BANDERA[3], J. CADE[1], R. KALLIECHARAN[4], L. H. KUSHI[5], J. MORETON[4] and D. FORMAN[4], [1]*Nutrition Epidemiology Group, University of Leeds, Leeds, UK, LS2 9PL,* [2]*Biostatistics Unit, University of Leeds, Leeds, UK, LS2 9LN,* [3]*Cancer Institute of New Jersey, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, USA,* [4]*School of Medicine, University of Leeds, Leeds, UK and* [5]*Division of Research, Kaiser Permanente, Oakland, CA, USA*

Given the known effects of dietary fibre on reducing endogenous oestrogen levels, a beneficial effect on endometrial cancer, a disease mostly attributed to exposure of the endometrium to increased levels of unopposed oestrogens, is also expected. However, the relationship between dietary fibre intake and cancer of the endometrium has not been extensively investigated. In support of the World Cancer Research Fund/American Institute for Cancer Research (1997) second report *Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective*, we conducted a series of systematic literature reviews on the associations between food, nutrition, physical activity and the risk of endometrial cancer. A total of seven studies were found to be suitable for inclusion within a meta-analysis of dietary fibre (one case–cohort and six case–control studies).

Women in the lowest categories of fibre intake had 25% lower odds of endometrial cancer than those in the highest categories (pooled estimated odds ratio (OR) 0.75, 95% CI 0.57, 1.00; P=0.05) (see Forest). However, there was significant heterogeneity (Q 13.2, df 6; P=0.04) with a moderate I^2 of 55%.



To explore any potential dose–response effect and summarise all the results on the same scale, we expressed these relationships in terms of OR per 5 g increment of dietary fibre intake per d. A 5 g increase in dietary fibre (equivalent to two large slices of wholemeal bread/d) was associated with a 3 (95% CI −5, 11) % reduction in risk of endometrial cancer (pooled estimated OR 0.97 (95% CI 0.89, 1.05) with significant heterogeneity). If this pooled estimate were reliable, this would be a very small association and not statistically significant. While sources of heterogeneity relating to dietary methodology, aspects of study design and adjustment for covariates were explored in the present analysis, no one source was able to explain the high degree of disparity between studies. Furthermore, we were unable to explore the differential effect of the various sources of dietary fibre (for example, fruit fibre, vegetable fibre, cereal fibre), as very few studies reported on this. Overall, the present results suggest a weak inverse relationship between dietary fibre intake and endometrial cancer.

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World Cancer Research Fund/American Institute for Cancer Research (1997) *Food, nutrition and prevention of cancer: a global perspective*. Washington, DC: American Institute for Cancer Research.

The effects of weight loss and weight gain on biomarkers of breast cancer risk. By M. HARVIE[1], T. MERCER[2], R. MALIK[3], J. ADAMS[3], A. FLYVBJERG[4] and A. HOWELL[1], [1]*South Manchester University Hospitals Trust, Manchester, UK, M20 2LR,* [2]*Department of Exercise and Sports Science, Manchester Metropolitan University, Manchester, UK,* [3]*Department of Clinical Radiology, University of Manchester, Manchester, UK and* [4]*Medical Research Laboratories, Institute of Experimental Clinical Research, Aarhus University Hospital, Aarhus, Denmark*

Premenopausal weight gain is associated with up to two-fold increases in postmenopausal breast cancer (Huang, 1997). Premenopausal weight gain is predominately abdominal, which is linked to breast cancer risk (Harvie *et al.* 2003), and to a series of endocrine changes including insulin resistance declines in sex hormone-binding globulin (SHBG) and insulin-like growth factor-binding protein (IGFBP)-1 and 2. We have recently shown that modest premenopausal weight loss (≥5% body weight) is linked to a 40% reduction in risk of postmenopausal breast cancer within the Iowa Women's Health Study (Harvie, 2003). The present paper examines the effects of an energy-restricted and exercise weight-loss programme as compared with standard diet and exercise advice on a range of biomarkers of breast cancer risk amongst premenopausal women (aged 35–45 years), with relatively large adult weight gains (>10 kg since the age of 20 years) and a family history of breast cancer (1 in 6 lifetime risk or greater).

Body weight, waist circumference, percentage body fat (measured using dual energy X-ray absorptiometry), total fat (TAF), subcutaneous fat (SAF) and intra-abdominal fat (IAF; measured using magnetic resonance imaging), insulin sensitivity (HOMA; Matthews *et al.* 1985), SHBG, IGFBP-1 and testosterone were determined at baseline and 6 months in seventy-four women. We report the effect of a weight loss of ≥5% (*n* 26) and of 0–5% (*n* 24) as compared with weight gain (*n* 25) on these parameters at 6 months.

	Weight loss >5%		Weight loss 1–5%		Weight gain 0–7%	
	Mean	SE	Mean	SE	Mean	SE
Weight (kg)	71.3*	0.5	76.6*	0.5	80	0.5
Waist (mm)	900*	9	966	10	982	11
% Body fat	34.5*	0.3	38.0*	0.3	39.0	0.3
TAF (cm^2)	247*	6.8	310*	7.0	332	6.8
SAF (cm^2)	172*	4.8	218*	5.0	235	4.8
IAF (cm^2)	69*	4.0	88	4.0	92	3.8
Testosterone (nmol/l)	1.4*	0.1	1.6	0.1	1.7	0.9
SHBG (nmol/l)	68*	3.1	64	3.1	56	3.1
Insulin sensitivity (μU/mmol per l)	1.05*	0.14	1.34	0.14	1.6	0.13
IGFBP-1 (μg/l)	39*	2.9	32.5	3.1	24.2	3.1

* Mean value is significantly different from that for weight gainers ($P<0.01$; ANCOVA adjusted for baseline levels).

After 6 months there were significant declines in percentage body fat, total and subcutaneous abdominal fat amongst women losing both >5% and 0–5% of their body weight. Only women losing >5% of their body weight experienced significant declines in waist circumference, intra-abdominal fat, testosterone and increases in insulin sensitivity, SHBG, and IGFBP-1. A weight loss of 5% had beneficial effects on biomarkers of breast cancer risk compared with the normal situation of weight gain amongst overweight women with a family history of breast cancer. We are currently examining the effects of the weight-loss intervention over 12 months to see whether these beneficial effects are maintained.

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Is dietary micronutrient intake a limiting factor in fetal growth? A comparison of Pakistani and European origin women in Britain. By J.C. OLDROYD[1], A. VYAS[1], C. LAMBERT[2], F. PRITCHARD[2], J. DRY[2], L. JAMES[1], A. KHAN[1], P. McELDUFF[3] and J.K. CRUICKSHANK[1], [1]*Clinical Epidemiology Group, Department of Medicine, Manchester University, M13 9PT,* [2]*St Mary's Hospital, Hathersage Road, Manchester, UK, M13 0JH and* [3]*Evidence for Population Health Unit, Manchester University, Manchester, UK, M13 9PT*

A recent Cochrane Review suggests only small effects of balanced energy and protein supplementation in pregnancy on mean birth weight (Kramer & Katuma, 2004). Recently, micronutrient intake (thought to reflect green leafy vegetable consumption) was found to be positively related to birth size in India (Rao *et al.* 2001). The influence of micronutrient intake together with energy and protein intake on birth weight in women in Britain, in particular of different ethnic origin, has not been investigated in detail. We tested the hypotheses that higher intakes of folate, vitamin B_{12} and vitamin C during pregnancy would be more common in European than Pakistani women and that higher intake of these nutrients would be associated with larger neonatal anthropometry.

Mothers attending antenatal clinic at 28 weeks recorded maternal age, pre-pregnancy weight, weight, parity as well as completed an interviewer-assisted 200-item food-frequency questionnaire (FFQ). Neonatal anthropometry (weight, length, head circumference and subscapular, triceps, and flank skinfolds) was measured within 72 h of delivery at St Mary's Hospital, Manchester. FFQ were analysed on WISP software.

Complete data are available on 116 Pakistani and 340 European origin women. Mean birth weight was 3406 (SD 509) g in European and 3183 (SD 560) g in Pakistani infants ($P<0.001$). Dietary energy and protein intakes at 28 weeks were not related to infant size at birth. Dietary folate (difference −39.4 (95% CI −63.3, −15.5) μg) and vitamin B_{12} intake (difference −0.9 (95% CI −1.6, −0.3) μg) were significantly lower in Pakistani than in European women (Vyas *et al.* 2004). After adjustment for dietary energy intake and ethnicity, dietary folate, vitamin B_{12} and vitamin C were not associated with size at birth.

Micronutrient intake in pregnancy does not appear to be a significant predictor of birth size in infants born to women in Britain. Validation against plasma folate concentrations is underway.

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Response of lambs fed maize stover treated with urea to supplementation of rumen-protected methionine. By E. SANCHEZ[1], M.E. ORTEGA-CERRILLA[1], L. LANDOIS[1], G. MENDOZA[1] and S. BUNTINX[2], [1]*Colegio de Postgraduados, Carr. Mexico-Texcoco km 36.5, 56230 Montecillo, Mexico and* [2]*Universidad Nacional Autonoma de Mexico, Mexico City, Mexico*

Maize stover is the only feed available for ruminants for part of the annual season cycle in many regions of Mexico. However, with such feed, nutrient intake may be insufficient to support even maintenance. Treatment of low-quality roughages with urea has shown to improve the feed intake and productive performance of ruminants (Flachowsky *et al.* 1996). But it is also necessary to supply S amino acids to the small intestine in order to achieve an adequate growth (Campbell *et al.* 1997). Therefore, the present study evaluated the effect of feeding maize stover untreated or treated with urea and supplemented with rumen-protected methionine to growing lambs on feed intake (FI), body-weight gain (BWG), digestibility of DM (DMD), crude protein (CPD), neutral-detergent fibre (NDFD), acid-detergent fibre (ADFD), ruminal pH and concentration of N-NH_3. Thirty-two male 'Criollo' lambs weighing 20 (SD 5) kg were housed in individual pens and randomly assigned to four treatments: UM (untreated chopped maize stover); TM (chopped maize stover treated with urea, 4 kg urea diluted in 20 litres water/100 kg maize stover, treated for 30 d); UMM (untreated chopped maize stover plus rumen-protected methionine, 5 g/animal per d at feeding time); TMM (chopped maize stover treated with urea plus rumen-protected methionine). Lambs were fed on a ration containing 700 g UM or TM/kg together with 300 g compound feed/kg (650 g ground sorghum, 300 g lucerne hay, 40 g molasses and 10 g mineral premix). Feed intake was recorded daily for a 60 d period, lambs were weighed and rumen fluid was collected by oesophageal probe at 45 and 60 d. Digestibility was evaluated at the end of the trial, feed was adjusted to 90% for 5 d and faeces were collected during the following 8 d. Data were analysed using a factorial design, with repeated measurements over a period of time for FI, BWG, pH and N-NH_3.

Treatments ...	UM	TM	UMM	TMM	SE	Probability
FI (kg/d)						
45 d	0.89	0.90	0.82	0.89	0.03	0.20
60 d	0.89	0.90	0.85	0.91	0.03	0.50
BWG (kg/d)						
45 d	0.08	0.05	0.11	0.13	0.02	0.07
60 d	0.07	0.06	0.05	0.08	0.02	0.47
pH						
45 d	6.45	6.65	6.76	6.35	0.10	0.05
60 d	6.54	6.54	6.60	6.46	0.07	0.52
N-NH_3 (mg/l)						
45 d	20.0^{a}	160.1^{b}	39.6^{a}	160.0^{b}	19.8	0.01
60 d	20.8^{a}	189.2^{b}	36.3^{a}	148.8^{b}	22.7	0.01
Digestibility						
DMD	0.57	0.61	0.54	0.54	0.02	0.29
CPD	0.44	0.48	0.40	0.40	0.04	0.43
NDFD	0.56^{b}	0.68^{a}	0.57^{ab}	0.62^{ab}	0.02	0.03
ADFD	0.28^{bc}	0.54^{a}	0.23^{c}	0.44^{ab}	0.04	0.01

^{a,b} Mean values with unlike superscript letters were significantly different.

The addition of rumen-protected methionine to maize stover either untreated or treated with urea did not improve FI or BWG. Significant differences were found for NDFD, ADFD and NH_3-N concentration in the rumen. However no beneficial effects were observed on the productive performance of the lambs, which indicates that although there were more N and S amino acids available, due to the high level of maize stover in the diet, animals were not able to get enough nutrients in order to achieve an adequate growth.

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Dietary organic acids for lactating sows. By N.K. AGROKIPIOTIS and S.P. ROSE, *Harper Adams University College, Newport, Shropshire, UK, TF10 8NB*

The restriction on the use of antibiotic growth promoters in animal feed in the European Union has led the industry to investigate alternatives in order to ensure good productive performance from farm animals. Organic acids may now be used in pig and poultry feeds to reduce the possibility of microbial contamination after manufacture but there is anecdotal evidence that they may also improve growth performance of the animals. The objective of the present trial was to investigate the effect of giving organic acids to lactating sows on their reproductive performance from parturition to weaning.

Sixty sows (a mixture of PIC F1 and Dalland F1) were used for the present on-farm experiment (Agrokipiotis Farm Ltd., Nicosia, Cyprus). Ten sows from each parity number group (parity numbers 1 to 6 inclusive) were randomly separated into two groups of five; the control and the treatment groups. At 3 d before their expected date of parturition, sows were given the following feeding regimen: 3 d to farrowing, 2.8 kg; 2 d to farrowing, 2.2 kg; 1 d to farrowing, 1.6 kg. From day 1 to day 5 of lactation sows were fed 1, 2, 3, 4, 5 kg respectively and from day 6 to day 25 they were fed 6 kg daily (divided into morning and afternoon feeds). The experimental feed (167 g/kg protein, 10.9 MJ/kg metabolisable energy and 9.5 g/kg lysine) was based on barley, maize, soyabean meal, wheat bran and a vitamin and trace mineral mix. The treatment group was additionally fed a pelleted acidifier (Acid Pig; Neofarma) that contained 450 g fumaric acid/kg, 210 g lactic acid/kg, 10 g propionic acid/kg, 10 g citric acid/kg and 10 g formic acid/kg. A total of 50 g of the acidifier was fed daily to the treated sows; 25 g was placed on top of each of the morning and afternoon feeds in the trough. On the first day of lactation the number of piglets that each sow was nursing was standardised to eleven. Each litter of piglets was weighed as a group on day 1 and day 25 of the lactation period and the sow was also weighed at this time. Piglets were fed creep feed from day 5 and their feed intake was measured. Data were compared using a randomised block ANOVA.

	Control (*n* 30)	Treated (*n* 30)	SED	*F* probability
Piglets weaned	9.83	10.43	0.279	0.036
Piglets total feed intake day 1 to day 25 (g)	976	1271	0.0608*	0.323
Piglets weight gain day 1 to day 25 (kg)	3.57	3.94	0.174	0.034
Sows weight loss day 1 to day 25 (kg)	7.3	9.4	2.65	0.424
Weaning to oestrus interval (d)	8.14	8.02	1.316	0.925
Sows total feed intake day −3 to day 25 (kg)	133.53	136.38	1.631	0.086

* Piglet feed intake data were skewed, so the data were transformed using $\log_{10}$. The untransformed treatment means are shown but the SED shows the transformed statistic.

There were 0.6 more piglets weaned per sow ($P<0.05$) and the weight gain of the piglets was 0.376 kg greater ($P<0.05$) in the acidified group. This is an important commercial difference and there are a number of possibilities that could explain this effect. Dietary organic acids increase gastric proteolysis and nutrient digestibility in growing pigs (Mroz *et al.* 1997) and this effect could have improved milk production in the sows. Second, dietary acidifiers reduce pathogenic bacteria in the gastrointestinal tract (Thomlinson & Lawrence, 1981) and this could have resulted in the sows excreting fewer pathogenic organisms to contaminate the environment of the piglets. Environmental microbial contamination tended ($P>0.05$) to be reduced when an acidifier was added to the drinking water of laying hens (Rose *et al.* 2003). In conclusion, the present experiment has given preliminary evidence that dietary organic acids for lactating sows give significant benefits to the survival and growth of their suckling pigs.

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Use of wheat straw upgraded by edible mushroom *Pleurotus ostreatus* in rations for sheep. By J. ACEVES, M.E. ORTEGA-CERRILLA, G. MENDOZA, R. BARCENA and C. GARCIA, *Colegio de Postgraduados, Carr. Mexico-Texcoco km 36.5, 56230 Montecillo, Mexico*

Straws are commonly used to cultivate edible mushrooms such as *Pleurotus*. After harvesting of the mushroom, because a substantial part of the cell-wall components are degraded in the straw, it can be used in ruminant rations (Adamovic *et al.* 1998). The present study investigated the effect of feeding wheat straw upgraded by *Pleurotus ostreatus* to sheep. Feed intake (FI), body-weight gain (BWG), *in situ* DM, neutral-detergent fibre (NDF), acid-detergent fibre (ADF) disappearance, and *in vivo* DM digestibility were evaluated. Twenty-four 'Criollo' sheep (twelve males, twelve females), weighing 25 (SD 2.75) kg were housed in individual pens and randomly assigned (six males, six females) to each of the following treatments; UWS (untreated wheat straw) or TWS (upgraded wheat straw by *P. ostreatus* for 60 d). Sheep were fed a ration containing 700 g UWS or TWS/kg together with 300 g compound feed/kg (10 g ground sorghum, 260 g soyabean meal, 440 g molasses, 230 g fish meal, 60 g mineral premix). Feed intake was recorded daily for a 60 d period. Sheep were weighed at 15, 30, 45 and 60 d. DM digestibility was carried out at the end of the trial, feed was adjusted to 90% for 5 d and faeces were collected during the following 8 d. Two Holstein cows fitted with rumen fistula were used for the *in situ* study. Animals were fed a ration containing 800 g maize stover/kg and 200 g lucerne hay/kg. Data were analysed for a completely randomised block design with repeated measurements over a period of time.

Days ...	15		30		45		60	
	UWS	TWS	UWS	TWS	UWS	TWS	UWS	TWS
FI (kg)	1.28	1.39	1.90	2.04	1.57	1.50	1.62	1.67
SE	0.05		0.08		0.08		0.07	
BWG (kg/d)	0.031	0.021	0.127	0.171	0.142	0.125	0.083	0.116
SE	0.24		0.01		0.02		0.02	

Proportionate disappearance		12 h	24 h	36 h	48 h	72 h
DM	UWS	0.18	0.25	0.30	0.35	0.42
	TWS	0.24	0.31	0.30	0.34	0.41
	SE	0.03	0.02	0.05	0.04	0.05
NDF	UWS	0.15	0.22	0.26	0.31	0.39
	TWS	0.22	0.29	0.26	0.32	0.40
	SE	0.02	0.02	0.03	0.03	0.05
ADF	UWS	0.17	0.23	0.28	0.34	0.35
	TWS	0.23	0.29	0.29	0.36	0.37
	SE	0.03	0.02	0.03	0.05	0.05

No significant differences ($P<0.05$) were observed for any of the variables evaluated in the present study, including DM digestibility (UWS 0.76, TWS 0.76, SE 0.02). These results showed that it is possible to include wheat straw that has been previously used to grow *P. ostreatus* in rations for sheep, obtaining similar results to those observed for wheat straw that has not been upgraded. However, the advantage of using upgraded straw is that it has also been used to produce a large amount of edible mushrooms, which might be another source of income (Henics, 1987).

Adamovic M, Grubic G, Milenovic I, Jovanovic R, Protic R, Stretenovic L & Stoicevic Lj (1998) *Animal Feed Science and Technology* **71**, 357–362.
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Overweight and obesity among women in the postpartum period. By P.H.C. RONDO[1] and A. TOMKINS[2], [1]*Nutrition Department, School of Public Health, University of Sao Paulo, Avenida Dr Arnaldo 715, Sao Paulo, Brazil and* [2]*Centre for International Child Health, Institute of Child Health, 30 Guilford Street, London, UK, WC1N 1EH*

In Brazil, as in some other developing countries, overweight/obesity has been an important issue in the last few decades, and the prevalence of malnutrition has decreased. In the present study, the anthropometric measurements of 712 Brazilian mothers were assessed in the postpartum period (from 12 to 72 h after delivery) in four hospitals in Campinas City, Sao Paulo, in 1991. The anthropometric measurements and the characteristics of the mothers are shown in the Table below.

	Mean	SD
Weight (kg)	60.76	10.25
Height (m)	1.57	0.0006
BMI (kg/m^2)	24.72	3.8
MUAC (mm)	255.7	30.9
Triceps skinfold thickness (mm)	15.40	4.97
Weight gain (kg)	11.84	4.19
Age (years)	23.98	5.79
Racial or ethnic origin (%)		
white	76.4	
mulatto and black	23.6	
Marital status (%)		
legitimate marriage	52.8	
others	47.2	
Residence (%)		
urban	89.6	
rural	10.4	
Per capita income/month (MBW)	1.44	1.42

MBW, minimum Brazilian wage (approximately US$65)

There were few mothers (n 43) with a BMI ≤20 kg/m^2. On the other hand, there were many (n 293) overweight (BMI 25–30 kg/m^2) and obese (BMI >30 kg/m^2) mothers. We advise further large epidemiological studies to assess the diet of pregnant women and its relationship to maternal weight, weight gain and birth weight. Although we did not find an association between BMI or weight gain in pregnancy and weight of the newborn babies, it is important to investigate this association in populations with a high prevalence of obesity. According to some authors (Luke & Petrie, 1980; Cogswell *et al.* 1995), there is a U-shaped curve when we assess maternal BMI and weight gain in pregnancy and weight of the newborn baby. Women who start pregnancy with high BMI and gain an excess of weight during pregnancy seem to be more prone to deliver a small baby.

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Improvements in mental wellbeing during weight loss on a slimming-on-referral programme. By A.J. AVERY[1], J.H. LAVIN[2], E. REES[3], J.H. BARTH[4] and S.M. WHITEHEAD[3], [1]*Greater Derby PCT and* [3]*formerly Southern Derbyshire Health Authority, 1 Stuart Street, Derby, UK, DE1 2EQ,* [2]*Slimming World, Clover Nook Road, Alfreton, UK, DE55 4RF and* [4]*Leeds General Infirmary, Leeds, UK, LS1 3EX*

Given the rising prevalence and escalating costs of obesity, innovative approaches should be sought to establish effective weight-management programmes which are both sustainable and address health inequalities. Self-esteem and mental wellbeing are important outcome measures in any weight-management programme.

A slimming-on-referral programme was set up in Southern Derbyshire to investigate the feasibility of referring patients to a local Slimming World group. Outcomes measured included attendance, weight loss, general wellbeing and participants' views of the scheme. Obese patients (*n* 107) from two general practitioner practices were offered vouchers covering membership and 12 weeks' attendance at a local Slimming World group. After the subsidised period, participants were able to continue attending and became responsible for the payment of weekly fees. Follow-up arrangements were made in the primary care setting at both 3 and 6 months. An important element of the slimming-on-referral scheme was that referred patients were not made to feel any different to paying members. The Slimming World approach is to empower members to have the skills and confidence to make healthy lifestyle changes over a sustained period of time. A motivating group process is used which recognises that individuals may need different levels of support and that each member is able to contribute to the group. Group consultants are local individuals who have themselves used the approach to lose weight and maintain weight loss.

Ninety-one patients (85 %) enrolled at a Slimming World group. Of these, sixty-two completed the 12-week subsidised period (defined as attending at least ten meetings). Forty-seven participants decided to pay the weekly fees themselves to continue after 12 weeks, with thirty-four completing 24 weeks. Participants' mental wellbeing was compared with a representative sample from a Southern Derbyshire health survey. Mental wellbeing was assessed using questionnaires measuring the following aspects of mood; feeling calm and peaceful, having a lot of energy, and feeling downhearted and low, at six levels of rating (all of the time, most of the time, a good bit of the time, some of the time, little of the time, none of the time). The data presented show values for 'most of the time'. Statistics represent χ^2 analysis run across all levels. Compared with the Southern Derbyshire sample, the obese patients generally had low ratings of wellbeing before enrolment. However, improvements in all aspects measured were seen in those completing both 12 weeks (calm, $P=0.001$; energy, $P=0.001$; downhearted $P=0.015$) and 24 weeks (calm, $P=0.02$; energy, $P=0.001$; downhearted, $P=0.001$). The mean weight loss of those completing 12 weeks was 6.4 (SD 3.0) % and in those completing 24 weeks it was 11.3 (SD 4.6) %. Qualitative data support these findings, with patients viewing slimming on referral very positively; for example, 'I was helped at a time when I had a very low self-esteem, the group consultant was excellent and very supportive' and 'The other slimmers were brilliant, a good laugh was had by all and we lost weight!'

		Slimming-on-referral participants		
			12-week completers (*n* 62)	
	South Derbyshire sample	Enrolled (*n* 91)	Enrolment	12 weeks
Feeling calm and peaceful (%)	38	28	29	46*
Having a lot of energy (%)	41	9	12	28*
Feeling downhearted and low (%)	8	23	19	7†

Significantly different from enrolment: *$P<0.01$, †$P<0.02$.

Overweight and obese individuals need to be recognised as being particularly vulnerable in that they generally have a low self-esteem and self-confidence. The recommended target of 5–10% weight loss for health improvement can be achieved in a significant percentage of patients participating in slimming on referral. Furthermore, the study shows that referring patients to the Slimming World programme can significantly improve mental wellbeing.

The study was funded jointly by the Southern Derbyshire Health Authority and Slimming World.

Weight loss and long-term health outcomes in obese individuals. By L. AUCOTT[1], A. POOBALAN[2], W.C.S. SMITH[1], A. AVENELL[3], R. JUNG[4], J. BROOM[5] and A.M. GRANT[3], [1]*Department of Public Health, University of Aberdeen, Aberdeen, UK,* [2]*Department of Child Health, University of Aberdeen, Aberdeen, UK,* [3]*Health Services Research Unit, University of Aberdeen, Aberdeen, UK,* [4]*Tayside University Hospitals NHS Trust, Dundee, UK and* [5]*Grampian University Hospitals NHS Trust, Aberdeen, UK*

Weight loss in obese patients has been associated with clinical improvements but this is mostly based on short-term studies. The hope is that the weight loss achieved by short-term interventions is maintained with sustainable benefits. However, there is a lack of evidence of the long-term effects of weight loss in obese individuals.

The aim here was to systematically review the evidence for the long-term effects of weight loss on the development of diabetes, lipid levels and blood pressure changes in obese individuals, and investigate if the weight loss benefits are sustained in practice.

A systematic review of long-term studies published between 1966 and 2001 was carried out. This included searching Medline, Embase, Cinahl and Health STAR bibliographic databases. Published studies were predominantly for Caucasian obese adults populations. A follow-up of at least 2 years for the weight and health outcomes was required.

For the development of diabetes, seven studies were included in the review. These showed a dose–response effect even at 2 or more years of follow-up. The risk of developing diabetes was substantially reduced with weight loss and the greater the weight loss, the bigger the risk reduction. The large weight losses achievable with surgical interventions reduced risk by at least 63 %.

The changes in cholesterol with weight loss from thirteen studies with a minimum follow-up of 2 years again showed a strong dose–response relationship. LDL-cholesterol had the strongest positive linear relationship with weight change (correlation r 0.903; $P<0.001$) followed by total cholesterol and triacylglycerols (correlations r 0.851 and r 0.760, respectively; both with $P<0.001$).

With respect to hypertension, seven studies with more than 2 years of follow-up and a weight loss of up to 10 kg showed that a diastolic blood pressure decrease of about 1.6 mmHg may be expected from a weight reduction of 3.4 kg, which is about half of the predicted value from the short-term trials. However, three additional studies with larger weight losses, that is the surgical interventions, had blood pressure reductions that were proportionately even less. There was a strong relationship with initial blood pressure that effectively made weight loss non-significant.

While the response to weight loss in the long term persists and shows a dose–response effect for both the development of diabetes and lipid levels, hypertension would appear to be more complex. The response to extreme weight loss, made possible by surgical interventions, do not, on their own, give sustained hypertension reductions of the expected effect size. Hence, lifestyle and diet changes induced by the non-surgical interventions should be investigated further for long-term improvements in hypertension.

Is elevated body mass index associated with an increased risk of mortality in individuals over 75 years old? By G.M. PRICE[1], R. UAUY[1], E. BREEZE[1], C.J. BULPITT[2] and A.E. FLETCHER[1], [1]*Centre for Ageing and Public Health, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK, WC1 E7HT and* [2]*Section of Care of the Elderly, Faculty of Medicine, Imperial College, Hammersmith Campus, Du Cane Road, London, UK, W12 0NN*

Studies in other countries indicate that the risk of increased mortality at higher BMI is less pronounced in older than in younger adults (Seidell & Visscher, 2000), but there are fewer studies in the oldest age group or from the UK.

We examined the association of BMI with mortality in 8394 women and 5358 men aged over 75 (mean age 81; maximum 108) years from fifty-three general practices throughout mainland UK participating in the MRC trial of assessment and management of older people living in the community (Fletcher *et al.* 2002). As part of an in-depth health assessment (between March 1995 and April 1999), trained nurses took anthropometry measurements, including weight and height, and collected information on smoking history, current morbidity, recent weight loss, activities of daily living (ADL) and self-perceived relative physical activity. All participants were registered with the Office for National Statistics for fact and cause of death, or embarkation. The results are based on deaths up to 31 July 2003. Median follow-up was 6 years per person, 72 620 person-years in total. BMI was categorised by sex-specific quintiles, and was related to mortality risk using Cox regression models adjusted for height (significantly negatively predictive) and age. Significant statistical interactions were found between BMI (as a continuous variable) and respectively sex (P=0.005) and, in women, current smoking status (P=0.003); hence the relative risk (RR) of death from all causes for categories of BMI are reported separately for the four sex and smoking groups. The upper fifth of BMI distribution in both men and women, regarded as 'obese' by the usual definitions, was the reference category in the analyses.

	Women					Men			
	Non-smokers		Smokers			Non-smokers		Smokers	
BMI (kg/m^2)*	*n* (% died)	RR (95% CI)	*n* (% died)	RR (95% CI)	BMI (kg/m^2)*	*n* (% died)	RR (95% CI)	*n* (% died)	RR (95% CI)
14.7–22.2	1454 (47.2)	1.17 (1.05, 1.31)	225 (63.1)	2.60 (1.69, 4.01)	15.9–22.9	846 (57.4)	1.30 (1.15, 1.48)	222 (70.7)	1.41 (1.00, 1.98)
22.3–24.5	1538 (41.4)	1.06 (0.97, 1.16)	142 (52.1)	1.85 (1.15, 2.99)	23.0–24.9	918 (48.4)	1.04 (0.89, 1.2)	153 (54.9)	0.95 (0.65, 1.38)
24.6–26.7	1555 (33.1)	0.82 (0.72, 0.93)	124 (43.5)	1.70 (1.07, 2.69)	25.0–26.6	946 (45.3)	1.00 (0.87, 1.16)	126 (46.0)	0.76 (0.49, 1.17)
26.8–29.6	1545 (33.7)	0.87 (0.77, 0.98)	133 (37.6)	1.23 (0.73, 2.08)	26.7–28.9	976 (42.4)	0.94 (0.83, 1.07)	98 (57.1)	0.96 (0.65, 1.43)
29.7–45.2	1573 (36.6)	1.00 (reference)	105 (30.5)	1.00 (reference)	29.0–40.4	976 (44.6)	1.00 (reference)	97 (58.8)	1.00 (reference)
All women	7665 (38.3)		729 (48.3)		All men	4662 (47.4)		696 (59.2)	

* Lower and upper bounds of sex-specific fifth of BMI distribution.

Mortality risk was significantly lower than that of the obese category only in non-smoking women with BMI between 24.6 and 29.6 kg/m^2; traditionally considered to be 'overweight'. In male smokers a RR of 0.76 at BMI 25.0 to 26.6 kg/m^2 was not significant. Risks were consistently highest for those in the lowest fifth of BMI and this was most pronounced for smokers. In women smokers, risk increased steadily with decreasing BMI (P of linear trend <0.001). In analyses of non-smokers (data not shown), further adjustment for unexplained weight loss, history of diabetes, cancer, or CVD at baseline, or by excluding deaths in the first 6 months of follow-up had little impact on the RR. Adjusting for ADL and physical activity increased the RR in all BMI categories, resulting in a more consistently monotonic inverse relationship. The present results confirm a different pattern of BMI and overall mortality risk for over-75-year-olds from those of younger adults and highlight an adverse effect of low BMI compared with elevated BMI.

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Seidell JC & Visscher TL (2000) *European Journal of Clinical Nutrition* **54**, Suppl. 3, S33–S39.

Trends in nutrient intake in the Irish population between 1998 and 2002. By C.C. KELLEHER[1], C.M. MURRIN[1], S. FRIEL[2] and G. NOLAN[1], [1]*National Nutrition Surveillance Centre, Department of Public Health Medicine and Epidemiology, Woodview House, University College Dublin, Belfield, Dublin 4, Republic of Ireland and* [2]*Department of Health Promotion, Clinical Sciences Institute, National University of Ireland Galway, Costello Road, Shantalla, Galway City, Republic of Ireland*

Analysis of the changing pattern in dietary habits of the Irish population is key to understanding the factors affecting overweight and obesity (National Nutrition Surveillance Centre, 2003). In 1998 baseline data were obtained in the Survey of Lifestyle, Attitudes and Nutrition (SLÁN) to examine a range of lifestyle-related health behaviours, including diet, in relation to various social factors (Kelleher *et al.* 2002). The survey was repeated in 2002 and comprised of a validated self-administered postal questionnaire (Hayes *et al.* 2004; J Harrington, unpublished results), including a 149-food item semi-quantitative food-frequency section, from which nutrient intakes were estimated based on McCance and Widdowson's food composition tables. A stratified probability sample of adults aged 18 years and over on the electoral register was drawn across each of the Irish Republic's twenty-six counties, proportionately distributed according to the urban–rural breakdown. Questionnaires were removed from the data set if the energy intake was ±2.5 standard deviations from the sample mean leaving a sample of 6465 for 1998 and 5938 for 2002.

Significant differences in nutrient intake were found over the 4-year period (P<0.001). The contribution of fat to daily total energy was significantly lower in 2002 (33.2%) than in 1998 (34.2%). This was also the case for saturated fat. The intake for carbohydrate as a percentage of energy was significantly higher in 2002 (49.8%; P<0.001) as were alcohol (2.1%; P<0.005), and total sugar (23.7%; P<0.001). There was no significant difference in total energy intake between 1998 and 2002.

In both years, males had significantly higher total energy intake than females (P<0.001). This was also the case for energy from fat, protein, alcohol, saturated fat, and MUFA (P<0.001). Females had significantly higher intakes of energy from carbohydrate, PUFA, sugars, starch and total fibre (P<0.001).

In 1998 and 2002 total energy intake for males decreased significantly with age (P<0.001). Energy intakes for females aged 35–54 years were higher than other age groups. The percentage of energy from fat decreased significantly with age (P<0.001) but the gradient was not as steep for women as for men since women aged 18–34 years had similar values to those in the middle age group (35–54 years). The energy from carbohydrates increased with age for males (P<0.001) but females from the middle age group showed intakes significantly lower than other age groups (P<0.001). Energy intakes from protein and starch increased with age (P<0.001). Intakes of energy from alcohol decreased significantly with age for females but peaked for males aged 35–54 years in both 1998 and 2002 (P<0.001). Energy from saturated fat and sugar decreased significantly from the young (18–34 years) to the middle age group in both sexes but tended to increase in the older age groups (P<0.001). While MUFA intakes decreased significantly with age (P<0.001), PUFA peaked for both males and females in the middle age group (P<0.001). In both sexes the middle age group showed higher intakes of fibre (P<0.005).

The daily intake pattern of nutrients in the Irish population has changed over 4 years. These differences vary significantly with the changing demographics of our society.

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National Nutrition Surveillance Centre (2003) *Dietary Habits of the Irish Population: results from SLÁN* Dublin: Department of Health and Children.

A comparison of self-reported and clinically measured body mass index in Irish adults. By K. HAYES[2], F. SHIELY[1], C.M. MURRIN[1], G. NOLAN[1] and C.C. KELLEHER[1], [1]*National Nutrition Surveillance Centre, Department of Public Health Medicine and Epidemiology, Woodview House, University College Dublin, Belfield, Dublin 4, Republic of Ireland and* [2]*Department of Mathematics and Statistics, University of Limerick, Plassey, Limerick, Republic of Ireland*

Typically, accurate measurements of height and weight cannot be made on subjects participating in large (*n* 1000+) epidemiological studies. Instead, self-reported values of these variables must suffice, which, in turn, are less precise and have no guarantee of accuracy. Problems of precision and accuracy are further compounded when the variables height and weight are converted into biomedical measures such as BMI, associated with mortality as far back as Quetelet (1841) and obesity throughout the twentieth century.

Measurements for this analysis were conducted on an examination sub-sample (*n* 402) of the main Survey of Lifestyle, Attitudes and Nutrition (SLÁN) data (Kelleher *et al*. 2002; National Nutrition Surveillance Centre, 2003). Subjects also completed a questionnaire including self-reported height and weight measurements. Exact determinations and self-reported estimates of height and weight were available for 337 subjects. Measured BMI and self-reported BMI were calculated from these height and weight values. BMI groups were categorised as normal (<25 kg/m^2), overweight (25–30 kg/m^2) and obese (>30 kg/m^2).

Self-report and measured BMI are compared in the Table. Of 108 subjects measured as normal, 94.4% reported themselves as normal. The probability discordant classification, that is a classification made by self-report not agreeing with the classification made by measurement, is higher for overweight and obese subjects. Of eighty-six subjects who were measured as obese, only fifty-five (64.0%) reported themselves as obese and thirty-one (36.1%) reported themselves as overweight. Similarly, of the 143 subjects measured as overweight, only ninety-four (65.7%) reported themselves as overweight and forty-three (30.1%) reported themselves as normal.

	BMI groups (self-report)			
BMI groups (measured)	Normal (*n*)	Overweight (*n*)	Obese (*n*)	Total (*n*)
Normal (*n*) %	102	4	2	108
	94.4	3.7	1.9	100
Overweight (*n*) %	43	94	6	143
	30.1	65.7	4.2	100
Obese (*n*) %	0	31	55	86
	0	36.1	64.0	100
Total (*n*) %	145	129	63	337
	43.0	38.3	18.7	100

The percentage of overweight females reporting themselves as normal weight (34.3%) is higher than the corresponding percentage for males (26.3%). The percentage of obese males reporting themselves as overweight (40.5%) is higher than the corresponding percentage of misclassifications for female subjects (31.8%). In both cases, the percentage of normal-BMI subjects reporting themselves as normal is high, 100% for males and 92.3% for females.

We conclude that quantification of the underestimation affords the development of a correction factor that may be interpolated to the main SLÁN datasets in interpreting obesity trends, taking into account the effects of the covariates age, education, smoking and sex.

Kelleher C, Friel S, Nolan G & Forbes B (2002) *Proceedings of the Nutrition Society* **61**, 527–536.
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Quetelet MA (1841) *A Treatise on Man and the Development of his Faculties*. New York: Burt Franklin.

Consumption patterns of junk foods in young Irish individuals in relation to body mass index. By G. NOLAN[1], C.M. MURRIN[1], F. SHIELY[1], H. CORRIGAN[2], S. NIC GABHAINN[2], S. FRIEL[2] and C.C. KELLEHER[1], [1]*National Nutrition Surveillance Centre, Department of Public Health Medicine and Epidemiology, Woodview House, University College Dublin, Belfield, Dublin 4, Republic of Ireland and* [2]*Department of Health Promotion, Clinical Sciences Institute, National University of Ireland Galway, Costello Road, Shantalla, Galway City, Republic of Ireland*

Dietary intake during childhood and adolescence influences the risk of major chronic diseases. Promotion of healthy eating has long been at the fore of health promotion campaigns among schoolchildren given that dietary habits established in youth continue into adulthood. The aim of the present study was to examine the relationship between BMI and intake of foods with low nutritional value in young Irish individuals. The data were obtained from the Health Behaviour in School-aged Children study, which is a World Health Organization (European) collaborative study. The sample was selected from the Department of Education School lists and was representative of the geographical distribution of school students. Self-administered questionnaires were completed by 8424 pupils. Data for BMI based on self-reported height and weight were only available for 2469 respondents.

The daily consumption of sweets and fizzy drinks increased significantly with age ($P<0.001$) while the proportion of 15–17-year-olds consuming chips ($P=0.012$) and hamburgers ($P=0.046$) on a daily basis was lower than younger ages (10–11 and 12–14 years). Girls aged between 12 and 14 years (51.6%; $P=0.006$) consumed significantly more sweets daily than boys of the same age (47.0%). Similar findings were evident for 15–17-year-olds ($P=0.021$). Significantly more boys ($P<0.001$) aged 12–14 years and 15–17 years consumed more fizzy drinks on a daily basis than of girls of the same ages (43.0% compared with 33% and 49.2% compared with 35.1% respectively). Boys of all ages (except 10–11 years) were significantly more likely to consume chips and hamburgers on a daily basis ($P<0.001$). While daily consumption of chips was higher in boys than girls aged 10–11 years, this difference was not statistically significant.

The prevalence of overweight and obesity was 6.0 and 1.5% respectively (*n* 2469). Sex differences were not evident but an age effect emerged ($P=0.031$). Young individuals aged 12–14 years were more likely to be overweight, however levels of obesity did not differ.

An ordinal regression was conducted with BMI groups (normal, <20 kg/m^2; overweight, 20–25 kg/m^2; obese, >25 kg/m^2) as the dependent variable and the consumption of sweets, fizzy drinks, burgers, chips, cakes and crisps as factors. The negative coefficient for the daily consumption of fizzy drinks shows that drinking fizzy drinks on a daily basis increases the probability of being in the overweight and obese categories ($P=0.018$). None of the other factors yielded significant *P* values.

These data suggest that targeting fizzy drinks among Irish school children, particularly boys, may be an important focus for dietary health promotion activities.

The effect of snack composition on energy intake and body weight. By S. WHYBROW[1], C. MAYER[2], T. KIRK[1] and R.J. STUBBS[3], [1]*Queen Margaret University College, Clerwood Terrace, Edinburgh, UK, EH12 8TS,* [2]*Biomathematics and Statistics Scotland, Greenburn Road, Aberdeen, UK, AB21 9SB and* [3]*Rowett Research Institute, Greenburn Road, Aberdeen, UK, AB21 9SB*

The present study investigated the effects of mandatory ingestion of commercial snack products differing in macronutrient composition, on energy intake and body weight in free-living adults.

Thirty-six males (mean age 35 (SD 7.6) years; mean BMI 25 (SD 4.6) kg/m^2) and thirty-six females (35 (SD 8.3) years; 25 (SD 4.1) kg/m^2) each consumed 0 MJ (control), 1.5 MJ (low-energy) and 3.0 MJ (high-energy) of snack products per d for 14 d in a counter-balanced cross-over-design study. The macronutrient compositions of the three types of snack treatments were, as percentage energy from carbohydrate (CHO), fat and protein respectively, 59:34:7 (high-CHO), 47:46:7 (mixed) and 37:57:6 (high-fat). Each subject received one type of snack at three manipulations of energy. Each intervention period comprised 7 d of equilibration where the only manipulations were measurement of body weight and consumption of the prescribed snacks. During the following 7 d subjects also self-recorded their food intakes using the weighed food record method. Body weight, corrected to nude, was investigator-recorded at the beginning, middle and end of each 14 d intervention period. Dietary intakes were analysed using Diet5 (Robert Gordon University, Aberdeen, UK). BMR was measured under standard conditions by indirect calorimetry. Suspected low-energy-reporters (<1.2·BMR) were excluded from the study and recruitment continued until seventy-two subjects had successfully completed all parts of the study.

Mean total daily energy intake increased as the intervention snacks were titrated into the diet (F(2,60) 12.19; P<0.001). However, the increases were less than the energy content of the intervention snacks, suggesting that subjects were partially compensating for energy. Compensation amounted to 54 and 64% on the low-energy, and high-energy treatments, respectively. There were no significant differences among the intervention snack types in their effects on energy intake. There was a significant effect of the amount of snacks consumed on change of body weight (F(2,116) 4.70; P=0.011), but no interaction between type of intervention snack and change in body weight; subjects neither gained nor lost more weight on one type of snack compared with the others. However, there was considerable variation in the responses; not all subjects gained weight over the intervention periods. The Table shows the mean daily energy intakes and changes in body weights over the intervention periods.

	Intervention snack treatment (energy level)					
	Control		Low-energy		High-energy	
Intervention snack type	Mean	SEM	Mean	SEM	Mean	SEM
Energy intake (MJ/d)						
High-CHO	10.9	0.58	11.0	0.50	11.5	0.52
Mixed	10.4	0.61	11.0	0.59	11.2	0.50
High-fat	10.1	0.42	11.3	0.57	11.7	0.53
Body-weight change (kg/14 d)						
High-CHO	−0.2	0.26	−0.0	0.17	+0.2	0.15
Mixed	−0.4	0.14	−0.4	0.16	+0.2	0.20
High-fat	−0.3	0.25	+0.1	0.16	+0.0	0.13

The results of the present intervention study suggest that subjects partially compensated for energy when their diets were supplemented with commercial snack products over 2-week periods, but this was insufficient to prevent an elevation of energy intake sufficient to increase body weight.

This work was funded by the Biscuit, Cake, Chocolate and Confectionery Association. S.W. received a studentship from Queen Margaret University College, Edinburgh.

Effects of snacks on energy intake as a function of habitual level of snack consumption. By S. WHYBROW[1], C. MAYER[2] and R.J. STUBBS[1], [1]*Rowett Research Institute, Greenburn Road, Aberdeen, UK, AB21 9SB and* [2]*Biomathematics and Statistics Scotland, Greenburn Road, Aberdeen, UK, AB21 9SB*

Laboratory-based studies show that energy-dense snacks can elevate total daily energy intakes over the very short term. In contrast, this is not seen when these snacks are consumed by 'habitual snackers' over the longer term (Lawton *et al.* 1998). The present study examined the differences in the effects of ingesting commercial snack products (CSP) on energy intake as a function of habitual snack consumption.

Thirty-six males (mean age 35 (SD 7.6) years; mean BMI 25 (SD 4.6) kg/m^2) and thirty-six females (mean age 35 (SD 8.3) years; mean BMI 25 (SD 4.1) kg/m^2) each consumed 0 MJ (control), 1.5 MJ (low-energy) and 3.0 MJ (high-energy) of snack products per d for 14 d in a counter-balanced cross-over-design study. Subjects self-recorded their food intakes using the 7 d weighed food record method during each intervention period. Dietary intakes were analysed using Diet5 (Robert Gordon University, Aberdeen, UK). Energy intake was calculated from all CSP (biscuits, cakes, chocolate, confectionery, nuts, and crisps and other similar savoury snack products) consumed during the control period, assumed to be representative of habitual diet. The mean change in energy intake during the low-energy and high-energy treatments relative to the control period was calculated for each subject. BMR was measured under standard conditions by indirect calorimetry. Suspected low-energy-reporters (<1.2·BMR) were excluded from the study and recruitment continued until seventy-two subjects had completed the study.

Fig. 1 shows that the frequent consumers of CSP compensated more completely for the additional energy content of the intervention snacks than did infrequent consumers of CSP. However, the relationship was weak (R^2 0.116; P=0.003).

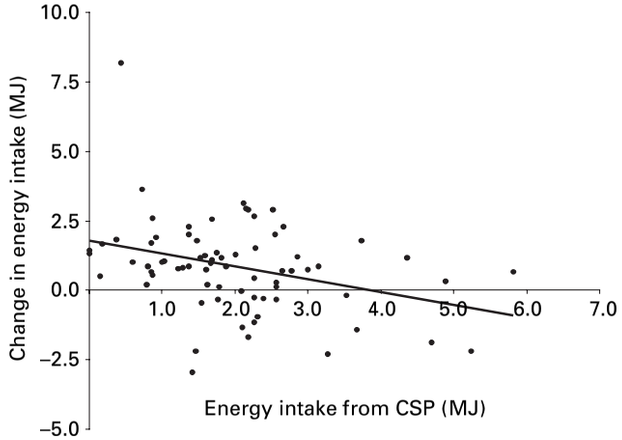

Fig. 1. Mean daily energy intake from CSP recorded during the control week and difference in mean daily energy intake between the control and intervention periods. R^2 0.116; P=0.003.

The regression line suggests that subjects habitually consuming around 4 MJ CSP per d would compensate completely for the intervention snacks' energy content (mean of 2.25 MJ).

The greater compensatory trends appear to be reflective of habitual patterns of feeding behaviour, which become established over time, and are often not apparent during acute interventions. This may explain some of the apparent differences between short-term and longer-term studies in the effects of snacks on energy intake.

This work was funded by the Biscuit, Cake, Chocolate and Confectionery Association. S.W. received a studentship from Queen Margaret University College, Edinburgh.

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Food insecurity in the UK and attitudes to healthy eating. By H.M. BUKHARI, B.M. MARGETTS and A. JACKSON, *Public Health Nutrition, Institute of Human Nutrition, FOAD Division, School of Medicine, University of Southampton, Southampton, UK*

Food insecurity exists where food availability is uncertain, or where financial or physical limitations limit access to potentially available food. Sustained food insecurity leads to poor health. Worldwide, about 815 million are considered to be food insecure (Food and Agriculture Organization, 1996); and for the USA about 12% of the general population (Bickel *et al.* 2000). There are few data on food insecurity in the UK. Food insecurity is multidimensional and in the present study, we have tested the hypothesis that the risk of food insecurity is higher in the most deprived individuals who also hold negative attitudes to healthy eating. The study population was derived from a representative sample of individuals living in a deprived area of Leeds, UK (*n* 459 households). The individual responsible for food preparation in the household completed a detailed questionnaire, including an assessment of food insecurity using a six-item scale (Bickel *et al.* 2000). Deprivation was defined according to Townsend (1987). Attitudes were assessed using a nine-item questionnaire, with "Do you think healthy food is expensive?" being most discriminatory (Margetts *et al*. 1998; Stockley, 2001). Binary logistic regression was used to analyse the data (SPSS, version 11.0), setting the least deprived with a positive attitude as the reference group.

			Deprivation				Attitude					
			Least deprived (0–1 level)		Most deprived (2–4 level)		Positive attitude		Negative attitude		Crude OR of deprived with negative attitude to healthy eating	
Food security groups	Proportion (%)	*n*	%	*n*	%	*n*	%	*n*	%	*n*		95% CI
Food secure	70	324	74.9	245	59.8	79	76.2	128	67.4	196	1	
Food insecure	30	135	25.1	82	40.2	53	23.8	40	32.6	95	2.813*	1.576, 5.022
Total (*n*)		459		324		135		168		291		

*Significantly different from least deprived with positive attitude ($P<0.001$).

The Table shows that 30% of this population were defined as being food insecure, similar to a recent study in a deprived area of the UK (Tingay *et al.* 2003). Food insecurity, 40%, was significantly more common in those who were most deprived, compared with those less deprived, 25% (odds ratio (OR) 2.004 (95% CI 1.306, 3.077); $P=0.001$). Food insecurity was 33% in those who had a negative attitude, compared with 24% in those who held a positive attitude (OR 1.551 (95% CI 1.008, 2.388); $P=0.046$). The risk of being food insecure was nearly three times higher in those who were deprived and had a negative attitude to healthy eating compared with those who were not deprived and had positive attitudes (crude OR 2.813 (95% CI 1.576, 5.022); $P<0.001$), suggesting a potentially important interaction between these two factors.

The present study shows that in a deprived area of the UK, food insecurity affects many individuals and is an important issue. The causes appear more complex than simply limited resources, and any programme aimed at alleviating food insecurity will need to understand and address these complex interactions.

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Leptin entry into the brain from the peripheral circulation *in vivo*. By C.L. ADAM[1], P.A. FINDLAY[1] and D.W. MILLER[2], [1]*Division of Energy Balance and Obesity, Rowett Research Institute, Bucksburn, Aberdeen, UK, AB21 9SB and* [2]*School of Biological Sciences, University of Aberdeen, Aberdeen, UK, AB24 4FA*

The entry of leptin from circulating blood to the brain is of physiological relevance to the regulation of food intake and body weight. It has been suggested that a reduced blood–brain transport of leptin contributes to the phenomenon of central leptin resistance seen in most obese subjects. Here we have developed an animal model in which simultaneous sampling of peripheral blood and ventricular cerebrospinal fluid (CSF) is undertaken repeatedly during increasing adiposity in order to study the relative concentrations of leptin in plasma and CSF *in vivo*. We tested the hypothesis that leptin transport into the brain is reduced as adiposity and endogenous leptin concentrations are increased.

Castrated, oestradiol-implanted, male sheep (*n* 9) were surgically prepared with intracerebroventricular cannulae and kept for 15 weeks with *ad libitum* food in artificial long days (LD; 16 h light–8 h dark). During weeks 5, 10 and 15, the sheep were injected intravenously (IV) at 08.00 hours with leptin (5 mg recombinant ovine leptin in 5 ml saline; leptin kindly donated by Professor A. Gertler, The Hebrew University of Jerusalem, Israel). Blood and CSF were sampled from the jugular vein and lateral cerebral ventricle, respectively, immediately before IV leptin injection and at hourly intervals over 8 h for leptin analysis by homologous radioimmunoassay (Marie *et al.* 2001). The sheep had body condition score 2.5 at week 5 (thin), 3 at week 10 (medium) and 3.75 (fat) at week 15, which equated to 24, 29 and 35% body fat, respectively (Russel *et al.* 1969). Mean endogenous plasma leptin before IV injection increased from 5.4 (SEM 1.47) to 7.8 (SEM 2.09) to 8.9 (SEM 1.17) ng/ml, accordingly in weeks 5, 10 and 15. Corresponding mean CSF leptin values were 0.5 (SEM 0.18), 1.1 (SEM 0.35) and 2.2 (SEM 0.67) ng/ml, respectively. Overall, endogenous CSF leptin concentrations were linearly related to concurrent plasma values (r^2 0.575; $P<0.001$); the CSF:plasma concentration ratio averaged 0.16, with no evidence of a decrease at the higher endogenous blood leptin concentrations (r^2 0.116). Following the leptin bolus administered IV, peak plasma leptin values at 1 h were 45.0 (SEM 7.04), 48.5 (SEM 3.64) and 50.6 (SEM 7.04) ng/ml at 5, 10 and 15 weeks, respectively. This peak was followed by a decay curve to approximately 10 ng/ml after 7 h on each occasion. CSF leptin values also peaked at 1 h at 3.7 (SEM 0.94), 4.6 (SEM 1.31) and 4.3 (SEM 1.11) ng/ml, respectively, and decayed to approximately 2 ng/ml after 7 h. There was no difference in these patterns between 5, 10 and 15 weeks. The CSF:plasma concentration ratio was 0.09 at peak values 1 h after IV leptin but increased thereafter to average 0.16 for the following 6 h.

The data indicate that a constant proportion of endogenous leptin enters the CSF from the blood across a range of blood concentrations in thin to fat sheep. Also, whereas the proportion of circulating leptin entering the CSF was temporarily reduced when circulating concentrations were acutely increased into the pharmacological range, the entry of exogenously administered leptin into the CSF was not affected by adiposity and endogenous leptin status. We therefore reject the hypothesis that leptin transport into the CSF is decreased when adiposity and endogenous leptin concentrations increase and suggest that a reduced blood–brain transport is unlikely to be the cause of central leptin resistance.

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Body-mass regulation in the mouse appears to be driven by a lipostat. By C. HAMBLY[1] and J.R. SPEAKMAN[1,2], [1]*Aberdeen Centre for Energy Regulation and Obesity (ACERO), Division of Energy Balance and Obesity, Rowett Research Institute, Bucksburn, Aberdeen, UK, AB21 9BS and* [2]*School of Biological Sciences, University of Aberdeen, Aberdeen, UK, AB24 2TZ*

It is often asserted that body mass is a regulated phenomenon. However, the exact nature of the regulation remains uncertain and several different models are available (for a review, see Speakman, 2004). Separating between these alternatives is complex. To assist this process a series of computer-based models were designed using existing data for the mouse (strain MF1).

Model 1 presumes that food intake is a stochastically variable parameter that is unregulated. Body mass is a consequence of the interaction between energy intake and energy expenditure and the ratio of partitioning of energy into fat and lean tissue (p-ratio).

Model 2 assumes that there is active cross-talk between the phenomena of intake and expenditure on a daily basis.

Model 3 is as model 2 but includes an additional regulatory component dependent on fat mass (a lipostat), which feeds back into both intake and expenditure.

Model 4 is as model 2 but involves feedback from an accumulated memory of energy deficit that is encoded separately from the level of fat storage.

We used these models to predict the responses of male MF1 mice to periods of energy restriction in an experiment explicitly designed to separate between models 3 and 4. Model 3 predicts that during *ad libitum* feeding following a period of food restriction mice would show hyperphagia because of their depleted fat stores. The same response is predicted from model 4, but because of an accumulated memory of the energy deficit. The computer models highlighted that if animals are clamped in energy balance following the period of restriction, then model 4 predicts that the magnitude of hyperphagia on release to *ad libitum feeding* would decline in relation to the duration of the clamp, but model 3 predicts no diminution of hyperphagia because fat mass would always be below the lipostatic target. We performed the present experiment by placing four groups of male MF1 mice (n 10) on a restricted diet (80% of *ad libitum*) for 50 d, followed by clamping energy balance for 0, 10, 30 or 50 d (one group at each duration), followed by *ad libitum* feeding (all groups). When mice were released from the clamps they all showed a marked hyperphagia, however this only lasted for 1 d before they stabilised their rate of feeding. The extent of the hyperphagia did not differ depending on the amount of time that the mice had remained clamped in energy balance (ANOVA $F_{3,37}$ 0.37; P=0.78). If the amount of food consumed was combined over the first 5 d of release onto *ad libitum* feeding and compared, there was also no significant difference between the groups (ANOVA $F_{3,37}$ 2.02; P=0.13). Before release onto *ad libitum* feeding, body mass differed from that of the controls by an average of 2.3 g for the 10 d clamp, 2.5 g for the 30 d clamp and 3.5 g for the 50 d clamp. This later value was significantly lower (ANOVA using a Tukey pairwise comparison $F_{1,18}$ 6.16; P=0.02). The response in body mass to release from restriction was extremely rapid, showing a marked increase by day 2 of release from restriction, which by this stage was not significant from the controls. Of this increase, only 39% was caused by an increase in gut fill and therefore the majority of the weight gain was due to increases in the lean or fat tissue depots. By the final measurement period none of the restricted groups differed significantly in body mass from a control group or each other (ANOVA $F_{3,49}$ 1.18; P=0.33). These data strongly suggest that a lipostatic regulation system (model 3) occurs in the mouse.

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Decreased adiposity in mice lacking inducible nitric oxide synthase supports the involvement of nitric oxide in body fat regulation. By J. GÓMEZ-AMBROSI[1], S. BECERRIL[1], P. OROZ[1], S. ZABALZA[1], F.J. MURUZÁBAL[2], M. ARCHANCO[2], M.J. GIL[3], M.A. BURRELL[2], J. SALVADOR[4] and G. FRÜHBECK[1,4], [1]*Metabolic Research Laboratory & Departments of* [2]*Histology,* [3]*Biochemistry and* [4]*Endocrinology, Clínica Universitaria de Navarra, University of Navarra, Pamplona, Spain*

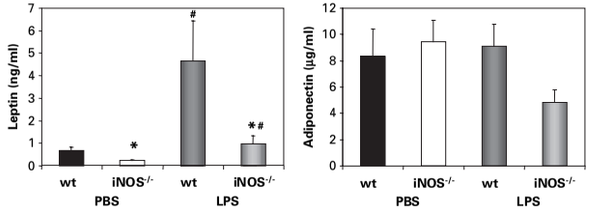
The identification of genes that cause obesity, leanness or provide resistance against obesity development has added new clues to the understanding of body weight control (Frühbeck & Gómez-Ambrosi, 2003). Stimulation of inducible NO synthase (iNOS) in skeletal muscle and white adipose tissue (WAT) has been proposed as a link between obesity, inflammation and insulin resistance. In fact, increased iNOS expression in adipocytes of genetic and dietary models of obesity has been described. Moreover, targeted disruption of iNOS has been shown to protect against diet-induced insulin resistance in muscle (Perrault & Marette, 2001). The aim of the present study was to evaluate the impact of the lack of iNOS on body weight and adipose tissue mass as well as on serum leptin and adiponectin concentrations in basal conditions and after lipopolysaccharide (LPS) administration.

Six-week-old male wild-type (w/t) and iNOS$^{-/-}$ mice were used in the study (six per group, twenty-four mice in total). LPS was administered intraperitoneally at 5 mg/kg body weight to half of the mice while the remaining were injected with PBS. All mice were killed 6 h later. Fasting plasma analyses included the determination of NO, leptin and adiponectin. NO was quantified as the concentration of nitrates+nitrites. Leptin and adiponectin were measured by ELISA. Epididymal WAT (EWAT) iNOS mRNA expression in was measured by RT-PCR.

	PBS				LPS			
	w/t		iNOS$^{-/-}$		w/t		iNOS$^{-/-}$	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight (g)	20.2	1.4	20.2	3.3	20.1	1.1	20.0	2.2
EWAT weight (g)	0.215	0.106	0.114*	0.028	0.193	0.050	0.158*	0.049
Nitrates+nitrites (μM)	123	37	128	19	523***	79	110	11
WAT iNOS mRNA (relative units)	100	14	ND		120	40	ND	

* P<0.05 main effect of genotype by two-way ANOVA. *** P<0.001 *v.* any other group by two-tailed unpaired Student's t test (after interaction genotype–LPS observed by two-way ANOVA). ND, not detected.

Body weight was not different among the groups. However, EWAT weight was significantly reduced in iNOS$^{-/-}$ mice. LPS significantly increased NO in w/t mice, but had no effect on iNOS$^{-/-}$ mice. iNOS mRNA expression was undetectable in the EWAT of iNOS$^{-/-}$ mice and was slightly increased by LPS in w/t mice, although these differences were not significant.



Data are means ± SD. * P<0.05 main effect of genotype; # P<0.05 main effect of LPS by two-way ANOVA.

Plasma leptin concentrations were significantly reduced in iNOS$^{-/-}$ mice, but were significantly increased by LPS treatment in both genotypes. Neither genotype nor treatment changed plasma adiponectin. In rodents, LPS injection is followed by an increase in leptin concentrations via a NO-independent pathway (Mastronardi *et al.* 2000). We have further confirmed these data showing that the LPS-induced rise in leptin circulating concentrations is independent of the presence of functional iNOS. In summary, iNOS$^{-/-}$ mice exhibited a normal body weight but reduced adipose mass accompanied by hypoleptinaemia. Leptin responsiveness to LPS is preserved. In addition, iNOS deficiency or LPS do not influence circulating concentrations of adiponectin. Our data support the involvement of NO and iNOS in adipose mass regulation.

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Peptide $YY_{3–36}$: its effects on rate of eating and meal duration. By S.E. ELLIS[1], R.L. BATTERHAM[2], S.R. BLOOM[3], M.A. GHATEI[3] and G.S. FROST[1], [1]*Department of Dietetics, Imperial College London, Hammersmith Hospital Campus, London, UK, W12 ONN,* [2]*Department of Medicine, University College London, Rayne Building, 5 University Street, London, UK, WC1E 6JJ and* [3]*Department of Metabolic Medicine, Imperial College London, London, UK, W12 ONN*

Peptide $YY_{3–36}$ ($PYY_{3–36}$) is a naturally occurring peptide, released into the circulation by intestinal L-cells following food ingestion. Recent peripheral infusion studies in human volunteers, which achieved physiological plasma concentrations of $PYY_{3–36,}$ demonstrated a reduction in preprandial hunger intensity and food intake without feelings of nausea (Batterham *et al.* 2002, 2003). These findings suggest that exogenous $PYY_{3–36}$ can directly influence the onset of satiety and thus the amount eaten. A decrease in meal duration and rate of eating are further measures of feeding behaviour taken as evidence that an experimental manipulation contributes to satiety (Kissileff, 1984). In the present randomised double-blind placebo-controlled cross-over study we measured the effect of intravenous $PYY_{3–36}$ on duration and rate of eating in humans subjects.

Twelve lean (mean BMI (SEM 20.5) 0.1 kg/m^2, mean age 27.3 (SEM 0.4) years) and twelve obese (BMI 33.0 (SEM 0.9) kg/m^2, age 27.3 (SEM 0.4) years) individuals participated. Subjects were asked to standardise their food intake 48 h before each study day and refrain from alcohol and strenuous exercise the day before. They attended the investigation unit on two occasions 1 week apart, having fasted for 12 h and were infused for 90 min with either saline or $PYY_{3–36}$ (2 nmol/m^2 body surface area). Subjective feelings of appetite and nausea were assessed every 30 min throughout the study period (09.00–17.00 hours) using visual analogue scales. At 2 h following infusion cessation, the test meal was presented. Food and water were served in excess, and subjects were instructed to eat until comfortably full. The duration of the meal, defined as the length of time from the first mouthful of food to final placement of utensils on the plate or tray, was measured surreptitiously using a digital stopwatch and subsequently the rate of eating (kJ/min) was calculated.

In both lean and obese groups, $PYY_{3–36}$ infusion reduced feelings of hunger before the test meal and energy intake during the test meal (mean percentage reduction 31.1 (SEM 4.5) %, $P<0.0001$ and 29.9 (SEM 4.4) %, $P<0.0005$ in lean and obese group respectively) compared with saline, as reported previously (Batterham *et al.* 2003). Fig. 1 shows that meal duration was significantly decreased following $PYY_{3–36}$ by 5 min on average in the lean (saline 24.5 (SEM 2.0) min and $PYY_{3–36}$ 19.7 (SEM 1.6) min; $P<0.001$) and in the obese (saline 24.4 (SEM 1.5) min and $PYY_{3–36}$ 19.4 (SEM 1.2) min; $P=0.01$) individuals. Furthermore, rate of eating was reduced in the both groups (lean: saline 197 (SEM 16) kJ/min and $PYY_{3–36}$ 167 (SEM 13) kJ/min, $P=0.03$; obese: saline 223 (SEM 18) kJ/min and $PYY_{3–36}$ 203 (SEM 24) kJ/min, $P=0.25$); however, this did not reach significance in the obese.

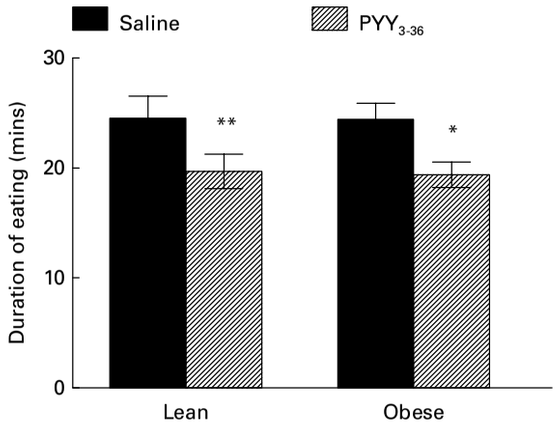

Fig. 1. Duration of eating following $PYY_{3–36}$ and saline infusions.

We have provided evidence that the reduced energy intake reported with PYY infusion may be in part explained by a reduction in the length of eating period and in the rate of eating. This is in keeping with observations made with cholecystokinin.

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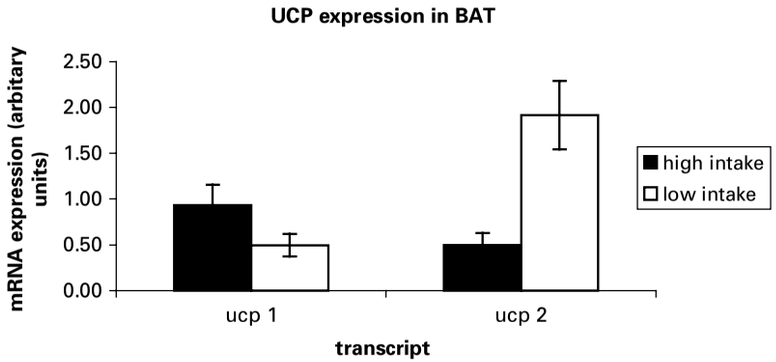
Metabolic rate and uncoupling protein (UCP) gene expression in mice selected for high and low food intake. By G.D. DALGLEISH[1], J. DUNCAN[2], L. BUNGER[3] and J.R. SPEAKMAN[1,2], [1]*Aberdeen Centre for Energy Regulation and Obesity (ACERO), University of Aberdeen, Aberdeen, UK, AB24 2TZ,* [2]*ACERO, Division of Appetite and Energy Balance, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 9SB and* [3]*Animal Breeding and Genetics Department, Animal Biology Division, SAC, Sir Stephen Watson Building, Bush Estate, Midlothian, UK, EH26 0PH*

RMR is the component of energy expenditure that explains the largest proportion of total daily energy needs in individuals; however the contribution to the development of obesity remains controversial. A low RMR for a given body size and composition is partially genetically determined (Bouchard *et al.* 1989) and has been suggested as a risk factor for weight gain. Mitochondrial uncoupling protein (UCP) 1 is suggested to make a major contribution to RMR in rodents but the contribution (if any) of UCP2 and 3 remains uncertain. Mice which have been divergently selected for high and low food intake, corrected for body mass, differ by 30% in food intake with no significant differences in body weight (Hastings *et al.* 1997). High-intake mice have also been reported to have elevated BMR (Selman *et al.* 2001). We investigated RMR at thermoneutral (30 °C) in ten male mice from each line and found the high-intake line to have significantly greater RMR (VO_2 0.79 (SE 0.8) ml/min) when compared with the low-intake strain (VO_2 0.63 (SE 0.09) ml/min). These mouse lines therefore provide a good model to investigate the potential contribution of the three UCP to the variability in RMR. We examined the expression levels of the UCP in brown adipose tissue (BAT) and white adipose tissue (WAT) by Northern blotting. In WAT although there was a tendency for the high-intake strain to have higher levels of UCP2 there were no significant differences between strains. In BAT, however, the expression of UCP1 was significantly elevated (22%) in the high-intake strain, in accordance with the higher RMR. UCP2 expression in BAT, however, showed a contrasting profile and was highly significantly reduced (74%) in the high-intake strain.



UCP1 functions by uncoupling the respiratory chain from oxidative phosphorylation and generating heat. Elevated expression of UCP1 may affect the production of reactive oxygen species (ROS) at complex III of the respiratory chain by decreasing the proton motive force. Recent studies suggest that UCP2 and 3 may be induced by ROS (Echtay *et al.* 2002), therefore the decrease in UCP2 mRNA levels when UCP1 is up regulated may be due to a decreased ROS production. These data provide further evidence that UCP2 does not function as a respiratory uncoupler.

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Peripherally administered α-melanocyte stimulating hormone (αMSH) increases resting metabolic rate, while peripheral agouti-related protein has no effect, in wild-type C57BL/6 and *ob/ob* mice. By N. HOGGARD[1], D. VERNON RAYNER[1], S.L. JOHNSTON[1] and J.R. SPEAKMAN[1,2], [1]*Aberdeen Centre for Energy Regulation and Obesity (ACERO), Division of Energy Balance and Obesity, Rowett Research Institute, Aberdeen, UK, AB21 9SB and* [2]*ACERO, School of Biological Sciences, University of Aberdeen, Aberdeen, UK, AB24 2TZ*

During energy restriction weight loss is not sustained at the same initial rate primarily because of adaptive reductions in components of expenditure including resting metabolism. Strategies to keep RMR high may therefore significantly augment traditional dieting approaches to weight loss. During restriction leptin declines and is believed to signal elevated appetite and reduced expenditure. Recent studies indicate that agouti-related protein (AgRP) is also elevated in the periphery during food restriction. As there is also an indication that the central administration of AgRP depresses metabolism we wondered if the peripheral rise in AgRP was involved in signalling the depression of RMR during food restriction.

To test this hypothesis we measured RMR using a continuous flow indirect calorimetry system which permits the continuous logging of O_2 consumption and CO_2 production every 10 s. The detailed time course of response allowed us to identify periods when the animals had elevated metabolism due to physical activity and exclude these from the analysis; a procedure generally impossible with devices that alternate between several chambers attached to a single analyser, and of importance because previous studies have suggested agonists and antagonists of melanocortin receptors may modulate activity. We measured leptin-deficient obese *ob/ob* (*n* 15; five per group) or lean C57BL/6 mice (*n* 15; five per group) before (1.5 h) and after (4 h) intraperitoneal administration of saline, [Nle^4, D-Phe^7]αMSH (150 μg; a potent analogue of αMSH) and agouti-related protein (AGRP) (82–131)-NH_2 (13 μg), the natural antagonist of αMSH, at melanocortin receptors. We calculated the lowest consecutive thirty measurements (5 min) both before and after injection to reflect the resting metabolism and then calculated the difference between these to determine the treatment effect. Each individual therefore acted as its own weight-matched control. We explored the effects of treatment on the change in metabolism using one-way ANOVA and probed the pair-wise differences using Tukey tests. Mice were not fed while in the respirometry chamber. We rejected data for one saline-injected lean C57BL/6 animal, which did not settle in the chamber sufficiently to measure a good pre-dose RMR.

In lean C57BL/6 mice there was a significant effect of saline, AgRP and αMSH on resting O_2 consumption (ANOVA $F_{2,11}$ 11.28; $P<0.002$). In saline- and AgRP-treated lean mice their O_2 consumption declined between pre- and post injection periods (by −0.14 and −0.11 ml O_2/min respectively) while in αMSH-treated mice O_2 consumption was significantly increased (0.15 ml O_2/min). Respiratory exchange ratio (RQ) in the post-injection phase was not significantly affected by treatment.

The pattern of response in obese *ob/ob* mice was similar. There was a significant overall effect of saline, AgRP and αMSH on the change in resting O_2 consumption between pre- and post-injection (ANOVA $F_{2,12}$ 5.67; $P=0.018$). In saline- and AgRP-injected *ob/ob* mice, O_2 consumption declined between pre- and post-injection periods by −0.106 and −0.38 ml O_2/min respectively, but in αMSH-injected *ob/ob* mice, O_2 consumption increased (0.274 ml O_2/min). As with lean C57BL/6 mice, RQ was unaffected.

αMSH significantly increased O_2 consumption when compared with the effect of saline or AgRP in both lean C57BL/6 and *ob/ob* mice (Tukey $P<0.05$). The effect of AgRP in decreasing O_2 consumption was not significantly different from that of the saline control.

These data suggest that peripherally administered AgRP at supra-physiological concentrations does not depress metabolic rate, possibly because it crosses the blood–brain barrier very slowly. In contrast, peripheral αMSH produced a large and sustained increase in resting energy expenditure independent of the status of leptin production in the periphery. αMSH analogues that cross the blood–brain barrier may significantly augment dietary-restriction strategies by sustaining elevated RMR.

Gene expression for appetite regulatory neuropeptides in the fetal sheep hypothalamus. By B.S. MÜHLHÄUSLER[2], I.C. McMILLEN[2], G. ROUZAUD[1,3], P.A. FINDLAY[1], E.M. MARROCCO[2], S.M. RHIND[3] and C.L. ADAM[1], [1]*Energy Balance and Obesity Division, Rowett Research Institute, Bucksburn, Aberdeen, UK, AB21 9SB,* [2]*Discipline of Physiology, Research Centre of the Early Origins of Adult Health, School of Molecular and Biomedical Science, University of Adelaide, Australia and* [3]*Macaulay Institute, Craigiebuckler, Aberdeen, UK, AB15 8QH*

In the adult, a hypothalamic neural network acts to maintain energy balance in response to nutritional feedback from the periphery. Whilst there is an immediate requirement for this system to be functional at birth, it is unknown whether the components of this central neural network are expressed in the developing brain before birth. We therefore examined in the fetal sheep hypothalamus during late gestation gene expression for leptin receptor (OB-Rb) and neuropeptides that regulate energy balance in the adult.

Brains were collected from fetal sheep at 110 d (*n* 12) and 140 d of gestation (*n* 5) (term being 150 d) and gene expression was detected in all hypothalami using *in situ* hybridisation with radiolabelled riboprobes for OB-Rb, neuropeptide Y (NPY), agouti-related peptide, pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript (CART). All mRNA were expressed in the arcuate nucleus of fetuses at both time points. Additional sites of mRNA expression were the dorsomedial hypothalamus (DMH) for NPY, the paraventricular nucleus (PVN), ventromedial hypothalamus (VMH) and lateral hypothalamic area for CART, and the DMH, PVN and VMH for OB-Rb.

We have therefore demonstrated that adult-like localisation of gene expression for OB-Rb and key appetite regulatory neuropeptides is established in the ovine hypothalamus before birth. Thus the fetus possesses a central appetite regulatory neural network with the potential to respond to changes in nutrient supply, which could impact on energy-balance regulation both before and after birth. The present study provides the foundation for further investigations of the potential short- and long-term consequences of altered nutrient supply *in utero* on the central neural network regulating energy balance. Such investigations are required to understand the mechanisms by which such altered states of nutrition may act to predispose the offspring to disorders of energy-balance regulation, such as obesity, in later life.

Phyto-oestrogen compounds for antioxidant studies: acute toxicity and hypoalbuminaemia. By M.C.Y. WONG[1], V.R. PREEDY[1], R. SHERWOOD[2] and H. WISEMAN[1], [1]*Nutritional Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, UK, SE1 9NN and* [2]*Clinical Biochemistry Unit, King's College Hospital, Denmark Hill, London, UK, SE5 9RS*

Phyto-oestrogens, such as genistein and daidzein, have been shown to protect against oxidative stress *in vivo* (Wiseman *et al.* 2000). It is our intention to further investigate the effects of these compounds at the tissue and molecular level using suitable animal models of oxidative stress. As a prelude, it is important to show that therapeutic treatment regimens with phyto-oestrogens *per se* do not cause overt tissue damage. This is particularly relevant, as recent studies have suggested that, at high doses, phyto-oestrogen administration in rats may cause organ toxicity such as hepatomegaly (Okazaki *et al.* 2002). However, we hypothesised that a treatment regimen encompassing the intraperitoneal injection of moderate doses of phyto-oestrogens does not cause overt organ toxicity, as reflected by changes in organ weights and blood analytes. To test this, male Wistar rats (about 0.1 kg body weight) were ranked and assigned to four groups (six rats per group) as follows: group A, uninjected; group B, carrier; group C, carrier+daidzein; group D, carrier+genistein. The carrier was Intralipid (20% fat emulsion). Daidzein and genistein were mixed with the carrier and homogenised ultrasonically before injection at a dose of 100 mg/kg body weight. All rats in groups B to D were injected intraperitoneally (to ensure complete bioavailability of phyto-oestrogens) for 4 d. At the end of the study, the rats were killed and blood was collected in heparinised tubes for the subsequent extraction of plasma. Plasma analytes were measured by routine laboratory procedures. Tissues were dissected, blotted and weighed. The data were analysed using one-way ANOVA, followed by a *post hoc* least significant differences test, where a P value <0.05 was considered to be significant. The Table shows the mean organ weights and plasma protein for each group.

	Organ weight (mg)		Plasma protein (g/l)	
Treatment	Liver	Gastrocnemius	Albumin	Globulin
Group A: uninjected (n 6)	4517	977	29.5	21.0
Group B: carrier (n 6)	4311	951	29.0	21.2
Group C: carrier+daidzein (n 6)	4179	952	27.2*	21.2
Group D: carrier+genistein (n 6)	4290	953	26.8*†	23.5**††

Mean values were significantly different from that for uninjected rats: * $P<0.05$, ** $P<0.01$.
Mean values were significantly different from that for carrier-injected rats: † $P<0.05$, †† $P<0.01$.

The results showed that plasma albumin concentrations decreased, and the calculated globulin fraction increased, in response to phyto-oestrogens (see Table). However, the activities of plasma aspartate aminotransferase and alkaline phosphatase were unaffected by any of the treatments when compared with either carrier or uninjected rats (data not shown for brevity). There were no significant effects of phyto-oestrogens on organ weights and hepatomegaly was not indicated (see Table). Neither food intakes nor body-weight gains were affected by any of the treatments (data not shown).

We conclude that intraperitoneal administration of genistein and daidzein causes a small, but statistically significant, hypoalbuminaemia. However, the mechanisms and long-term implications of this phenomenon are unknown at present.

We wish to thank Hangzhou FST, Republic of China, for their generous gift of phyto-oestrogen.

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Suitability of the three-component model to track body composition changes following insulin treatment in type 2 diabetes. By I.C. PACKIANATHAN[1,2], N.J. FULLER[3], D.B. PETERSON[2], A. WRIGHT[4], W.A. COWARD[4] and N. FINER[1,2], [1]*Wellcome Trust Clinical Research Facility, Addenbrookes Hospital, Cambridge, UK, CB1 2QQ,* [2]*Department of Diabetes and Endocrinology, and Centre for Obesity Research, Luton and Dunstable Hospital, Luton, UK, LU4 0DZ,* [3]*MRC Childhood Nutrition Research Centre, Institute of Child Health, London, UK, WC1N 1EH and* [4]*Stable Isotope Group, MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge, UK, CB1 9NL*

The aim of the present study was to assess and compare the ability of a number of techniques to estimate body composition changes in type 2 diabetes after 6 months of insulin therapy. Nineteen type 2 diabetes patients (mean age 60 (SD 8.3) years; weight 69.8 (SD 10.3) kg), poorly controlled despite maximal oral hypoglycaemic agents, received insulin (mean dose 40 (SD 12.2) units/d). Body-composition changes were assessed between baseline and 6 months using two-component model techniques, including dual-energy X-ray absorptiometry (DXA; Lunar model DPX-IQ), ^{2}H dilution (^2H_2O) and air-displacement plethysmography (ADP; the 'BODPOD'), a three-component model (3-CM), based on ADP and ^2H_2O, and typical examples of prediction equations based on anthropometry (Garrow & Webster, 1985) and bioelectrical impedance analysis (BIA; Segal *et al.* 1988). A four-component model (4-CM; Fuller *et al.* 1992), based on ADP, DXA and ^2H_2O, was the reference against which the validity of the alternative techniques was assessed using bias and 95% limits of agreement.

Body weight increased by 5.2 (SD 2.7) kg, of which 2.9 (SD 2.7) kg was fat, and the remaining 2.3 (SD 1.5) kg consisted entirely of total body water, with no change in protein or mineral as assessed by the 4-CM.

Change in body fat (kg) compared with 4-CM	Bias (kg)	95% Limits of agreement (kg)
3-CM	−0.02	−0.34 to 0.30
ADP	1.11	−0.46 to 2.68
DXA	0.21	−3.75 to 4.17
^2H_2O	−0.87	−2.43 to 0.69
BIA (Segal *et al.* 1988)	−0.83	−6.19 to 4.52
Anthropometry (Garrow & Webster, 1985)	0.85	−2.52 to 4.22

The 3-CM model was the most valid and least variable technique for assessing changes in body fat. Although less reliable, ^2H_2O, DXA and ADP provided generally better estimates of such changes than did prediction equations based on BIA or anthropometry, which were uncertain for both the sample group and individuals within it. This inaccuracy may have arisen from the inability of these techniques to account for concomitant changes in the hydration of fat-free mass (HF_{ffm}), the assumed constancy of which is essential to their valid use. Therefore, the 3-CM is recommended for tracking changes in type 2 diabetes patients in whom HF_{ffm} is likely to change, and where there is no change in protein or mineral.

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A ^{2}H-labelled mixed triacylglycerol test for intraluminal fat digestion. By C. SLATER[1], T. PRESTON[2] and L.T. WEAVER[1], [1]*University of Glasgow, Division of Developmental Medicine, Yorkhill Hospitals, Glasgow, UK, G3 8SJ and* [2]*Isotope Biochemistry Laboratory, Scottish Universities Environmental Research Centre, East Kilbride, UK, G75 0QF*

The ^{13}C-mixed triacylglycerol (MTG) breath test is a non-invasive measure of intraluminal fat digestion (Vantrappen *et al.* 1989). It is a useful measure of exocrine pancreatic insufficiency in children with cystic fibrosis and could be used to optimise pancreatic enzyme replacement therapy in these children (Amarri *et al.* 1997). The test has not been widely adopted in children because it lacks specificity and requires frequent breath sampling over a period of 5–6 h. Recovery of ^{13}C in breath CO_2 is influenced by factors that affect CO_2 production rate (VCO_2), such as physical activity, diet-induced thermogenesis and elevated resting energy expenditure. In addition, ^{13}C is sequestered into organic molecules via the tricarboxylic acid cycle. Therefore tracer recovery is incomplete (<50%), even in healthy subjects (Slater *et al.* 2003).

MTG (1,3-distearyl, 2-octanoyl glycerol) has long-chain fatty acids (18:0) at the *sn*-1 and -3 positions and a medium-chain fatty acid (octanoic acid, 8:0) at the *sn*-2 position. The fatty acids at the *sn*-1 and -3 positions are removed by pancreatic lipase. Octanoic acid and the monoacylglycerol are rapidly absorbed and oxidised in the liver. The final products of fatty acid oxidation are CO_2 and water. If 8:0 is labelled with ^{2}H, the label appears in body water, which can be sampled in urine or saliva. ^{2}H sequestration into organic molecules is low (about 4%) and is not influenced by food intake or physical activity. Therefore recovery should be almost complete in pancreatic-sufficient subjects. The use of ^{2}H-labelled MTG avoids the problems associated with the lack of knowledge of VCO_2 during ^{13}C breath tests (Slater *et al.* 1999, 2003) and could provide a simple non-invasive measure of fat digestion, which avoids the need for stool sampling and the regular sampling of breath.

[^{2}H]MTG (1,3-distearyl,2-[$^{2}H_{15}$]octanoyl glycerol 97 atom % ^{2}H; 20 g) was custom synthesised by Cambridge Isotope Laboratories Inc. (MA, USA). This was sufficient for two adult subjects. The [^{2}H]MTG (0.2 mmol/kg body weight) was baked in a biscuit (Slater & Preston, 2002), which was consumed after an overnight fast. Total body water (TBW) was measured by ^{18}O dilution (Schoeller *et al.* 1980) and estimated from height and weight using the equations of Hume & Weyers (1971) and Slater & Preston (2002). Urine and saliva were sampled at baseline and for 10 h after the consumption of the test meal. The abundance of ^{2}HOH and $H_2{}^{18}O$ in urine and saliva was measured by continuous-flow isotope ratio MS. Cumulative percentage dose recovered (PDR) of ^{2}H and ^{18}O was calculated from the plateau enrichment, which was reached by 6 h in both saliva and urine. Recovery of ^{2}H in urine calculated using measured TBW was compared with that using an estimated value of TBW.

	Subject 1 (female, age 46 years, wt 59.1 kg, ht 1.59 m)			Subject 2 (male, age 48 years, wt 80.8 kg, ht 1.83 m)		
TBW estimate	Measured by isotope dilution	Hume & Weyers	Slater & Preston	Measured by isotope dilution	Hume & Weyers	Slater & Preston
TBW (kg)	30.9	30.3	29.7	42.9	45.6	45.4
Cumulative PDR	94.2	92.5	90.5	98.7	105.0	104.4

[^{2}H]MTG could provide a simpler, more robust test of intraluminal fat digestion compared with the ^{13}C breath test, if supplies of this novel tracer were available. Only a baseline sample and a second sample 6 h later are required to calculate percentage ^{2}H dose recovered in body water. TBW could be estimated from height. Further studies are required in pancreatic insufficient subjects.

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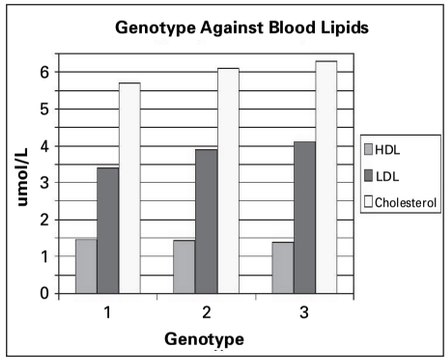
Apolipoprotein E genotype and blood lipids in EPIC Norfolk. By K.K.H. WU[1], R. BOWMAN[1], R. LUBEN[2], K.T. KHAW[2], N. WAREHAM[2], N.E. DAY[2] and S.A. BINGHAM[1], [1]*MRC Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge, UK, CB2 2XY and* [2]*EPIC, Institute of Public Health and Strangeways Research Laboratory, Cambridge, UK*

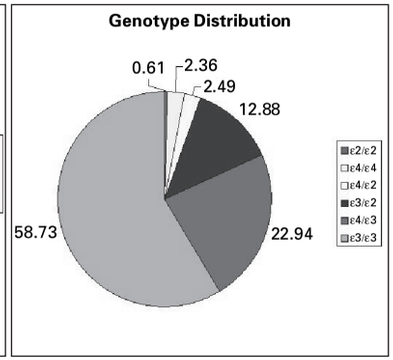
CHD is a complex multifactorial condition involving hereditary and environmental influences. There may be important gene–environment interactions. Attempts have been made to associate polymorphisms of candidate genes with LDL and HDL, cholesterol levels and susceptibility to CHD with conflicting results. However most studies were small, and few studies have included detailed analyses of diet and other known risk factors for CHD (Eichner *et al.* 2002).

The major lipids found in the plasma, cholesterol and triacylglycerols, are carried in the plasma as part of lipoprotein particles. Apo E is one of the constituents of lipoprotein particles. It acts as a ligand for the LDL receptor directing and regulating the uptake and metabolism of lipoproteins from the plasma. Apo E is a polymorphic protein. The apo E alleles ε2, ε3, and ε4 give rise to six different genotypes. The polymorphism arises from single nucleotide polymorphism at residues 112 and 158. These polymorphisms have different effects on serum levels of LDL, HDL and cholesterol.

In the Norfolk arm of the European Prospective Investigation of Cancer (EPIC), detailed information on diet, and on other lifestyle factors, family history, and blood lipids has been gathered from 25 000 participants and is being related to later development of CHD (Day *et al.* 1999). DNA has been extracted from blood samples collected from 15 000 individuals attending a second follow-up visit. Using a novel fast throughput Pyrosequencing protocol (Pyrosequence Application Note 208, 2001), these samples are being genotyped for apo E which will be examined for interactions between diet and other environmental variables and blood lipids, and CHD risk.

To date 11 712 samples have been genotyped for apo E. The genotype distributions from the 11 712 samples and effects on blood cholesterol levels were as expected and in agreement with other literature studies (see Figures) (Mahley & Rall, 2000).





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