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Effects of forage legumes on the fatty acid composition of beef. By N.D. SCOLLAN[1], M. ENSER[2], R.I. RICHARDSON[2] and J.D. WOOD[2], [1]*Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth SY23 3EB and* [2]*Division of Food Animal Science, School of Veterinary Science, University of Bristol, Langford, Bristol BS40 5DU*

Grasses and legumes are important to the livestock industry in developing more sustainable and environmentally friendly production systems. Grass is rich in α-linolenic acid and α-tocopherol, which enhances the content of *n*-3 polyunsaturated fatty acids (PUFA) in beef muscle and has important benefits for colour and shelf life in comparison with feeding grain-rich diets (Wood *et al*. 1999). The effect of legumes such as white and red clover on meat quality attributes such as fatty acid (FA) composition is not well established. The objective of this study was to determine the effects of forage type (grass or grass plus white or red clover) on the FA composition of beef from two breeds, the Welsh Black and Hereford.

Forty-eight pure-bred Welsh Black and Hereford steers, average live weight 230 kg, were allocated to each of three forage systems, resulting in eight animals per breed per treatment. Animals grazed a grass sward, a grass/white clover sward or a grass/red clover sward from May to October in the first year and from May in the second year and received silage prepared from the same forage during the intervening winter. Across the study the proportions of grass : clover were approximately 60:40 on a dry matter basis. Animals were slaughtered in the second summer period to achieve a similar level of carcass finish (fat class 4L) across all treatments. Lipids were extracted from *longissimus dorsi* muscle and fatty acids were analysed by GC.

	Forage					Breed			
	Grass	Grass/red clover	Grass/white clover	SED	*P*	Welsh Black	Hereford	SED	*P*
	(mg per 100 g muscle)								
C14:0	75.2	73.1	83.9	12.90	NS	72.5	82.3	10.54	NS
C16:0	747	747	824	99.7	NS	726	820	81.4	NS
C18:0	483	438	503	58.0	NS	467	483	47.4	NS
C18:1*n*-9	934	924	1023	115.4	NS	884	1037	94.2	NS
C18:1 trans	87.7	70.9	98.1	16.02	NS	82,2	89.0	13.08	NS
CLA	16.3	15.1	18.9	3.02	NS	15.3	18.2	2.47	NS
C18:2*n*-6	55.0	74.2	66.3	2.67	0.001	63.3	67.1	2.18	0.09
C18:3*n*-3	42.9	48.9	50.0	2.74	0.027	48.2	46.3	2.23	NS
Total fatty acids	2955	2877	3203	339.7	NS	2848	3175	196.1	NS
P:S	0.058	0.079	0.065	0.0054	0.001	0.070	0.065	0.0044	NS
n-6:*n*-3	1.31	1.53	1.34	0.043	0.001	1.33	1.46	0.035	0.001

Total intramuscular lipid contents were similar across diets and breeds and averaged 3012 mg/100 g muscle. Muscle contents of trans C18:1 and conjugated linoleic acid (*cis*-9, *trans*-11 CLA) did not differ significantly between diet or breed with overall means (mg/100 g muscle) of 85.6 and 16.8, respectively. Similarly the saturated FA, C14:0, C16:0 and C18:0 were not different. Muscle content of C18:2*n*-6 and C18:3*n*-3 were increased by 0.35 and 0.21 and 0.14 and 0.17 on inclusion of red and white clover in the diet, respectively, in comparison to grass alone. This contributed towards beneficially higher ratios of polyunsaturated : saturated fatty acids (P:S) on the legumes compared with grass. In contrast, the *n*-6 : *n*-3 ratio was lower on grass than on legumes, although on all treatments the ratio (mean 1.39) was less than the recommended target of <2.

It is recognised that feeding legumes results in higher dry matter intakes and this, along with reduced feed retention time in the rumen, helps to increase the flow of dietary C18:2*n*-6 and C18:3*n*-3 to the small intestine, and consequently higher contents of these fatty acids in beef muscle. The higher amounts of PUFA are highly desirable in terms of human nutrition.

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Wood JD, Enser M, Fisher AV, Nute GR, Richardson RI & Shear PR (1999) *Proceedings of the Nutrition Society* 58, 363–370.

The fatty acid composition of muscle and liver of lambs fed concentrates alone or with grass silage. By E. KASAPIDOU[1], J.D. WOOD[1], L.A. SINCLAIR[2], R.G. WILKINSON[2] AND M. ENSER[1], [1]*Division of Food Animal Science, University of Bristol, Langford, Bristol BS40 5DU and* [2]*ASRC, School of Agriculture, Harper Adams University College, Edgmond, Newport, Shropshire, TF10 8NB*

Meat from ruminants finished on fresh forage is a significant source of *n*-3 polyunsaturated fatty acids (PUFA) in the UK diet and has a good ratio of *n*-6: *n*-3 PUFA. Feeding cereal based concentrates when grass is not available decreases the nutritional quality by increasing linoleic (18:2 *n*-6) relative to α-linolenic (18:3 *n*-3) (Enser *et al.* 1998). High quality silage retains the grass fatty acid composition but fed alone results in a low daily liveweight gain. This study compared the fatty acid composition of muscle and liver from lambs fed concentrates alone or a mixture of concentrates and grass silage.

Two groups of eight Suffolk x Charollais lambs were fed one of two diets: (1) concentrates *ad libitum* (C) and (2) grass silage *ad libitum* supplemented with 400 g/day concentrates (CG) 2 months before slaughter. The concentrate consisted of wheat, soya hulls, soya bean meal, rape seed meal, oatfeed and molasses. Diets were supplemented with 500 mg/kg DM vitamin E which is recommended for extending the shelf life of meat. The ratio of 18:2 *n*-6: 18:3 *n*-3 in the diet consumed was approximately 8.9:1 and 0.9:1 for concentrates and grass silage plus concentrates respectively.

	M. *semimembranosus*			Liver		
Fatty acid (mg/100g muscle or liver)	C	CG	SEM	C	CG	SEM
SFA[1]	1024	1112	71.3	1646	2214	123.5
MUFA cis[2]	1213	1081	74.9	1312	1702	135.8
18:1 trans	83	57**	5.6	193	122*	21.0
18:2 *n*-6 (linoleic)	148	97***	7.1	493	295***	26.4
18:3 *n*-3 (α-linolenic)	23	39***	2.0	36	109***	4.9
CLA (conjugated linoleic)	20	20	1.7	31	65**	5.9
20:4 *n*-6 (arachidonic)	41	36	1.8	330	194***	16.0
20:4 *n*-3	9.1	9.3	0.5	50	22***	3.4
20:5 *n*-3 eicasapentaenoic (EPA)	20	30**	1.7	52	122***	3.6
22:4 *n*-6 (docosatetraenoic)	2.1	1.1***	0.1	41	15***	2.8
22:5 *n*-3 (docosapentaenoic) (DPA)	21	26*	1.2	136	217***	6.6
22:6 *n*-3 (docosahexaenoic) (DHA)	6.0	8.3**	0.4	92	189***	11.2
Total fatty acids	2610	2517	150.2	4412	5266*	281.8
P:S	0.17	0.12**	0.0	0.32	0.18***	0.0
18:2 *n*-6 / 18:3 *n*-3	6.48	2.52***	0.4	14.70	2.74***	1.1
Total *n*-6 / *n*-3	2.44	1.20***	0.1	2.36	0.77***	0.0

*P<0.05, ** P <0.01, *** P <0.001. [1]Sum of 12:0, 14:0, 16:0 and 18:0; [2] sum of 16:1, 18:1 *n*-9, 18:1 *n*-7 and 20:1 One way ANOVA (One way ANOVA with covariates: tissue vitamin E and/or fatness levels did not change the results significantly).

The relative concentrations of linoleic and α-linolenic acids in muscle and liver reflected the dietary ratios, with more linoleic in C lambs and more α-linolenic in CG lambs. Thus the 18:2 *n*-6:18:3 *n*-3 ratio in muscle and liver of C lambs was above the maximum of 4.0 recommended for the complete human diet compared with the acceptable lower values in the CG lambs. Dietary effects on the valuable EPA and DHA PUFA were less than for linolenic acid and greater in liver than muscle. Lambs fed diet C had better P:S ratios compared with those on the CG diet but they remained below the recommended 0.4. Trans 18:1 was higher in the C fed lambs. Liver contained greater quantities of the *n*-3 PUFA and a 100 g serving from the CG lambs would meet the daily recommended intake. Adding some silage to the diet of concentrate fed lambs produces “healthier” meat.

Enser M, Hallett KG, Hewett B Fursey GAJ, Wood JD & Harrington G (1998) *Meat Science* **49**, 329–341.

The effects of breed and diet on the fatty acid content of the phospholipid fraction of bovine *longissimus dorsi*. By H.E. WARREN[1,2], M. ENSER[2], K. HALLETT[2], J.D. WOOD[2] and N.D. SCOLLAN[1], [1]*Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB and* [2]*Division of Food Animal Science, School of Veterinary Science, University of Bristol, Langford, Bristol, BS40 5DU*

Beef is an important source of the beneficial *n*-3 series polyunsaturated fatty acids (PUFA), which can be further enhanced by offering feeds, such as linseed, that contain high levels of α-linolenic acid (C18:3*n*-3) (Scollan *et al*. 2001). Grass is an important feed for ruminants and is also rich C18:3*n*-3, in comparison with the more traditional concentrate feeds that are high in *n*-6 fatty acids. This study compared the effect of forage and concentrates on the fatty acid composition of the phospholipid fraction of beef muscle.

Sixteen Aberdeen Angus (AA) and sixteen Holstein-Friesian (HF) steers were allocated to one of two dietary treatments (*ad libitum* grass silage plus sugarbeet pulp shreds at *a.* 15% of the total dry matter (DM) intake or a barley-based concentrate and chopped barley straw at a ratio of 70:30 on a DM basis), resulting in eight animals per breed per dietary treatment. Intake of the concentrate-fed animals was adjusted to ensure similar growth rates between diets within breed. Animals were slaughtered at 14 months of age. Lipids for fatty acid analysis were extracted and fatty acids determined as methyl esters by gas chromatography. Data were analysed by ANOVA with diet and breed as the main effects.

Total fatty acid levels were higher (P=0.042) in the muscle phospholipids of silage-fed steers compared with their concentrate-fed counterparts. Also, levels of long-chain *n*-3 PUFA were much higher for C20:5*n*-3, C22:*n*-3 and C22:6*n*-3 (P<0.001) in the muscle phospholipids of the animals fed silage, and HF animals had higher (P=0.009) amounts of C22:6*n*-3. Feeding concentrate resulted in higher (P<0.001) amounts of C20:4*n*-6 in the muscle phospholipids. Changes in neutral lipid C18:2*n*-6 and C18:3*n*-3 levels (data not shown) reflected those in the phospholipid. Total lipid P:S ratio was reduced by feeding silage (P<0.001) and lower in the AA (P=0.003). The ratio of *n*-6 : *n*-3 in total lipid was markedly lower (P<0.001) in muscle from silage-fed animals.

	AA		HF					
	Concentrate	Silage	Concentrate	Silage	SED	Diet	Breed	Interaction
mg/100 g muscle								
Total FA	540	558	477	574	38.9	*	NS	NS
20:4*n*-6	13.8	5.8	13.5	6.9	0.78	***	NS	NS
20:5*n*-3	4.3	18.3	3.9	19.7	1.19	***	NS	NS
22:5*n*-3	11.0	25.1	10.1	25.9	1.58	***	NS	NS
22:6*n*-3	1.2	4.5	1.3	5.9	0.37	***	**	*
P:S and *n*-6:*n*-3 ratios (total fat)								
P:S†	0.23	0.08	0.26	0.10	0.011	***	**	NS
n-6:*n*-3‡	7.32	0.87	7.54	0.92	0.248	***	NS	NS

†P:S calculated as (18:2*n*-6 + 18:3*n*-3)/(12:0 + 14:0 + 16:0 + 18:0).
‡*n*-6:*n*-3 calculated as (18:2*n*-6 + 20:3*n*-6 + 20:4*n*-6)/(18:*n*-3 + 20:4*n*-3 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3).

This study demonstrates contrasting contributions of different beef feeding systems on muscle fatty acids in terms of human nutrition. The *n*-6 PUFA-rich concentrate diet increases the P:S ratio at the expense of the *n*-6 : *n*-3 ratio, whereas an all-forage diet had the opposite effect; namely, a decreased *n*-6 : *n*-3 ratio, but a more undesirable P:S ratio.

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Scollan ND, Choi NJ, Kurt E, Fisher AV, Enser M. & Wood JD (2001) *British Journal of Nutrition* **85**, 115–124.

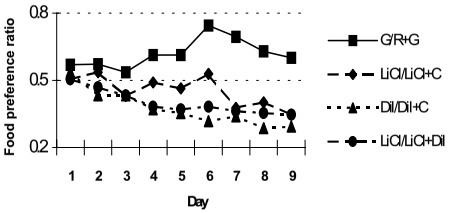
Development by chicks of selection against foods containing lithium chloride or diluted with wheat, or both. By D. LI and J.M. FORBES, *Centre for Animal Sciences, University of Leeds, Leeds LS2 9JT, UK*

Negative feedback and learned associations between metabolic consequences and the sensor properties of foods are important in the control of food intake and diet selection (Forbes, 2001). It has been suggested that the effects of discomfort generated by eating sub-optimal foods are additive (Forbes, 1999), in which case animals would develop aversion to a toxic food more slowly if the other food given in choice was nutritionally deficient (and therefore aversive itself) than if the other food was nutritionally balanced. This experiment investigated the development of choices between normal food (C), a food rendered mildly toxic by the addition of lithium chloride (LiCl) and a nutrient-diluted food (Dil), the pairs of foods being distinguished by colour.

Eighty-eight female broiler chickens were allocated to eight groups 21 d after hatching; each group was offered a pair of foods, one coloured green (G) the other red (R), for 9 d. The experiment was a 2 × 2 factorial design, replicated twice, with two levels of LiCl (0 and 6 mg/kg food; C and LiCl) and two levels of dilution of the ground standard starter food with ground wheat (0 and 420 mg/kg; C and Dil). For the second group within each treatment the colours were reversed between the two foods. The development of preference was described by the slope of the relationship between the amount of one food eaten as a proportion of total food intake (see Figure).

Treatment	C/C	LiCl/C	C/Dil	LiCl/Dil
Intake on days 7--9 (g/d)	127	83	118	92
Body weight on day 7 (g)	913	743	900	737

Food intake averaged 99 g/d at the start of the experiment and the mean weight of the birds was 457 g. The Table shows that intake and weight gain were depressed by the two treatments in which one of the foods was LiC1.



In the control groups there was an initial strong preference for G but this declined after day 6 and the slope was not significantly different from no choice (0.5) (see Figure). When the choice was between C and LiC1 an aversion for LiC1 developed progressively when LiC1 was R but when LiCl was G it was preferred for the first 6 d, then there was a sudden switch to C (R) so that from day 7 aversion to LiCl was similar in the two groups. Over the whole experimental period the slope of the relationship between the weight of LiC1 eaten as proportion of total intake was significant (–0.018 per day; P<0.05). Both groups given choice between C and Dil progressively developed an aversion to Dil, irrespective of colour pairing, at a rate of –0.028 per day (P<0.001). Those groups offered LiC1 and Dil similarly developed an aversion for LiCl (slope = –0.019; P<0.001). The differences between the slopes for LiCl/C and LiCl/Dil were not significant.

Although LiCI depressed food intake and body weight gain the birds did not avoid it completely. Their aversion to LiC1 developed at the same rate whether the choice was between foods containing LiC1 and normal food, or LiC1 and diluted food, even though Dil was strongly aversive when given in choice with C. Had there been additivity between the effects of LiCI and those of Dil on preference we would expect the aversion to LiC1 to develop more quickly when it was paired with C than with Dil.

Forbes JM (1999) *Appetite* 33, 371.
Forbes JM (2001) *Comparative Biochemistry and Physiology A* **128**, 461–468.

The effect of the biological selenium status on the oxidative stability of sheep meat By E. KASAPIDOU[1], J.D. WOOD[1], B.G. MERRELL[2], S.N. BROWN[1] AND M. ENSER[1], [1]*Division of Food Animal Science, School of Veterinary Science, University of Bristol, Langford, Bristol BS40 5DU* and [2]*ADAS Redesdale, Otterburn, Newcastle Upon Tyne NE19 1SB*

Vitamin E (α-tocopherol) is the main antioxidant extending the shelf life of muscle foods in terms of myoglobin and lipid oxidation. However, *in vivo* antioxidant protection is provided by a combination of vitamin E and the selenium containing glutathione peroxidases (GSHPx) (Ammerman and Miller, 1974). The activity of these enzymes is dependent on the dietary selenium intake and decreased activity as a result of selenium deficiency will increase the requirement for vitamin E, resulting in decreased levels at slaughter. Soil and plant selenium levels are considered to be generally low throughout the UK and the best indicator of biological status is the erythrocyte GSHPx activity. The purpose of this experiment was to investigate the quality of meat in terms of lipid oxidation from two groups of lambs differing in their selenium status based on the erythrocyte GSHPx activity.

Two groups of twelve Blueface Leicester × Scottish Blackface wether lambs grazing on pastures with reputedly marginally low or adequate soil selenium levels were selected. Lambs were slaughtered two months later. At slaughter, blood samples were collected for GSHPx activity, creatine kinase and plasma vitamin E levels. M. *semimembranosus* samples were collected for vitamin E, selenium, fatty acid composition analysis and for lipid oxidation as thiobarbituric acid reacting substances (TBARS) during simulated retail display in modified atmosphere packaging.

	High GSHPx	Low GSHPx	SEM		High GSHPx	Low GSHPx	SEM
GSHPx (u/ml RBCs at 37 C)	100.9	51.3***	4.99	TBARS Display day 3[1]	0.71	0.34**	0.09
Plasma vitamin E (μg/ml)	1.18	0.67**	0.09	TBARS Display day 6[1]	1.04	0.66	0.19
Muscle selenium (μg/g DM)	0.06	0.08*	0.00	Total *n-3* PUFA[2]	5.40	6.09	0.29
Muscle vitamin E (μg/g)	3.06	3.04	0.19	Total *n-6* PUFA[2]	5.85	6.06	0.34

One way ANOVA * $P<0.05$, ** $P<0.01$, *** $P<0.001$. [1] mg malonaldehyde/kg muscle; [2] g/100g fat

The GSHPx activity was in agreement with the designation of the pastures, with the "marginal" pasture producing lambs with half the GSHPx activity of the sufficient pasture although just above the recognised deficiency level of 50. The lower plasma vitamin E levels of the low GSHPx lambs suggests greater utilisation of plasma vitamin E, protecting the lambs from oxidative stress. However, this pattern was not observed in the muscle where the levels of vitamin E and selenium were similar. Lipid oxidation (TBARS) was higher for the high GSHPx lambs than for the low ones but this may reflect other differences in intrinsic factors between the groups since the increase in oxidation between days 3 and 6 was similar for both treatments indicating that oxidation was proceeding at the same rate in both. Tissue polyunsaturated fatty acid (PUFA) content was similar in the two groups, indicating no differences in endogenous peroxidation. The study shows that marginal selenium deficiency over the finishing period did not adversely affect the quality of sheep meat in terms of lipid oxidation.

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Ammerman CB & Miller SM (1974) *Journal of Dairy Science* **58,** 1561–1578.

The individual and combined effect of thymol and carvacrol on coccidia-infected broiler chickens. By F. IBRIR, H.M.R. GREATHEAD and J.M. FORBES, *Centre for Animal Sciences, Leeds Institute for Plant Biotechnology and Agriculture, University of Leeds, Leeds LS2 9JT*

Coccidiosis is a ubiquitous disease and one of the main problems affecting the poultry industry. The use of anticoccidial drugs has led to the occurrence of resistant strains of *Eimeria*. Alternatives are being sought to control the disease, one of which is the use of 'natural' products. Allen *et al.* (1998) identified products with antioxidant properties as being beneficial for the control of coccidiosis in poultry. Thymol and carvacrol are the major components of the essential oils of thyme and oregano, respectively, and have antioxidant properties (Aeschbach *et al.* 1994). The aim of this study was to test the individual and combined effects of thymol and carvacrol on the performance of broiler chickens infected with *Eimeria acervulina*.

Day-old female Cobb chicks (n=80) were randomly allocated to one of five treatments so that each group had eight replicates (two birds per cage): uninfected untreated (UU), infected untreated (IU), infected thymol-treated (IT), infected carvacrol-treated (IC) and infected thymol/carvacrol (1:1, w/w) treated (IT/C). Birds were fed *ad libitum* on a ground coccidiostat-free commercial finisher to which was added thymol (125 ppm) and/or carvacrol (125 ppm) to the relevant diets. Birds had free access to drinking water. At 28 d of age, birds in the appropriate treatment groups were infected with 50 000 sporulated *Eimeria acervulina* oocysts. During the experimental period (15 d), feed intake (FI), weight gain (WG) and faecal oocyst output were measured. One bird was killed from each cage at day 7 post-infection (PI) and the second at day 15 PI. Blood and duodenal tissue samples were taken from the birds post-slaughter for plasma carotenoid analysis and histological analysis (tunica muscularis thickness, crypt depths and villi heights), respectively.

Thymol treatment tended to increase food intake (P=0.087). It significantly (P<0.05) increased the weight gain of the infected birds and significantly (P<0.05) improved feed conversion efficiency (FCE). In contrast, carvacrol treatment significantly (P<0.05) reduced weight gain of the infected birds. Both thymol and carvacrol treatments significantly (P<0.05) decreased the crypt depth in the infected birds. There was a significant (P<0.01) interaction between thymol and carvacrol on the crypt depth and plasma carotenoids, with the effect of the combined T/C treatment being less than the effects of thymol and carvacrol when used individually. There was no significant (P>0.05) effect of either thymol or carvacrol on villi height, plasma carotenoids and oocyst output. Infection significantly (P<0.001) increased crypt depth and significantly (P=0.001) decreased plasma carotenoids.

Treatments	UU	IU	IT	IC	IT/C	SEM (n=8)	Probability			
							Infection	Thymol	Carvacrol	Thym.+Carv.
FI (g/bird/d) d1–15 PI	78	83	86	77	84	2.8	NS	0.087	NS	NS
WG (g/bird/d) d1–15 PI	30	33	35	29	34	1.6	NS	0.043	0.035	NS
FCE d1–15 PI	0.43	0.42	0.45	0.41	0.46	0.015	NS	0.043	NS	NS
Tunica muscularis (μm) d7PI	151	149	144	129	134	6.5	NS	NS	0.026	NS
Crypt depth (μm) d7PI	139	325	265	262	275	10.8	<0.001	0.031	0.017	0.001
Villi height (μm) d7PI	1039	1020	1076	1034	1018	28.7	NS	NS	NS	NS
Carotenoids (μg/ml) d7PI	2.13	1.52	1.91	1.87	1.74	0.085	0.001	NS	NS	0.004
Log 10 (total oocysts+1) d1–15PI		8.53	8.61	8.60	8.62	0.052		NS	NS	NS

Data from the infected birds were analysed as a 2×2 factorial ANOVA and the effect of infection by comparing UU *v.* IU groups as a one-way ANOVA. Statistical significance was set at P<0.05.

While thymol and carvacrol showed no effect on the output of *Eimeria acervulina* oocysts, thymol alone had a significant (P<0.05) beneficial effect on the performance of the coccidia-infected chickens, suggesting that it provided the birds with some level of protection from the disease.

Aeschbach R, Loliger J, Scott BC, Murcia A, Butler J & Halliwell B (1994) *Food and Chemical Toxicology* **32**, 31–36.
Allan PC, Danforth HD & Augustine PC (1998) *International Journal for Parasitology* **28**, 1131–1140.

Effects of quebracho tannins on nutrient utilisation of chickpeas by sheep. By M.H.L. BENTO[1,2], T. ACAMOVIC[1] and J.M.F. ABREU[2], [1]*ASRC, SAC, Ayr, KA6 5HW and* [2]*DPAA, ISA, Universidade Técnica de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal*

The objective of the present study was to assess the effect of quebracho tannins (QT) on rumen degradation and post-rumen digestion of chickpeas (*Cicer arietinum*). Chickpeas, like other legume seeds, degrade rapidly in the rumen, thus reducing nutrient availability in the duodenum. The use of tannins has been proposed as potential additives for improving the utilisation of protein-rich feeds in sheep.

Chickpeas (dry matter (DM) = 918 g kg^{-1}; nitrogen (N) = 35 g kg^{-1} DM) were ground (2mm), QT were then added to ground chickpeas (C) and the following treatments were obtained: C_0, C_{QT250} and C_{QT350}, containing 0, 250 and 350 g of QT kg^{-1} CP, respectively.

In experiment 1, C_0, C_{QT250} and C_{QT350} were incubated in nylon bags in the rumen of sheep for 3, 6, 9, 12, 16, 24, 48 and 72 h. *In situ* degradabilities were fitted to the $p=a+b(1-e^{(-ct)})$ model (p represents the loss from the bag after t hours, a is the rapidly degradable fraction, b the slowly degradable fraction and c the rate of degradation of fraction b; Ørskov & McDonald, 1979). Comparisons among treatments for the observed degradability were made using analysis of variance with the animal effect as a block.

A precision feeding study (experiment 2) was used in broilers (Ferraz de Oliveira *et al.* 1994) as a model for the post-rumen digestion of sheep. Broilers were tube-fed C_0, C_{QT250} and C_{QT350} and the coefficients of true DM digestibility (TDMD) and N retention (CNR) were calculated. Differences between treatments were analysed using analysis of variance.

In Experiment 1, QT decreased ($P<0.05$) zero time washing losses when compared with C_0. The disappearance of DM (g 100 g^{-1} DM) from chickpeas in the nylon bag was not significantly ($P>0.05$) affected by QT throughout the incubation period, except at 48 h ($P<0.05$). The disappearance of N (g 100 g^{-1} N) was lower in C_{QT250} at 3 h ($P<0.05$) when compared with C_0, and lower in C_{QT350} at 3 h ($P<0.05$) when compared with C_{QT250}. The exponential equation of Ørskov & McDonald (1979) poorly described the rumen degradability of chickpeas (R^2=0.87, SE 2.47) and a large deviation from the adjusted curves was observed (RSD=2.47, SE 1.10). The disappearance of N did not fit the exponential equation $p=a+b(1-e^{(-ct)})$.

In Experiment 2, TDMD decreased (P=0.066) from 0.71 (SE 0.047) for C_0 to 0.61 (SE 0.019) for C_{QT350}. The CNR was low and variable (0.16 (SE 0.226) for C_0, 0.37 (SE 0.076) for C_{QT250} and 0.17 (SE 0.087) for C_{QT350} ($P>0.05$)). The excretion of threonine increased from 0.08 g (C_0) to 0.19 g in (C_{QT350}) ($P<0.05$).

The rapidly degradable fraction (a) in chickpeas is mainly composed of starch (highly degradable) and this may interfere with the ability of tannin to reduce rumen degradability. Similarly, the dilution of the large rumen volume might have interfered with the ability of the tannin to reduce the disappearance of DM. In the lower gastrointestinal tract, tannins increased threonine excretion, which supports the hypothesis that there is an increased excretion of mucins from the mucosa of the gut. This may account for increased endogenous losses and poorer CNR.

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Ferraz de Oliveira MI, Hillman K & Acamovic T (1994) In *Plant-Associated Toxins: Agricultural, Phytochemical and Ecological Aspects*, pp. 195–200 [SM Colegate and PR Dorling, editors]. Wallingford, UK: CAB International.

Ørskov ER & McDonald I (1979) *Journal of Agricultural Science* **92**, 499–503.

Influence of grass silage fermentation and concentrate composition on the fatty acid composition of bovine muscle. By A.P. MOLONEY[1], D. McGILLOWAY[1] and C. STANTON[2], [1]*Teagasc, Grange Research Centre, Dunsany, Co. Meath, Ireland and* [2]*Teagasc, Dairy Products Research Centre, Moorepark, Fermoy, Co. Cork, Ireland*

An increase in the polyunsaturated (P):saturated (S) fatty acid ratio and a decrease in the n-6:n-3P ratio would enhance the nutritive value of beef. Ruminant fat is a relatively enriched source of conjugated linoleic acid (CLA), which has many human health-enhancing effects. The n-3P and CLA concentrations are higher in muscle from grass-fed than from concentrate-fed or grass-silage-fed cattle (French *et al.* 2000). Concentrates differ in their content and profile of fatty acids, which may also be influenced by grass ensiling procedures. Little information is available on the extent to which such differences are subsequently reflected in muscle composition. Groups of fifteen Friesian steers were offered for 20 weeks either: extensively fermented grass silage (25 kg molasses/t grass), restricted fermentation grass silage (9 l (48% formic acid, 16% ammonium tetraformate/l)/t grass), a starchy concentrate (895 g barley/kg), a fibrous concentrate (880 g unmolassed sugarbeet pulp/kg), or zero-grazed grass. Concentrate allowances were restricted and the forages supplemented with a minimum quantity of a common concentrate to ensure similar carcass growth for all groups. *Longissimus dorsi* fatty acid composition was measured as described by French *et al.* (2000) and data were subjected to ANOVA.

	Silage		Concentrate				
Fatty acids (g/100 g FAME)	Extensive	Restricted	Starch	Fibre	Grass	SED	Sig.
C16:0	284.1	280.0	285.1	277.1	264.3	5.13	***
C18:0	118.0	115.8	114.1	113.9	115.9	4.54	NS
C18:1	374.9	384.2	373.3	388.3	385.8	7.90	NS
C18:2	36.7	34.3	35.5	34.7	77.6	2.58	NS
C18:3	7.3	7.5	6.5	6.5	10.2	0.69	***
C18:2 CLA	3.7	4.2	3.8	4.3	6.9	0.49	***
C20:4	9.1	7.9	9.0	8.2	8.5	1.20	NS
S	448.8	441.9	447.2	435.6	423.1	7.95	*
P	61.9	59.1	60.2	58.9	68.9	4.36	NS
P:S	0.10	0.10	0.10	0.10	0.12	0.008	*
C18:2:C18:3	5.14	4.67	5.66	5.55	3.86	0.397	***
n-6:n-3P	4.35	4.05	4.72	4.76	3.38	0.258	***

*$P<0.05$; **$P<0.01$; ***$P<0.001$.

Restricting fermentation tended to decrease the proportion of C18:3 (506 *v.* 456 g/kg) and to increase the proportion of C16:0 (219 *v.* 254 g/kg) of fatty acids, in agreement with Dewhurst & King (1998). The grass contained 503 g C18:3 and 212 g C16:0/kg fatty acids. The starchy concentrate had 453g C16:0, 335g C18:1 and 203g C18:2 and the fibrous concentrate 470g C16:0, 418g C18:1 and 79g C18:2/kg fatty acids.

On average, grass silage resulted in lower n-6:n-3P and C18:2:C18:3 in muscle than concentrates (see Table). There was little effect of grass silage fermentation or concentrate composition on fatty acid composition of muscle. Muscle from grass-fed cattle had higher ($P<0.05$) C18:3 and CLA proportions and P:S and lower ($P<0.05$) n-6:n-3P and C18:2:C18:3 than all other diets.

It is concluded that manipulating silage fermentation or selection on C18:2 concentration within conventional, non-oilseed concentrates offers little opportunity to enhance the fatty acid composition of muscle. That a similar muscle CLA concentration was observed for the fibrous concentrate despite lower C18:2 consumption suggests a role for this ingredient in optimizing CLA production from concentrate diets.

Dewhurst RJ & King PJ (1998) *Grass and Forage Science* **53**, 219–224.

French P, Stanton C, Lawless F, O'Riordan EG, Monahan FJ, Caffrey PJ & Moloney AP (2000) *Journal of Animal Science* **78**, 2849–2855.

In-vitro evidence for plant enzyme mediated lipolysis in the rumen. By M.R.F. LEE[1], M.K. THEODOROU[1], T.T. CHOW[2], M. ENSER[3] and N.D. SCOLLAN[1], *[1]Institute of Grassland and Environmental Research, Aberystwyth, UK, SY23 3EB, [2]Ghent University, Department of Animal Production, 9090 Melle, Belgium, [3]University of Bristol, School of Veterinary Science, UK, BS18 7DY.*

It is generally accepted that microbial enzymes are involved in lipolysis and are thus responsible for the destruction of plant membranes in the rumen. However, we question this assertion and tested the hypothesis that in ruminants grazing fresh pastures, the first stages of lipolysis could be mediated by plant lipases. These enzymes are ubiquitous in plants and their regulation might be altered as a consequence of the dual stress of elevated temperature and anoxia imposed on the plant metabolism of intact plant cells ingested by ruminants. If the hypothesis is proven, it might be possible to reduce the rate and extent of lipolytic activity in the rumen by selectively breeding forage plants with reduced lipolytic activity, with potential benefit to both the livestock producer and the consumer.

Leaf blades of *Lolium perenne* (var. AberElan), grown under controlled conditions (25^{o} and 14h artificial light d^{-1}), were harvested (3cm above ground level) after 5 weeks, cut into 5mm segments and incubated in 50ml anaerobic phosphate buffer (50 mM Na_2HPO_4 and 50 mM KH_2PO_4) at 39^{o}C for up to 360 min. At each time point, (0, 15, 30, 60, 120 and 360 min) leaf segments were removed from each of 3 replicate incubations together with a 1ml sample of the buffer solution, taken for volatile fatty acid and lactate analysis. Internal standard was added to the buffer and leaf blades (1ml of 2.5mg C19:0/ml chloroform) and 20ml isopropanol to deactivate any plant enzymes. The sample was blended and filtered through a glass fibre filter. An isopropanol : chloroform (1:1 v/v) extraction was carried out on the filtered residue which was then recombined with the filtrate, rotary evaporated and resuspended in chloroform : methanol : saline (8:4:3) and extracted using the method of Folch *et al.* (1957). The lipid fractions were separated by thin layer chromatography, the individual layers methylated and analysed by gas chromatography. Both linoleic and linolenic acids were analysed to determine the level of biohydrogenation of the acids during the incubation period. The individual fractions are represented as percentage of total fatty acids and analysed by regression analysis and Student's t-test between individual time points using Genstat (Lawes Agricultural Trust, 1997).

	Incubation time in minutes							P	
	0	15	30	60	120	360	SED		
Lipid Fraction (%)								Linear	Quadratic
Triacylglycerol	3.6	5.1	5.0	6.4	8.1	13.6	1.35	0.001	
Free Fatty Acids	3.5	4.1	4.5	3.9	3.5	9.8	0.78	0.001	
Polar Fraction	67.9	64.7	63.3	73.2	73.2	50.2	5.37	0.045	0.008
Monoglycerides	1.7	2.7	1.7	2.2	3.2	5.1	1.25	NS	
Diglycerides	1.6	1.8	1.9	2.2	5.6	7.0	0.37	0.001	
Total Lipid (g/kg DM)	24.9	24.0	24.1	22.9	23.7	22.2	2.49	NS	

The results show a change in the lipid fractions towards the end of the incubation period, with no change in total lipid recovery or percentage linoleic and linolenic acids (12.4 and 64.5, respectively). After 120 min there was a significant decline in the polar fraction and an increase in free fatty acids, triacylglycerol and diglycerides. It was concluded that a proportion of the polar fraction underwent lipolysis resulting in an increase in free fatty acids, which were then used for lipogenesis resulting in an increase in the triacylglycerol- and diglyceride fractions. Although trace amounts of acetate (propionate and butyrate were not detected) and lactate (0.71 and 1.21 mmol/l, respectively) were found in the buffer at the end of the incubation, their low levels made it unlikely that activity from contaminating bacteria (brought in with the leaf blades) could be responsible for the observed changes. Moreover as the linoleic or linolenic acids were not biohydrogenated, it appears that the lipolysis and lipogenesis was indeed plant-mediated and that the trace levels of acetate and lactate were of plant origin.

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Folch J, Lees M & Stanley GHS (1957) *Journal of Biological Chemistry* **226,** 497–509.

Colour of subcutaneous adipose tissue and _longissimus dorsi_ muscle of heifers fed grass, grass silage or concentrate-based diets. By P.G. DUNNE[1,2], A.P. MOLONEY[1], F.J. MONAHAN[2] and F.P. O'MARA[3], *[1]Teagasc, Grange Research Centre, Dunsany, Co. Meath, Ireland, [2]Department of Food Science and [3]Department of Animal Science and Production, University College Dublin, Dublin 4, Ireland*

Accumulation of the carotenoids, β-carotene and lutein, is responsible for the yellow subcutaneous (SC) adipose tissue of bovines produced in grass-based beef systems (Knight *et al.* 2001). Cattle that graze pasture are reported to have darker muscle colour (Muir *et al.* 1998). The objective of this experiment was to determine the effect of dietary composition and duration of feeding on SC adipose tissue and *M. longissimus dorsi* (LD) colour and pigment concentrations of heifers.

Fifteen heifers were permanently housed (PH) and fed a concentrate diet (PH-LC). Fifty-four heifers were grazed for 3 months (PA), housed and offered concentrates (PA-SC), 20% grass silage (PA-GS20), 50% grass silage (PA-GS50) or zero-grazed grass (PA-GRA). Heifers (n=3/treatment (T)) were slaughtered at housing and 28, 56, 91 and 120 days (D) thereafter. Yellowness ('b' value) of SC adipose tissue and lightness ('L' value) and redness ('a' value) of LD were recorded 48 h post-mortem using a Minolta Chromameter (model CR300). β-Carotene and lutein contents of SC adipose tissue were determined by reverse-phase HPLC and total LD haem pigments were determined spectrophotometrically at 525 nm. Data were analysed as a 5T × 5D factorial design.

At housing, 'b' values of the grazing group (mean=13.46) were higher (P<0.01) than those of the PH-LC group (mean=10.35) but there was no difference in β-carotene or lutein concentrations. A significant T×D interaction occurred (P=0.035) for SC adipose tissue 'b' value, whereby yellowness of PA-GS20 and PA-GS50 heifers decreased (P<0.01 and P<0.05, respectively) from housing to day 28, but then increased to day 120, significantly so for PA-GS50. Yellowness of PA-SC heifers decreased (P<0.01) from housing to day 28, and tended to decrease to day 120, similar to PH-LC heifers. The PH-LC heifers had the least yellow SC adipose tissue (P<0.05) on all dates.

	Treatment					
Pigment	PH-LC	PA-SC	PA-GS20	PA-GS50	PA-GRA	SED
β-carotene (μg/g)	0.17^{a} †	0.28^{ab}	0.31^{b}	0.36^{bc}	0.45^{c}	0.06
Lutein (μg/g)	0.14^{a}	0.21^{b}	0.23^{bc}	0.23^{bc}	0.29^{c}	0.03
Haem (mg/g)	5.84^{a}	6.62^{b}	6.73^{b}	6.7^{b}	6.4^{ab}	0.33

†Within row, means with different superscripts differ significantly (P<0.05).

Only T had a significant effect on SC adipose tissue β-carotene and lutein (both P<0.001) (see Table). The effect of T on LD haem pigments tended towards significance (P=0.058) whereby PH-LC heifers, which did not graze pasture before housing, tended to have lower haem pigments than other heifers. The effect of D on LD lightness was significant (P<0.001) with the LD of PH-LC heifers tending to become lighter than other T after 56 d housing.

It is concluded that, while concentrate feeding (PH-LC and PA-SC) led to a time-dependent decrease in SC adipose tissue yellowness relative to PA-GRA, partial removal of forage from the diet (PA-GS20, PA-GS50) did not lead to a consistent reduction in yellowness. Removing the grazing opportunity for 3 months may have led to production of paler muscle with a lower pigment content, but further research is required to confirm whether such an effect occurs and how it may be mediated.

Knight TW, Death AF, Lambert MG & McDougall DB (2001) *Australian Journal of Agricultural Research* **52**, 1023–1032.

Muir PD, Smith NB, Wallace GJ, Cruickshank GJ & Smith DR (1998) *New Zealand Journal of Agricultural Research* **41**, 517–526.

Use of distortions of the jaws and the skull during eating and ruminating to measure strength of chewing in sheep fed roughage and concentrate diets. W.L. GROVUM[1], J.J. THOMASON[1], W.W. BIGNELL[1] and A.G. DESWYSEN[2] (deceased), [1]*Department of Biomedical Sciences, University of Guelph, Ontario, Canada, N1G 2W1 and* [2]*Université Catholique Louvain, Belgium*

The central question is whether ruminants, which thrive on roughages, will chew harder on a poor quality hay than on good quality hay to try to sustain their energy intakes. Although they will generally compensate by chewing the poorer roughage longer during both eating and ruminating, in the end, intakes are often positively associated with digestibility (Dulphy *et al*. 1980). In this context, the critical question is whether ruminants will chew the poorer hay harder as well as longer to try to maintain their intakes. Ultimately, chewing produces small particles which will either be fermented in the forestomachs to produce useful metabolites or passed to the lower gut for further digestion and elimination. These actions reduce distension and promote throughput in the stomach to facilitate roughage intake.

The answer was sought by using strain gauges to measure the extent of the bending of the bones in the jaws and the skull while sheep were eating hays differing in toughness. The focus was on how the sheep used facial bones to chew rather than on bone strength *per se*. Mastication during rumination was studied briefly to compare this grinding process to that of chewing. The strength of chewing was measured for a pelleted diet, since it differed markedly from the roughages in texture.

Five sheep were used to study the strain energy developed during chewing and how this was transmitted through the jaws and skull to fragment food. With the sheep under sodium pentobarbital anaesthesia, three simple strain-gauges were glued to the underside of each jaw bone and four rosette gauges to the maxilla and the frontal bones on each side of the skull. Upon recovery, each sheep was fed randomly either bromegrass hay (*Bromus mollis*; 64% NDF) which was tough, lucerne hay (*Medicago sativa*; 53% NDF) or finisher pellets (31% NDF) twice while recording strains (με) for 22.5 s periods. The sheep chewed on either their left or right side but never on both sides together (side changed frequently). The underside of the jaw used for chewing bent down in the middle, creating gauge tension under the bolus. It was bent upward at the front on this side by forces transferred from the opposite jaw whose underside was always bent upwards in the middle by unopposed masseter muscle action (no bolus here). The mean peak strains recorded in the mandibular gauges during chewing were not significantly different for the two hays (+476 and +485 for tensions in the middle gauges on chewing side and –524 and –607 for compressions in these gauges on the balancing side; $P>0.05$) but they were greater than the maximum values for the pelleted diet (+80 on the front chewing side; –145 on the front balancing side, $P<0.05$). Not unexpectedly then, the mean chewing effort (mean area under chewing strain curves) was similar for the hays, being 61 *v.* 68 ($P>0.05$) and exceeded that for pellets (29; $P<0.004$). With right- or left-sided chews, both frontal bones were compressed since the maxillae were rotated caudally and dorsally. The compressions were once again similar for the two hays (–264 and –290; $P>0.05$) and exceeded that for pellets (–103; $P<0.01$). The maxillae in the nose rotated slightly toward the non-chewing side due to the compression of the bolus during chewing and the unopposed downward pull by the masseter on the opposite side. Again, hay effects were similar ($P>0.05$) on the chewing (–124 *v.* –115) and non-chewing sides (341 *v.* 399) and exceeded corresponding values for pellets (–56 *v.* 131; $P<0.04$). Since chewing frequency was similar for all diets (118–128/min; $P>0.05$), a tougher roughage must be chewed longer with more chews/kg dry matter eaten. Similar grinding processes were used for chewing during eating and ruminating, since the two sets of strain curves were similar in shape. The differences between animals in their chewing efficiencies and levels of food intake should now be studied. This may help to increase the productivity of ruminants at pasture.

The eating data will be presented elsewhere (Grovum *et al*. 2002).

Dulphy JP, Remond B & Theriez M (1980) In *Digestive Physiology and Metabolism in Ruminants*, pp. 103–122 [Y Ruckebusch and P Thivend, editors]. Lancaster: MTP Press.
Grovum WL, Thomason JJ, Bignell WW & Deswysen AG (2002) *Journal of Animal Science* (in press).

A study of gas pressure receptors in the reticulorumen which may evoke eructation in sheep. By W.L. GROVUM and W.M. SHAIK MOSSADEQ, *Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, N1G 2W1*

This is the first report of receptors which specifically sense gas pressure in the reticulorumen and, possibly, elicit eructation via a vago-vagal reflex. Although the gas pressure sensors have not been investigated before, there is some information on the centres in the brain which coordinate the gastric contractions and oesophageal events enabling eructation (Grovum & Gonzalez, 1998). Ruminants must eructate, as carbon dioxide and methane gases are produced during microbial fermentation of food in their forestomachs. A failure of this mechanism will result in bloat and, frequently, death by asphyxiation. A lesser consequence is a reduction in food intake. Previous studies showed that eructation could be evoked in conscious animals by insufflating the rumen with air, carbon dioxide, nitrogen, methane or hydrogen (Dougherty, 1940; Louvier *et al*. 1979) whereas it was inhibited by distending the reticulorumen with fluid (Dougherty *et al*. 1958). The sensors triggering the reflex exist in the cranial reticulorumen, as insufflation of a gastric pouch surrounding the cardia elicited eructation (Dougherty *et al*. 1958).

The gas pressure sensors were studied using a computerized version of the single fibre technique in twenty-eight sheep anaesthetized to effect with sodium pentobarbital. Their forestomachs were exposed to 0, 5, 10, 15, 20 and 25 mmHg air pressure, tactile stimulation and manual stretch. Each sheep was in dorsal recumbency with its head secured in a stereotaxic apparatus, its cardia blocked with an air-filled balloon, its left vagus in a pool of silicone oil and its rumen emptied and washed through a rumenotomy in the ventral sac. Impulses from single gas sensors were isolated from impulses in several fibres in a strand of the vagus connected to the reticulorumen using spike templates and counters in Multi-Spike Detection software (Alpha Omega, Israel). Seven receptors in seven different sheep responded with increasing spike frequencies between pressures of 6.7 and 19.0 mmHg. Thereafter, the spike frequencies decreased. The background activities of three of these receptors were also inhibited by manual stretch. This enabled their localization to the cranial sac's dorso-medial wall (two receptors) and dorsal wall. One other receptor devoid of background activity was localized to the dorsal sac just cranial to the dorsal blind sac when the receptor responded slightly to manual stimulation. Despite this latter feature of the receptors, they did not behave like in-series tension receptors or epithelial receptors (Leek, 2001) since they failed to respond in a graded manner to manual stretching and to light brushing of the wall, respectively. Recordings made simultaneously from overlapping in-series tension and gas sensor units in one sheep proved conclusively that the in-series receptor was excited by manual stretch of the wall, whereas the gas sensor was inhibited.

The threshold pressure activating the gas sensors (6.7 mmHg) also evoked eructation in conscious sheep (Ruckebusch & Tomov 1973; Louvier *et al*. 1979). This fact and the localization of the sensors in the dorsal parts of the cranial and dorsal sacs, where gas normally accumulates, lend credibility to the findings. Further, the inhibition of gas sensor activity at high pressures may explain in part why eructation is diminished during the terminal stages of bloat (Siegmund, 1979). Froth covering the area around the cardia, like fluid, may also contribute to this inhibition (Dougherty, 1961).

A preliminary report of the above work was written earlier and will be presented elsewhere (Shaik Mossadeq & Grovum, 2002).

Dougherty RW (1940) *Journal of the American Veterinary Medical Association* **96**, 43–46.
Dougherty RW, Habel RE & Bond HE (1958) *American Journal of Veterinary Research* **19**, 115–128.
Dougherty RW (1961) In *Digestive Physiology and Nutrition of the Ruminant*, pp. 79–87 [D Lewis, editor]. London: Butterworths.
Grovum WL & Gonzalez JS (1998) *European Journal of Neuroscience* **10** (Suppl 10), 250 (Abstr. 97.01).
Leek BF (2001) *Veterinary Quarterly* 23, 26–31.
Louvier JA, Colvin HW, Ishizaki G, Iwamoto GA & Parker HR (1979) *Journal of Animal Science* **48**, 934–940.
Ruckebusch Y & Tomov T (1973) *Journal of Physiology* **235**, 447–458.
Shaik Mossadeq WM & Grovum WL (2002) *Journal of Animal Science* (in press).
Siegmund OH (1979) *The Merck Veterinary Manual*, 5th Edn, pp. 145–148. Rahway, NJ: Merck and Co.

Reducing dietary fat intake: strategies employed by subjects in the UK Women's Cohort Study. By C. GOLDING[1], J. CADE[1], S. KIRK[1], C. LAWTON[2] and D. GREENWOOD[1], [1]*Nutrition Epidemiology Group, Nuffield Institute for Health, 71–75 Clarendon Road, Leeds LS2 9PL and* [2]*School of Psychology, University of Leeds, Leeds LS2 9JT*

Despite continuing recommendations, the UK population's average intake of dietary fat still remains high (MAFF, 1999). Since little is known about the mechanisms underlying *successful* fat reduction in the free-living population, this study identifies possible strategies to help reduce fat intake.

A repeat food frequency questionnaire was mailed to a random sample (n=2200) of the UK Women's Cohort Study (response rate 87%). Using dietary intake data collected at two time points (up to 6 years apart), subjects who reduced their fat intake by 5% or more (RF) were compared to those whose fat intake increased by 5% or more (IF) or remained constant (–1 to +1%) (NC). The percentage contribution of fat for the top ten foods in the RF and IF groups is presented in the Table.

Reduced fat (RF)				Increased fat (IF)			
Baseline	**%**	**Repeat**	**%**	**Baseline**	**%**	**Repeat**	**%**
Butter	5.1	Cheddar type cheese	3.5	Cheddar type cheese	4.4	Butter	5.4
Polyunsaturated marg	4.9	Half-fat milk	3.4	Polyunsaturated marg	3.9	Cheddar type cheese	4.0
Cheddar type cheese	4.7	Butter	3.3	Half-fat milk	3.0	Mini chocolate bars	3.9
Fromage frais	2.9	Polyunsaturated marg	2.8	Wholemeal bread	2.6	Polyunsaturated marg	3.7
Half-fat milk	2.7	Tomatoes	2.5	Butter	2.4	Mayo/salad cream	2.5
Pistachio/peanuts	2.7	Scrambled egg	2.1	Mini chocolate bars	2.3	Half-fat milk	2.4
Mayo/salad cream	2.3	Wholemeal bread	2.1	Tomatoes	2.2	Pistachio/peanuts	2.1
Scrambled egg	2.1	Mini chocolate bars	2.1	Boil/mashed potatoes	2.1	Scrambled egg	2.1
Mini chocolate bars	2.1	Porridge	2.0	Peppers	2.1	Fromage frais	1.9
Quiche	1.8	Chips	2.0	Mushrooms	2.0	Oily fish	1.9
Total % contribution	**31.3**		**25.8**		**27.0**		**29.9**
Mean fat intake (g)	**93.5**		**68.0**		**67.0**		**90.1**

Butter, polyunsaturated margarine, cheddar type cheese and half-fat milk were top contributors to fat in each group at baseline and repeat. In the RF group, three out of these four foods reduced their percentage contribution to fat over the two time points, of which polyunsaturated margarine had the highest percentage reduction for any food, –2.1%, followed by butter, –1.8%. This was significantly better than the NC and IF groups (P<0.01). The percentage contribution of half-fat milk and soya-milk increased, +0.7% and +0.4%, respectively. These foods appeared to be substitutes for whole milk, which was reduced by –1.2%. Similarly, the percentage contribution of cereals, vegetables and low-fat yoghurts were increased (total +3.4%), some of which appeared to be substitutes for higher fat foods which were significantly reduced, for example, a variety of fat spreads, nuts, mayonnaise and fromage frais (total –6.5%). In the NC group, seven out of the top ten contributors were the same at both time points. The total percentage difference of the top ten foods which increased in frequency between baseline and repeat was 4.2% and the top ten foods with the highest percentage reduction was –4.0%, the lowest difference across the three groups. In the IF group, butter more than doubled its percentage contribution to fat, +3.0%, the highest percentage increase for any food. The percentage contribution of monounsaturated and soft margarine increased, whilst low-fat spread was reduced, –0.5%. Mini chocolate bars, a top contributor to fat at both time points, increased its percentage contribution along with larger chocolate bars, 1.6% and 0.6%, respectively. Four different vegetables (including potatoes) were top contributors at baseline. However, these appeared to be replaced with higher fat foods, for example mayonnaise, pecan/walnuts and pistachio/peanuts, all of which had significant changes.

Successful RF consumers appeared to reduce the frequency of consumption of high-fat foods and substitute them with reduced-fat alternatives, cereals and vegetables. These findings have important implications for future food policy in relation to encouraging the population to reduce their fat intakes.

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MAFF (1999) National Food Survey 1998: Annual Report on Food Expenditure, Consumption and Nutrient Intakes. London: The Stationery Office.

The stability of nutrient intake between adolescence and adulthood: a 21-year follow-up. By A.M. CRAIGIE, A.A. LAKE, C. WOOD, M. GIBBONS, S. WEBSTER, A.J. ADAMSON, A.J. RUGG-GUNN and J.C. MATHERS, *University of Newcastle, Human Nutrition Research Centre, Wellcome Research Laboratories, RVI, Queen Victoria Road, Newcastle upon Tyne NE1 4LP*

Cardiovascular disease and cancer are major causes of death in Western societies and links with diet are well established. Evidence of poor dietary habits in childhood and adolescence (Gregory *et al*. 2000), has led to recommendations that healthy eating be promoted at an early age (HEA, 1995). This assumes that diet tracks from adolescence into adulthood, but evidence for this is scarce. Previous work in this study has provided evidence that both adult BMI (Lake *et al.* 2001) and intake of some food groups (Craigie *et al*. 2002) can be predicted from adolescent levels. The aim of this analysis was to determine whether this is reflected at the level of macronutrient and selected micronutrient intakes.

Longitudinal dietary information and body weight were collected from 202 individuals surveyed initially at 11–13 years old (mean 11.6 years) (Hackett *et al*. 1984) then again at 32–34 years (mean 32.5 years). At each time point, participants completed two 3 d estimated food diaries followed by an interview with a nutritionist to clarify details and estimate portion sizes using the method considered most suitable at the time. These were calibrated food models at 11.6 years and a photographic food atlas (Nelson *et al*. 1997) at 32.5 years. Nutrient intake was determined using standard UK food tables with additions. The stability of relative intake was then determined using Pearson correlation analysis.

	11.6 years		32.5 years			11.6 years *v.* 32.5 years	
	Mean	SD	Mean	SD	Mean change	Pearson correlation coefficient	*P*
Energy (MJ)	8.68	1.57	9.41	2.75	+0.73	0.211	<0.01
Fat (g)	94.3	19.7	86.8	32.2	–7.5	0.155	0.03
Carbohydrate (g)	262.9	52.3	251.7	70.6	–11.2	0.163	0.02
Total sugar (g)	118.0	35.0	97.9	37.3	–20.1	0.246	<0.01
Total starch (g)	144.9	30.7	150.1	44.6	+5.2	0.158	0.02
Southgate fibre (g)	13.7	3.7	18.5	7.0	+4.8	0.166	0.02
Protein (g)	57.9	11.6	85.6	32.6	+27.7	0.263	<0.01
Vitamin C (mg)	39.0	20.8	83.8	56.1	+44.8	0.292	<0.01
Iron (mg)	9.7	2.1	12.4	4.9	+2.7	0.223	<0.01

Between 11.6 and 32.5 years, reductions in sugar and fat intake had occurred, equivalent to 3.5% and 3.8% food energy from sugar and fat, respectively, replaced mainly by protein and starch. At the individual level, the correlation coefficients indicate that intakes of energy, macronutrients, vitamin C and iron tracked moderately, yet significantly, between the two time points. Particularly notable were vitamin C (r=0.292) and protein (r=0.263) intakes, with slightly weaker, but similar, correlations for the other macronutrients and iron. In addition, these correlations strengthened further when expressed on a per kilogram body mass basis, ranging between r=0.182 and r=0.341.

These findings suggest that adult intakes of energy, macronutrients and selected micronutrients, namely vitamin C and iron, are moderately, though significantly, predictable from adolescent intakes. This reflects the similarly moderate predictability found previously for fruit and vegetables, bread, other cereals and potatoes, and meat, fish and alternatives in the sample. How tracking is influenced by socio-demographic characteristics and life events will be the focus of future investigations.

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Craigie AM, Lake AA, Wood C, Gibbons M, Webster S, Adamson AJ, Rugg-Gunn AJ & Mathers JC (2002) *Proceedings of the Nutrition Society* (in press).
Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LJ, Smithers G, Wenlock R & Farron M (2000) *National Diet and Nutrition Survey: Young People Aged 4–18 Years.* London: HMSO.
Hackett AF, Rugg-Gunn AJ, Appleton DR, Eastoe JE & Jenkins GN (1984) *British Journal of Nutrition* **51**, 67–75.
Health Education Authority (1995) *Diet and Health in School Age Children*. University of London, Institute of Education.
Lake A, Craigie A, Gibbons M, Wood C, Adamson A & Rugg-Gunn A (2001) *Proceedings of the Nutrition Society* **60**, 210A.
Nelson M, Atkinson M, Meyer J (1997) A *Photographic Atlas of Food Portion Sizes*. London: MAFF Publications.

Parental change in starch and fat intake after a "starchy food" intervention. By P.J. CURTIS, S. WEBSTER, A.J. ADAMSON and J.C. MATHERS, *University of Newcastle, Human Nutrition Research Centre, Wellcome Laboratories, RVI, Queen Victoria Road, Newcastle upon Tyne NE1 4LP*

Reduction of total fat intake to <35% food energy (FE) and replacement of energy with low-fat starchy foods is central to the UK public health strategy to lower the risk of cardiovascular disease (Department of Health, 1992). The Newcastle "*Family Food and Health Project*" (FFHP) is testing the hypothesis that positive advice to increase consumption of starchy foods will lower fat intake. 176 families with middle-range incomes, identified using the Townsend Deprivation Index (1987), were randomized into one of three interventions (INT). The INTs were: (1) "knowledge", (2) "cook & eat" skills and (3) "1+2 and personal advice". The data presented focus on the 175 mothers and 117 fathers recruited to take part in the INT. Food intake data were collected using a 3 d estimated food diary, including one weekend day, at baseline (T_0), 3 months post-INT (T_1) and 6 months post-INT (T_2) and quantified at interview, using a *Photographic Atlas of Food Portion Sizes* (MAFF, 1997). Nutritional composition was analysed using standard UK food tables, and entered into an ACCESS database to generate average daily intake. Mothers' and fathers' data were analysed separately, due to likely correlation of couples' dietary intake. Overall means were calculated at T_0, T_1 and T_2, and ANCOVA performed to identify dietary changes of retained participants with validated intake (grouped by INT). Dietary intake was "valid" when energy intake was >1.1× predicted BMR (Schofield *et al*. 1985).

At T_0, the overall mean for mothers' intake of starch and fat was 26.8 and 36.4%FE. Fathers' mean intake of starch and fat at T_0 was 27.5 and 37.3%FE.

	Mothers: T_0 to T_1 (n=71), T_0 to T_2 (n=46)				Fathers: T_0 to T_1 (n=46), T_0 to T_2 (n=33)			
	Change in %FE starch		Change in %FE fat		Change in %FE starch		Change in %FE fat	
	T_0–T_1	T_0–T_2*	T_0–T_1	T_0–T_2*	T_0–T_1	T_0–T_2	T_0–T_1	T_0–T_2
INT 1	3.59	0.76	–0.18	0.03	–0.40	–0.49	0.46	–0.78
INT 2	4.24	3.75	–3.95	–3.04	2.97	3.22	–1.07	–0.66
INT 3	2.24	–4.01	–0.40	3.05	2.00	–1.07	–0.94	–0.59

*Significant difference between interventions (P=0.02).

In the short term (T_0–T_1), % FE starch increased in all INTs for mothers, yet in the longer term (T_0–T_2) the degree and direction of change differed significantly between INTs (P=0.02). INT 2 maintained the largest starch change. Mothers in all interventions also reduced %FE fat between T_0 and T_1, with INT 2 being the most successful. Between T_0 and T_2 only INT 2 maintained reduced %FE fat intake. A significant difference (P=0.02) between INTs was found for the degree of change in %FE fat from T_0 to T_2.

Fathers in INTs 2 and 3 increased %FE starch from T_0 to T_1. During the same period, INT 1 reduced %FE starch intake. Maintained increases in %FE starch (T_0 to T_2) were only observed in INT 2, whilst INTs 1 and 3 decreased starch below pre-INT levels. Fathers in INTs 2 and 3 reduced %FE fat from T_0 to T_1, whilst INT 1 reported an increase in %FE fat. Between T_0 and T_2 all INTs had modest %FE fat reductions. The SD of the mean percentage change in nutrient intake from T_0 to T_1 and T_0 to T_2 were generally high (between 2.7 and 7.8) for both mothers and fathers.

In general, parents in the FFHP were able to increase %FE starch at 3 months post-intervention. Yet maintenance of these increases over the longer term proved more difficult, with only INT 2 consistently increasing starch intake from T_0 to T_2 in both gender groups. INT 3 performed poorly in maintaining starch increases at T_2 for both mothers and fathers. Increases in %FE starch were not directly proportional to reductions in %FE fat. Only INT 2 successfully reduced and maintained lower fat intake. Changes from T_0 to T_1 were generally greater than at T_0 to T_2, possibly due to a "participation-fatigue" effect at T_2. A skills-based approach (INT 2) appears to have been the most successful INT at increasing starch and reducing fat, whilst a traditional knowledge-based INT (INT 1) had little impact. INT 3, designed as the "gold standard", may have suffered from imposing a higher burden on volunteers.

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Department of Health (1992) *The Health of the Nation: A Strategy for Health in England*. London: HMSO.
MAFF (1997) *A Photographic Atlas of Food Portion Sizes*. London: MAFF Publications.
Schofield WN, Schofield C & James WPT (1985) *Human Nutrition – Clinical Nutrition* **39c**, 5–41.
Townsend P (1987). *Health and Deprivation: Inequality and the North*. London: Croom Helm.

Promoting dietary change in primary school children: parental perspectives. By K.H. HART, L.V. RYDER, L.A. BENNETT, J.A. BISHOP and H. TRUBY, *Centre for Nutrition and Dietetics, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH*

Our previous work has uncovered potential weaknesses inherent in the traditional approach to nutritional guidance for primary-school-aged children. A cognitive mismatch between message and audience (Hart *et al*. 2002) and negative parental perceptions of existing guidelines serve to inhibit the uptake of positive dietary change. This study aimed to enhance our understanding of parental attitudes and beliefs towards healthy eating and to assess the psychosocial factors that influence parents' ability and desire to provide a healthy diet for their children.

Parents were recruited from various socio-economic status (SES) backgrounds, categorized by occupation, through schools, community groups and parent centres in the South East (n=346) and the North West (n=62) of England and were asked to complete a previously validated questionnaire (personal data). The questionnaire was based on the key constructs of the "Theory of Planned Behaviour" (Ajzen, 1998), namely intention, attitudes, behavioural beliefs, normative pressure, subjective norm and perceived control, along with items to assess parental nutrition knowledge, subjective behaviour, perception of children's diet-related disease risk and responsibility for diet and exercise provision. Analysis was completed on 199 returned questionnaires (49% response rate).

Results indicated that, in general, parents possess strong positive attitudes, intentions and behavioural beliefs with regard to healthy eating. However, barriers remain with regard to the time, financial and skill demands perceived to be associated with a healthy diet. Interestingly, a substantial number of parents believe that providing their children with healthy food will, to some extent, reduce their enjoyment of eating. Comparing responses between SES groups indicated that high SES parents possessed significantly greater nutrition knowledge than parents classified as medium or low SES (χ^2=18.3, P<0.001). High SES parents were also more likely to believe that healthy eating was good, wise and beneficial (z= –3.60, P<0.001; –4.64, P<0.001; and –4.42, P<0.001, respectively) and more likely to feel guilty if they did not provide a healthy diet for their child (z= –3.13, P=0.002) than their low SES counterparts. Parents in the lowest SES group showed a trend towards more negative behavioural beliefs about healthy eating (χ^2=5.76, P=0.06), yet perceived their children to be at greater risk of diet-related heart disease (z= –2.46, P=0.01) and of becoming overweight (z= –2.64, P=0.008) than parents classified in the highest SES group.

Stepwise multiple regression analysis was used to investigate the prediction of parental intention to provide a healthy diet for their children. Across the whole sample, attitudes and the subjective norm accounted for 40% of the variation in intention (F=64.2, P<0.001). Among low SES parents the subjective norm was independently predictive of intention (Adj.R^2=0.80, F=93.6, P<0.001). In contrast, perceived control was rejected as a significant determinant of intention in all models tested. This supports our previous qualitative findings, which have identified a high level of parental self-efficacy with regard to the provision of a healthy diet for children.

In order to enable real dietary change in primary-school-aged children, nutritional messages need to be cognisant of the constructs of greatest subjective and objective relevance to parents. By focusing on these, capitalizing on existing positive attitudes and beliefs and recognizing that different SES groups may have different needs, we can inform the tailoring of future education schemes to maximize uptake and efficacy. In particular, altering the subjective norm by such strategies as peer modelling may represent a key approach to improving health behaviours among lower SES populations.

Ajzen I (1998) *Attitudes, Personality and Behaviour*. Milton Keynes: Open University Press.
Hart KH, Bishop JA & Truby H (2002) *Journal of Human Nutrition & Dietetics* **15**, 129–140.

Trends of overweight and obesity in 11–12-year-old adolescents between 1980 and 2000, measured using different BMI cut-offs. By E.S. FLETCHER[1], A.J. ADAMSON[1], A.F. HACKETT[2] and A.J. RUGG-GUNN[1], *[1]Human Nutrition Research Centre, University of Newcastle, Newcastle upon Tyne NE1 4LP, [2] Liverpool John Moores University, School of Education, Liverpool L17 6BD*

Obesity is associated with chronic diseases such as cardiovascular disease and diabetes. In 1996, prevalence of obesity in adolescence was reported to be 14% in the UK (Reilly *et al.* 1999). Comparing reported incidence of obesity between studies is difficult due to the range of methods of determining obesity in adolescents. The aim of this study was to determine the trends of overweight and obesity among 11–12-year-olds in 1980, 1990 and 2000, using four different BMI cut-offs.

In 2000, children in Year 7 (aged approx. 11.5 years), attending seven middle schools in Northumberland, were invited to participate in the study, and 424 (68%) children took part, 379 and 405 participated in 1990 and 1980 respectively. Height and weight were measured in each survey and BMI and *Z*-scores were calculated. The prevalence of overweight and obesity was determined at each time point using the following criteria:
(a) adult cut-offs (WHO, 2000); (b) international BMI cut-offs for 11.5-year-olds (Cole *et al.* 2000); (c) BMI reference curves, 85th and 95th (Barlow & Dietz, 1998); (d) *Z*-scores 85th centile and 95th centile (Barlow & Dietz, 1998). Prevalence of overweight and obesity in 1980 and 2000 reported.

Method	% Overweight				% Obesity			
	1980		2000		1980		2000	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Adult BMI	3	2	9	9	1	1	2	1
International definition	11	12	27	33	2	1	8	6
BMI reference curves	22	15	44	38	9	5	22	16
Z-score	25	18	41	36	11	8	21	18

In 1980, the prevalence of overweight 11-12-year-olds ranged between 2% and 25%, in 2000 the prevalence ranged between 9% and 44%, depending on which BMI cut-off was used. Between 1980 and 2000, all BMI cut-offs showed an increase in the prevalence of overweight children. Adult BMI-cut-offs found a similar percentage of children were obese in 1980 and 2000. International adolescent BMI cut-offs found 2% of children in 1980 were obese which increased to 7% in 2000. This compares with data from Chinn and Rona (2001), using the same international cut-offs, which showed 1.4% of English 9-11-year-olds were obese in 1974 and 2.6% were obese in 1994. BMI reference curves and Z-score cut-offs gave the highest prevalence of obesity in 2000, identifying one in five children were obese.

All the methods used above indicate an increase in the prevalence of overweight and obesity in Northumbrian adolescents. Depending upon which method is used, the level of obesity in adolescents in 2000 could be reported as being from 2% to 21% for boys and from 1% to 18% for girls. Compared with previous UK figures for obesity (Reilly *et al.* 1999; Chinn and Rona, 2001), adolescents living in Northumberland in 2000 had higher percentages of overweight and obesity. Obesity in adolescence has serious health implications, as well as being predictive of adult obesity (Mascarenhas *et al.* 1999); recent findings of Drake *et al.* (2002) have associated adolescent obesity with type II diabetes in white UK teenagers. Monitoring the prevalence of obesity in adolescents is fundamental in assessing the effectiveness of public health messages in targeting obesity. The use of a common definition of overweight and obesity is essential to ensure that this can be done effectively.

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Barlow SE & Dietz WH (1998) *Pediatrics* **102**, E29.
Chinn S & Rona RJ (2001) *British Medical Journal* **322**, 24–26.
Cole TJ, Bellizzi MC, Flegal KM & Dietz WH (2000) *British Medical Journal* **320**, 1240–1243.
Drake AJ, Smith A, Betts PR, Crowne EC & Shield JPH (2002) *Archives of Diseases in Childhood* **86**, 207–208.
Mascarenhas MR, Tershakovec AM & Stettler N (1999) *Current Opinion in Pediatrics* **11**(6), 598–604.
Reilly JJ & Dorosty AR (1999) *Lancet* **354**, 1874–1875.
WHO (2000) Obesity: Preventing and Managing the Global Epidemic. Geneva: WHO.

Low folate status, high alcohol intake and genomic DNA hypomethylation predict the risk of colorectal adenoma and cancer: a case control study. By M. PUFULETE[1], R. AL-GHNANIEM[2], A.J.M. LEATHER[2], P.W. EMERY[1] and T.A.B. SANDERS[1], *[1]Nutrition Food and Health Research Centre, King's College London, Stamford Street, London SE1 9NN and [2]Academic Department of Surgery, King's College Hospital, Denmark Hill, London SE5 9RS*

A low dietary intake of folate may increase the risk of colorectal cancer by inducing genomic DNA hypomethylation (Mason & Choi, 2000). A case control study was conducted to test the hypothesis that habitual folate status and markers of DNA methylation influence the risk of developing adenoma and cancer. Three groups of patients were recruited for the study: thirty-five patients with histologically confirmed colorectal adenoma (aged 41–82 years), twenty-eight patients with histologically confirmed colorectal cancer (age 39–89 years) and seventy-six controls (aged 38–89 years) found to be free from polyps or cancer at colonoscopy. Exclusion criteria were previous history of adenoma or cancer, hyperplastic polyps, inflammatory bowel disease, pregnancy, alcoholism, pernicious anaemia and the use of anti-folate medication.

Dietary folate and alcohol intakes were assessed by a short food frequency questionnaire. Prior to colonoscopy, fasting venous blood samples were obtained for determination of serum and erythrocyte folate, plasma homocysteine and leucocyte DNA methylation. Tissue biopsies were obtained at colonoscopy for determination of DNA methylation in normal-appearing colonic mucosa. DNA methylation status was measured by the *in vitro* methyl acceptance assay based on the ability of DNA to incorporate [^{3}H] methyl groups from *S*-adenosylmethionine. A folate status score was calculated by categorizing subjects into quintiles of the distribution according to folate intake, serum and erythrocyte folate concentration and summing the category numbers for each variable. Odds ratios (OR) with 95% confidence intervals (CI) were calculated using a logistic regression model.

Low folate status was associated with increased risk of cancer (P=0.01). High alcohol intake was associated with increased risk of adenoma (P=0.02) and cancer (P<0.001). Colonic DNA hypomethylation was associated with increased risk of adenoma (P=0.01) and cancer (P=0.04). There was a trend for increased risk of adenoma (P=0.06) with decreasing leucocyte DNA methylation. These results suggest that a low folate status, high alcohol intake and DNA hypomethylation may play a role in colorectal neoplasia. The findings of this pilot study require confirmation in a larger prospective cohort study.

		Adenoma[§]		Cancer[§]	
		OR (95%CI)[§§]	*P* for trend	OR (95% CI)[§§]	*P* for trend
Folate status score	Middle third	1.04 (0.30–3.66)	0.95	0.47 (0.13–1.73)	0.25
	Top third	1.40 (0.47–4.8)	0.55	0.08 (0.02–0.54)	0.01
Alcohol intake (g/d)	Middle third	1.35 (0.32–5.73)	0.68	4.18 (1.05–16.67)	0.04
	Top third	9.48 (2.76–32.51)	0.00	5.99 (1.41–25.49)	0.02
Plasma homocysteine (μmol/l)	Middle third	0.98 (0.29–3.38)	0.98	1.84 (0.39–8.8)	0.44
	Top third	1.29 (0.39–4.29)	0.41	2.79 (0.60–12.9)	0.19
[^{3}H] methyl incorporation into colonic DNA (Bq/μg DNA)	Middle third	4.32 (0.97–19.29)	0.05	6.31 (0.64–61.78)	0.09
	Top third	6.57 (1.58–27.22)	0.01	9.55 (1.03–88.50)	0.04
[^{3}H] methyl incorporation into leucocyte DNA (Bq/μg DNA)	Middle third	2.18 (0.55–8.64)	0.27	1.87 (0.44–7.9)	0.39
	Top third	3.25 (0.91–11.57)	0.06	2.63 (0.66–10.40)	0.16

[§]Compared with control; [§§] Compared with bottom third (OR = 1.00). OR and their 95% CI adjusted for gender, age, body mass index, smoking and supplement use.

Mason JB & Choi SW (2000) Nestle Nutrition Workshop Series, Clinical Performance Programme **4**, 87–99.

The influence of the lipoprotein lipase S447X and HindIII polymorphisms on the association between habitual dietary saturated fat intake and lipid levels. By L.F. MASSON, A. CUMMING, C. TUYA and G. McNEILL, *Faculty of Medicine and Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD*

The S447X and HindIII polymorphisms in the lipoprotein lipase gene have been shown to influence the lipid response to changes in dietary fat intake, with carriers of the rarer X- and H- alleles showing greater low-density lipoprotein (LDL) cholesterol and total cholesterol responses, respectively (Wallace *et al*. 2000; Humphries *et al*. 1996). This study aimed to investigate whether these polymorphisms influence the relationship between plasma lipid concentrations and habitual saturated fat intake.

The subjects included 225 healthy men and women (91 M, 134 F) aged 18–50 years who were recruited for a study of coronary heart disease risk factors in twins. Habitual diet was assessed by a food frequency questionnaire, and a fasting blood sample was taken for analysis of lipids and DNA extraction. S447X and HindIII genotypes were determined by the polymerase chain reaction followed by HinfI and HindIII digestion, respectively. Digested products were visualized under UV light following ethidium bromide staining of an agarose gel. Regression coefficients for the relationships between total fat and saturated fatty acids (SFA), and total, LDL, and high-density lipoprotein (HDL) cholesterol levels were estimated by multiple regression with adjustment for age, sex, oral contraceptive use, percentage body fat, smoking status, physical activity level and energy intake for each genotype group.

In S447X S/S individuals (n=176), there were significant positive associations between SFA and total and LDL cholesterol, and in carriers of the X allele (S/X and X/X, n=49), total fat and SFA were significantly positively associated with HDL cholesterol. No significant associations were seen between total fat or SFA and lipid levels in HindIII H+H+ individuals (n=114), although SFA was significantly positively associated with total and HDL cholesterol in carriers of the H– allele (H+H– and H–H–, n=111).

The table shows the regression coefficients for the relationships between total fat and SFA and lipid levels in the combined genotype groups. The significant association between SFA and total cholesterol seen in all S/S individuals remained only in those who also carried the H– allele. The association between SFA and total cholesterol seen in all H– individuals remained only in those with the S/S genotype, whereas the association between SFA and HDL levels remained only in those who also carried the X allele.

Genotype			Total cholesterol		LDL-C		$\log_e$ HDL-C	
S447X	**HindIII**	***n***	Total fat	SFA	Total fat	SFA	Total fat	SFA
S/S	**H+H+**	114	0.000	0.009	0.002	0.010	0.001	0.003
S/S	**H–**	62	0.011	0.026*	0.005	0.016	0.000	0.003
X	**H–**	49	0.015	0.003	–0.001	–0.025	0.008**	0.015**

*P<0.05, **P<0.01.

The data suggests that in this population, S/S individuals carrying the rarer H– allele are more sensitive to the influence of SFA on total cholesterol levels, while individuals carrying the rarer X allele are more sensitive to the influence of SFA on HDL cholesterol levels.

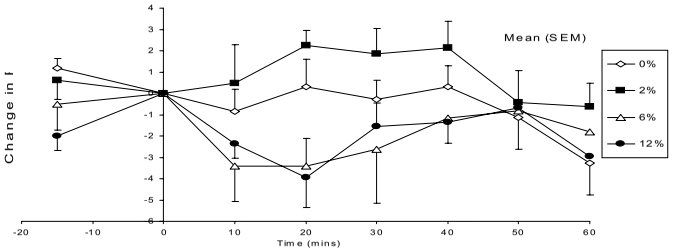
Humphries SE, Talmud PJ, Cox C, Sutherland W & Mann J (1996) *QJM* **89**, 671–680.
Wallace AJ, Mann JI, Sutherland WH, Williams S, Chisholm A, Skeaff CM, Gudnason V, Talmud PJ & Humphries SE (2000) *Atherosclerosis* **152**, 327–336.

Changes in blood, plasma and red cell volume after ingestion of hypotonic and hypertonic solutions. By S.J. MERSON, S.M. SHIRREFFS, J.B. LEIPER and R.J. MAUGHAN, *Biomedical Sciences, University Medical School, Foresterhill, Aberdeen AB25 2ZD*

Ingestion of a solution that is hypertonic to body fluids may result in a transient movement of water into the intestine by osmosis through the epithelium. This study investigated the effect of ingesting hypotonic and hypertonic solutions on blood, plasma and red cell volumes.

Six healthy male subjects participated in this study with local ethics committee approval. Subjects arrived at the laboratory following an overnight fast on four occasions. Subjects voided on arrival at the laboratory and a cannula was inserted into a superficial forearm vein to allow repeated blood sampling. Subjects ingested one of four test solutions (0%, 2%, 6% and 12% glucose) in a randomized order after 45 min seated rest. The drinks were provided in two 300 ml aliquots and subjects were allowed 2.5 min to drink each. Blood samples were collected 15 min before and immediately before ingestion of the test solution and every 10 minutes for 1 h after cessation of drinking. Subjects remained seated throughout each trial and provided a urine sample after the last blood sample had been collected. Haemoglobin concentration (cyanmethaemoglobin method, in duplicate) and packed cell volume (by centrifugation, in triplicate) were measured to allow calculation of changes in blood, plasma and red cell volume (Dill & Costill, 1974). Data were analysed by repeated measures ANOVA, one-way ANOVA and *post hoc* Tukey test.

Blood, plasma and red cell volumes were unchanged in the two samples collected before drinking. Haemoglobin concentration and packed cell volume at the baseline time points were not significantly different between trials.



Blood and plasma volume tended to decrease over time after drinking the 6 and 12% glucose solutions but the difference was not statistically significant. Blood (P=0.007) and plasma (P=0.004) volumes were lower 20 min after ingesting the 6 and 12% solutions than when the 2% solution was ingested. There was no significant difference in the red cell volume between trials and no change over time (P>0.05). Blood glucose concentration increased after the 12% trial (P<0.001) and there was a tendency for it to increase during the 6% trial (P=0.052). Blood glucose concentration during the 12% trial was higher than on the 0 and 2% trials 30 min after drinking ceased and remained significantly elevated throughout the trial (P=0.020).

We conclude that ingestion of 6% or 12% glucose solutions causes a transient decrease in blood and plasma volume which may be due to a net flow of water into the intestine before subsequent reabsorption.

Dill DB & Costill DL (1974) *Journal of Applied Physiology* **37**, 247–248.

Effect of daily caffeine consumption on fluid balance. By C.L. MILLER and R.J. MAUGHAN, *Biomedical Sciences, University Medical School, Foresterhill, Aberdeen AB25 2ZD*

Daily fluid balance is achieved by matching intake of fluids and electrolytes with output via sweat, urine and respiration. Fluid losses will be altered by environmental factors e.g. heat, humidity, by exercise and by the ingestion of diuretic agents such as caffeine. Caffeine is found in a large number of beverages and foods including tea, coffee and some soft drinks. One of the most commonly cited side effects of caffeine ingestion is the stimulation of urine production. Large doses of caffeine (more than 300 mg) have an acute diuretic effect (see Neuhauser-Berthold *et al.* 1997), but there is very little evidence of any effect at lower doses. Grandjean *et al.* (2000), found that lower doses of caffeinated beverages (doses similar to those achieved by normal daily consumption of caffeine-containing beverages) did not affect urine production compared to caffeine-free beverages.

The present study investigated the effects of abstention from caffeinated beverages on water turnover over 7 d. With ethics committee approval, nine male subjects gave written informed consent before commencing the 3-week study. The subjects' mean age was 26 (SD 5) years, weight was 71.88 (SD 9.76) kg. Subjects had a habitual caffeine intake of two or more cups of tea, coffee or equivalent caffeine-containing soft drinks per day. During weeks 1 and 3, subjects consumed their normal intake of food and drink. In week 2 subjects abstained from caffeine-containing beverages. Weighed food and drink diaries were kept for the duration of the study. These were used to calculate total water intake and to estimate caffeine intake. Entire urine output was collected for 3 weeks. A sample of the first urine produced each day was collected and tested for osmolality and electrolytes. On the first and last day of each week body mass was measured and a blood sample drawn. Statistical significance ($P<0.05$) was determined using repeated measures ANOVA followed by Kruskal-Wallis and Mann-Whitney tests where appropriate.

Daily caffeine intake was estimated for each subject by assigning caffeine concentrations to the caffeine-containing beverages. Caffeine intake was 185 (SD 64) mg in week 1, and 179 (SD 62) mg in week 3. The number of caffeinated beverages consumed each day was 3.3 (SD 1.3) and 3.3 (SD 1.1) drinks in weeks 1 and 3, respectively. No significant difference in the volume of urine produced in trial weeks 1, 2 and 3 was seen ($P>0.05$). Water intake did not drop significantly in week 2 compared to weeks 1 and 3 ($P>0.05$). Osmolality did not significantly differ between weeks 1, 2 and 3 ($P>0.05$) (see Table). However there was a tendency for both urine volume and fluid intake to decrease and osmolality to increase in week 2 (see Table). There were no significant changes in body mass between the three weeks ($P>0.05$), nor did body mass change significantly between the beginning and end of each week.

	Water intake (ml/day)		Urine volume (ml/day)		Osmolality (mosmol/kg)	
	Median	Range	Median	Range	Mean	SD
Week 1	2663	1221–6823	1765	619–4531	674	136
Week 2	2424	959–6696	1343	460–5193	803	128
Week 3	2741	1024–5990	1650	541–4263	753	106

From these results, no significant decrease in urine volume or water intake was caused by the withdrawal of caffeine from the diet of regular caffeine drinkers, although a slight tendency towards reduced water intake and urine output was seen. Advising people to abstain from caffeinated beverages on the basis that they cause negative fluid balance is not supported by the results of this study.

Grandjean AC, Reiners KJ, Bannick KE & Haven MC (2000) *Journal of the American College of Nutrition* **19**, 591–600.
Neuhauser-Berthold BS, Verwied SC & Luhrmann PM (1997) *Annals of Nutrition and Metabolism* **41**, 29–36.

Branched chain amino acids and prolonged exercise capacity in a warm environment. By P. WATSON[1], A.T. STRACHAN[2], S.M. SHIRREFFS[1] and R.J. MAUGHAN[1], [1]*Biomedical Sciences and* [2]*Clinical Biochemistry, University Medical School, Foresterhill, Aberdeen AB25 2ZD*

Exercise capacity is significantly reduced in a warm environment (Galloway & Maughan, 1997). Performance may be primarily limited by thermoregulatory and fluid balance factors, but hyperthermia may also result in an acceleration of central fatigue leading to a reduced drive to continue exercise (Nielsen, 1992). Branched chain amino acids (BCAA) limit the entry of free tryptophan (f-TRP) into the central nervous system (CNS), attenuating the synthesis of serotonin, which has been proposed to contribute to the development of central fatigue (Blomstrand *et al*. 1991). The aim of this investigation was to assess the effect of BCAA supplementation on exercise capacity in the heat.

Eight healthy males (mean (SD); age 30.9 (8.6) years; mass 77.41 (8.38) kg; height 1.77 (0.05) m; VO_2 max 4.31 (0.26) l/min) volunteered to participate in this ethics committee-approved study. Each subject completed an incremental VO_2 max test, a familiarization trial and two experimental trials. Experimental trials were randomized in a double-blind crossover manner, separated by 7 d. Subjects entered the laboratory in the morning (8–9 am) following an overnight fast. Post-void, nude body mass was measured, skin and rectal temperature probes and a heart rate monitor were positioned. Baseline and exercise blood samples were drawn from a cannula inserted into a superficial forearm vein. Subjects remained seated in a thermoneutral (25.9 (0.2) °) environment for 45 min before commencing cycle exercise at 60 (2) % VO_2 max in the heat (35.0 (0.3) °) until volitional exhaustion. Each participant ingested either 500 ml of a BCAA+CHO solution (12 g/l BCAA, 65 g/l CHO) or CHO placebo (65 g/l CHO) 45 min prior to exercise, with an additional 250 ml consumed at 15-min intervals throughout exercise. Following the cessation of exercise, the probes were removed and nude mass was measured. A statistical difference ($P<0.05$) between the experimental trials was evaluated using repeated measures one-way ANOVA and paired *t*-tests as appropriate.

No difference in time to exhaustion was apparent between the BCAA+CHO (62.8 (15.7) min) and CHO (60.8 (18.8) min) trials ($P>0.05$). Rectal temperature was similar throughout both trials ($P>0.05$), with temperatures of 39.2 (0.6) ° and 39.1 (0.6) ° recorded, respectively, at exhaustion. Weighted mean skin temperature and body heat content were also unaffected by the supplementation ($P>0.05$). The subjects' perceived exertion (RPE) and thermal comfort were not different ($P>0.05$). The addition of BCAA to the ingested solutions produced a marked increase in plasma BCAA immediately prior to exercise (+866 µmol/l; $P<0.001$), with this difference maintained throughout. Consequently, a significant reduction in f-TRP : BCAA was observed during the BCAA+CHO trial when compared to the placebo ($P<0.001$). No effect on plasma glucose ($P>0.05$) or lactate ($P>0.05$) concentrations were apparent during the trials.

These results suggest that ingestion of a BCAA+CHO solution does not enhance exercise capacity in a warm environment over that of a CHO placebo. This occurred despite a marked reduction in the ratio of f-TRP to BCAA, which should limit the uptake of f-TRP into the CNS, questioning the importance of the serotonergic system in the development of fatigue during prolonged exercise in a warm environment.

Blomstrand E, Hassmen P, Ekblom B & Newsholme EA (1991) *European Journal of Applied Physiology* **63**, 83–88.
Galloway SD & Maughan RJ (1997) *Medicine and Science in Sports and Exercise* **29**, 1240–1249.
Nielsen B (1992) *Medicine and Sport Science* **34**, 207–217.

Altered plasma phosphatidylcholine *n*-3 polyunsaturated fatty acid concentrations in male colectomy patients. By A.N.J. MAY, D.H.L. NG, G.C. BURDGE, P.C. CALDER, M.A. STROUD and S.A. WOOTTON, *Institute of Human Nutrition, University of Southampton, Southampton General Hospital, Southampton SO16 6YD*

Patients suffering severe Inflammatory Bowel Disease (IBD) may have the entire large bowel removed (total colectomy). Changes in metabolic demands combined with alterations to intestinal function and availability may affect the extent to which the supply of fatty acids can satisfy demands. Altered plasma polyunsaturated fatty acid (PUFA) concentrations in colectomy patients has previously been reported (Esteve-Comas *et al*. 1993), although longer term consequences remain unclear. To address this issue, we measured plasma phosphatidylcholine (PC) *n*-3 PUFA concentrations in patients with established colectomy.

Subjects were twenty-two men (59 (SD 13) years, BMI 26.6 (SD 4.8) kg/m^2) who had undergone colectomy, as a result of severe IBD, at least 12 months (76.5 (15–252) months) prior to the study, and who were otherwise well. The reference group were healthy men (n=19) of similar age (53 (SD 11) years) and BMI (26.6 (SD 3.1) kg/m^2). Plasma C-Reactive Protein (CRP) was measured in the colectomy group at recruitment to characterize current inflammatory state. Plasma PC was isolated by solid phase extraction, fatty acid composition was measured by GC-FID and concentrations reported as absolute concentration and as a fractional concentration: sum of α-linolenic acid (18:3*n*-3, ALNA), eicosapentaenoic acid (20:5*n*-3, EPA), docosapentaenoic acid (22:5*n*-3, DPA) and docosahexaenoic acid (22:6*n*-3, DHA).

CRP was marginally elevated (6.4 and 11.5 mg/l) in two patients; all others were within the normal range (0–6 mg/l).

	Plasma phosphatidylcholine *n*-3 polyunsaturated fatty acid concentration							
	Absolute concentration (μg/ml)				Fractional concentration (% *n*-3 PUFA)			
	ALNA	EPA	DPA	DHA	ALNA	EPA	DPA	DHA
Reference	10.4 (1.8–11.8)	13.2 (3.9–31.3)	14.5 (3.6–20.9)	63.4 (12.8–134.8)	8.7 (2.0–23.6)	12.5 (6.8–26.8)	14.8 (3.2–20.3)	64.2 (30.6–74.7)
Colectomy	5.2† (1.8–18.4)	26.9** (6.4–58.6)	26.4† (14.0–46.3)	76.0 (26.3–143.3)	4.2† (1.8–9.5)	19.7** (3.4–27.2)	20.7† (12.2–30.6)	56.0* (42.2–68.9)

Significantly different from reference group: **P*<0.05, ***P*<0.01, †*P*<0.001 (Mann-Whitney *U*).

Plasma PC ALNA concentration in the colectomy patients was approximately half that of the reference group. In contrast, EPA and DPA concentrations in the colectomy patients were approximately twice that of the reference group. There was no significant difference in DHA concentration between the two groups. Similar findings were seen when results were expressed as fractional concentration: ALNA concentration in the colectomy group was roughly half that seen in the reference group, and EPA and DPA concentrations were also higher in the colectomy group. In contrast, fractional DHA concentration was found to be significantly lower in the colectomy group.

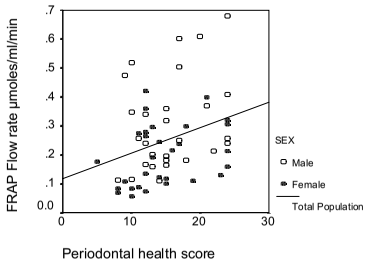
These data suggest that these patients may have an upregulation of ALNA conversion to EPA and DPA, suggesting that they may have higher demands for longer-chain PUFAs than the reference group. However, lower fractional DHA concentration in these patients could suggest that, despite this upregulation of ALNA conversion, DHA synthesis is insufficient to meet their demands.

Esteve-Comas M, Nunez MC, Fernandez-Banares F, Abad-Lacruz A, Gil A, Cabre E, Gonzalez-Huix F, Bertran X & Gassull MA (1993) *Gut* **34**, 1370–1373.

Association between salivary antioxidant status and periodontal disease. By D.V. SCULLEY[1] and S.C. LANGLEY-EVANS[2], [1]*Division of Health and Life Sciences, University College Northampton, Boughton Green Road, Northampton NN2 7AL and* [2]*School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD*

Periodontal disease, initiated by bacterial infection of the gingivae, is a common chronic condition in the adult population. Inflammatory responses to infection may result in damage to the gums, periodontal ligaments and underlying alveolar bone through the release of free radical species from activated neutrophils. Saliva is rich in antioxidants, particularly uric acid, albumin and ascorbate, and in most individuals contains greater total antioxidant capacity than blood plasma. Salivary antioxidants may provide a mechanism against oxidative attack in the periodontium (Sculley & Langley-Evans, 2002). In particular, the saliva flow rate plays an important role as a vector for antioxidant delivery to the periodontal area. Low saliva flow has been found to be a risk factor in poor oral health (Edgar, 1992). This study investigated the relationship between antioxidant delivery in saliva and the severity of periodontal disease.

Saliva was collected from fifty-eight dental patients attending for routine check-up examination (twenty-nine males and twenty-nine females), mean age 59.5 (SD 7.06) years. Periodontal disease status was determined by the dental examiner, using a graduated periodontal probe to measure gingival pocket depth (Ainamo *et al*. 1982). Whole, unstimulated saliva was collected over a 5-min period, measured for volume, and stored at –80°C until assayed. Total antioxidant activity (TAA) was determined using an adaptation of the ferric reducing ability of plasma (FRAP) assay (Benzie & Strain, 1996). Antioxidant flow rate was calculated from TAA and based on saliva flow over 5 min. In addition, protein carbonyls were analysed as an index of oxidative damage in the oral cavity.



The Figure shows that salivary antioxidant (FRAP) flow rate was significantly related to periodontal disease score. The score rates periodontal health from 0 (severe disease) to 24 (disease absent).

	FRAP flow rate (μmol/ml per min)		Disease score	
	Mean	SE	Mean	SE
Men	0.31	0.03	14.9	1.9
Women	0.20	0.02	14.4	1.0
P	0.004		0.716	

Antioxidant status and disease scores in men and women.

Increased salivary antioxidant flow rate was significantly related to the severity of periodontitis (R=0.312, P=0.017). The relationship persisted after adjustment for age and sex in a linear regression model (P=0.025). Salivary antioxidant flow rate was significantly greater in males than in females. The data are consistent with the hypothesis that an increase in salivary antioxidant delivery provides an improved protective mechanism against free radical mediated tissue degradation. This data may suggest a possible nutritional strategy in the treatment and prevention of periodontal disease.

Ainamo J, Barmes D, Beagrie G, Cutress T, Martin J & Sardo-infiri J (1982) *International Dental Journal* **32**, 281–291.
Benzie IFF & Strain JJ (1996) *Analytical Biochemistry* **239**, 70–76.
Edgar WM (1992) *British Dental Journal* **172**, 305–312.
Sculley DV & Langley-Evans SC (2002) *Proceedings of the Nutrition Society* **61**, 137–143.

Dietary intake and bone mass in the mothers of gymnasts and controls: preliminary analysis of the extent of genetic influences on peak bone mass development. By J.A. NURMI[1], J.A. BISHOP[1], P. TAYLOR[2], C. COOPER[2] and S.A. NEW[1], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH and* [2]*Osteoporosis Centre, University of Southampton, Southampton SO16 6YD*

Previous results from our longitudinal study on bone health in young gymnasts and controls have shown differences in anthropometric measurements, age at menarche, bone mass (density and quality) and body size adjusted energy intake, but similar intakes of macro- and micronutrients (Nurmi *et al.* 2001a,b). Since few data exist on the likely genetic impact on patterns of bone growth and body maturation in young athletes, the aim of this study was to undertake a dietary and bone mass study in the mothers of gymnasts and controls.

Subjects included a total of fifty-three mothers (twenty-seven gymnasts' (G-)mothers and twenty-six controls (C-)mothers) with a mean age of 43.0 *v.* 43.7 years, respectively. Dietary intake was assessed using 3 d diet records and analysed using the Diet-5 program (version 2000 for Windows). Total body (TB) bone mineral density (BMD) and content (BMC) were measured by dual energy x-ray absorptiometry (DXA, Lunar DPX). Broadband ultrasound attenuation (BUA) and velocity of sound (VOS) were measured by calcaneal quantitative ultrasound (McCue Ultrasonics Ltd, Winchester, version 3.5).

	G-mothers		C-mothers			G-mothers		C-mothers	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD
Height (m)	1.59[a]	0.05	1.65[b]	0.06	EI:BMR	1.26	0.2	1.26	0.3
Weight (kg)	61.5[a]	10.5	70.2[b]	14.6	Calcium (mg)	685	239	808	218
BMI (kg/m^2)	24.6	4.8	25.8	5.2	TB BMD (t-score)	0.64	0.9	0.68	1.1
Age at menarche (yrs)	13.3[a]	1.0	12.5[b]	1.4	BUA (dB/MHz)	83	14	83	14
Energy (MJ)	7.08	1.3	7.56	1.9	VOS (m/s)	1674[a]	43	1650[b]	32

[a,b] Different superscripts indicate $P<0.05$ (ANOVA).

As shown in the Table, the G-mothers were significantly shorter and lighter, with a later recalled age at menarche. Dietary intakes of macro- and micronutrients were similar between the two groups. BMD and BUA (bone density/structure) values did not differ between the groups, but G-mothers were found to have significantly higher VOS (bone density/elasticity). This was an unexpected finding, since past and present physical activity levels did not differ between the mothers. Higher VOS in gymnast-daughters has been assumed to result purely from impact through exercise, but whether heredity also has an effect is not known. The type of exercise undertaken by the mothers needs further examining.

Previously reported findings in young gymnasts, including small body size and later pubertal development, appear to exist in their mothers, thus supporting the suggestion of 'self-selection' into specific types of sports. Higher axial and peripheral bone mass shown previously in gymnast-daughters was not found in their mothers, supporting the suggestion that in the daughters, skeletal benefits result from vigorous weight-bearing training. Parental factors contributing to growth and development in young sportswomen require further investigation, including the interesting finding concerning bone elasticity as measured by VOS.

Financial support from the National Osteoporosis Society is gratefully acknowledged.

Nurmi JA, Bishop JA, Kolaghassi D, Simpson H & New SA (2001a) *Proceedings of the Nutrition Society* **60**, 190A.
Nurmi JA, Bishop JA & New SA (2001b) *Proceedings of the Nutrition Society* **60**, 201A.

Dietary patterns and alkaline phosphatase in the elderly in the UK. By M. SAFARIAN, B.M. MARGETTS and A.A. JACKSON, *Institute of Human Nutrition, Southampton General hospital, Southampton SO16 6YD*

Because of the complex interaction of the effects of components of diet on health, recent research has begun to focus on the effects of dietary patterns, rather than the effects of nutrients measured in isolation. To date, studies of diet and bone health in the elderly have mainly focused on single nutrients, with few studies assessing more than calcium, protein and vitamin D intake (Bonjour *et al.* 1996, 1997; Cumming & Klineberg, 1994; Nieves *et al.* 1992). The aim of the present study was to assess the relationship between dietary patterns and a marker of bone health, alkaline phosphatase (ALP).

Analysis is based on a secondary analysis of data from the National Diet and Nutrition Survey (NDNS) conducted on a UK nationally representative sample of 1687 men and women aged 65 years and over. Principal component analysis was used to describe dietary patterns; seven statistically independent eating patterns were generated. Correlations between dietary factor loading scores on each of the identified dietary patterns, nutrient intakes and alkaline phosphatase were examined for men and women separately.

The healthy dietary pattern, characterized by a high intake of vegetables, fruits, cereals, fish and other seafood, showed the strongest negative (beneficial) association with ALP (r –0.17, $P<0.001$). The dietary patterns that highlighted foods rich in calcium, vitamin D and protein were not correlated with ALP (patterns 2 and 3, r –0.03, $P=0.38$ and r –0.01, $P=0.65$, respectively,). Individual nutrients were weakly, but statistically significantly correlated with ALP (r –0.14 for protein, r –0.06 for vitamin D and r –0.08 for calcium $P<0.05$). Multiple regression analysis controlling for energy intake, identified the healthy diet principal component as the strongest predictor for serum ALP in elderly men and women, separately. Subjects in the highest fourth of the healthy diet in comparison to the lowest, were less likely to have high levels of plasma ALP (OR=0.4, 95% CI, 0.3–0.6) after adjustment for known confounders.

Prevalence odds ratios* (95% CIs) of high ALP (defined by median values of ALP distribution) and the median values of ALP by quartiles of healthy diet score in men and women (NDNS)

	Fourths of healthy diet score			
	First	Second	Third	Fourth
Women				
n				
OR[†] (95% CI)	Reference	0.89 (0.50–1.58)	0.81 (0.46–1.44)	0.45(0.25–0.81)
ALP[‡] (IU/l)	93.8	91.4	89.8	84.4
Men				
n				
OR[†] (95% CI)	Reference	0.64 (0.37–1.11)	0.54 (0.31–0.93)	0.38 (0.21–0.67)
ALP[‡] (IU/l)	93.6	86.6	84.6	75.9

*The model included age (10-year intervals), dietary scores on other dietary patterns (2–7), energy intake, smoking (never, former, current smoker), physical activity (thirds) and undernutrition risk (three levels, defined by MAG tool).
[†]P for trend < 0.001
[‡]Median values are presented.

These data suggest that the overall dietary pattern may be more important in predicting bone health, as defined by alkaline phosphatase, than any one nutrient on its own. These results have implications for preventive programmes aimed at improving and maintaining bone health in the elderly by focusing on the entire diet as a comprehensive approach.

Bonjour JP, Schurch MA & Rizzoli R (1996) *Bone* **18**, 139–144.
Bonjour JP, Schurch MA & Rizzoli R (1997) *Pathologie Biologie* **45**, 57–59.
Cumming RG & Klineberg RJ (1994) *American Journal of Epidemiology* **139**, 493–503.
Nieves JW, Grisso JA & Kelsey JL (1992) *Osteoporosis International* **2**, 122–127.

Indices of bone health and lifestyle characteristics in premenopausal and postmenopausal Saudi Arabian women. 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 GU2 7XH*

There are few data available concerning the bone health and lifestyle characteristics of women living in Middle Eastern countries. The aim of this study therefore was to identify the extent of osteopaenia and examine the prevalence of diet and lifestyle risk factors for poor bone health in Saudi Arabian women.

A total of 100 premenopausal and 112 postmenopausal women living in the city of Jeddah were involved in the study. They were aged 20–30 years and 45–60 years, respectively; they had not suffered from any known condition and were not taking any medication likely to affect bone metabolism. Bone mineral density (BMD) was determined at the lumbar spine (L2–L4) and femoral neck using dual x-ray absorptiometry (DXA) (Lunar Corp., DPX version 4.7). Calcaneal bone mass was measured by broadband ultrasound attenuation (BUA) (CUBA $^{plus+}$ softwareV4). All subjects were interviewed concerning their habitual dietary intake, physical activity levels and general lifestyle. Measurements were also made of weight and height.

As shown in the Table, bone health indices indicated a high prevalence of low bone mass in these groups. According to WHO criteria (WHO, 1994), a total of 37% of premenopausal women and 52% of postmenopausal women were osteopaenic at the lumbar spine. The percentages of women in the two groups defined as osteoporotic were 2% and 13%, respectively. Similar results were found for the femoral neck (23%, 27% osteopaenic) and calcaneous (36%, 62%). Physical activity levels were low and milk intake averaged one glass per day. Exposure to sunlight was low, which is likely to be compounded by the dress style used by the women.

	Premenopausal		Postmenopausal			Premenopausal		Postmenopausal	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD
Age (years)	23.11	3.6	49.6	5.01	Milk intake (glad/day)	1.0	0.8	1.0	0.8
Weight (kg)	61	15	75.7	14.6	Sunlight exposure (min/day)	16.2	17.0	11.2	16.8
Height (m)	1.59	0.06	1.56	0.06	Walking (time/week)	3.2	2.8	1.7	3.1
Lumbar spine t-score	–0.6	1.0	–1.0	1.3	Coffee intake (cup/day)	1.0	1.2	1.0	1.5
Femoral neck t-score	–0.2	1.1	–0.2	1.3	Tea intake (cup/day)	1.5	1.5	2.6	1.7
BUA t-score	–0.7	0.8	–1.2	0.9	Dairy product (portions/wk)	2.2	1.5	2.1	1.4
VOS t-score	–1.1	0.7	–2.1	0.7	Fatty fish (portions/wk)	1.0	1.3	0.9	1.1

These results are a cause for concern. The data indicate that bone health is poor in Saudi Arabian women and lifestyle factors are not favourable to skeletal integrity. Further investigations of dietary intake as well as markers of calcium and bone metabolism are under way to examine the extent of vitamin D insufficiency in these population groups and the resultant effect on indices of bone health.

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

World Health Organization (1994) *Assessment of Fracture Risk*. Geneva: WHO.

Investigation into the role of dietary phenolics in the acute modification of glucose tolerance and gastrointestinal hormone secretion in volunteers. By K.L. JOHNSTON, M.N. CLIFFORD and L.M. MORGAN, *Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH*

Phenolic compounds are ubiquitous in most sectors of the plant kingdom and thus form an integral part of the human diet. Several recent reports suggest that certain classes of dietary phenols have the ability to inhibit glucose uptake both *in vitro* (Welsch *et al.* 1989; Song *et al.* 2002) and *in vivo* in rats (Andrade-Cetto & Wiedenfeld, 2001). Their effect in humans, however, is unknown. Two separate, randomized, single-blind, within-subject intervention trials were therefore conducted to investigate whether different dietary sources of phenols affected glucose tolerance and circulating gastrointestinal (GI) hormone profiles in volunteers when administered orally with a glucose load.

Each trial used nine lean healthy volunteers (age 23–32 years; BMI <25 kg/m^2) who were studied on three occasions. Study 1 examined the effects of apple juice phenolics. The test beverages were apple juice containing either high or lower levels of naturally occurring phenolics. These and the control were equimolar for D-glucose and D-fructose (25 g each) and the final volume was 400 ml. Study 2 examined the effects of coffee phenolics using either decaffeinated or caffeinated coffee, both containing 2.5 mM caffeoylquinic acids (CQA). These and the control were equimolar for D-glucose (25 g) and the final volume was 400 ml. In both cases, the control was administered using water as a vehicle. Serial venous blood samples were taken over 3 h and plasma was analysed for glucose, insulin, glucose-dependent insulinotropic-polypeptide (GIP) and glucagon-like peptide-1 (GLP-1).

The results of both experiments suggest that dietary phenols present in both apple juice and coffee significantly affect the profiles of glucose absorption and/or GI hormone secretion in volunteers. In study 1, two-way repeated measures ANOVA showed a significant treatment × time interaction for plasma glucose with concentrations peaking later and remaining high for longer after consumption of the high-phenols apple juice compared with both the lower-phenols apple juice and the control (P<0.05). This delayed pattern of absorption was also reflected in the GI hormone profiles. GIP secretion, which occurs in the proximal region of the gut, was suppressed after consumption of the high-phenols apple juice (P<0.05) whereas GLP-1 secretion, which occurs in the distal region of the small intestine was enhanced (P<0.005). Similar differences in the patterns of GIP (P<0.005) and GLP-1 (P<0.005) secretion were also observed in study 2. However, the differences in insulin and glucose profiles between the treatments were less pronounced, possibly due to caffeine-mediated attenuation of peripheral glucose uptake.

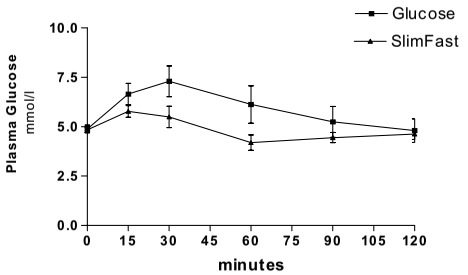
These preliminary data suggest that phenols have an active role in determining the glycaemic index of plant foods that may be of relevance for functional foods. In conclusion, it had been demonstrated that dietary phenols in apple juice and coffee cause significant attenuation of both plasma glucose and insulin concentrations, and alterations in circulating GI hormone profiles. The specific components of this and the exact mechanisms involved need to be further investigated.

Andrade-Cetto A & Wiedenfeld H (2001) *Journal of Ethnopharmacology* **78**, 145–149.
Song J, Kwon O, Chen S, Daruwala R, Eck P, Park JB & Levine M (2002) *Journal of Biological Chemistry* **277**(18), 15252–15260.
Welsch CA, Lachance PA & Wasserman BP (1989) *Journal of Nutrition* **119**, 1698–1704.

The glycaemic index of a meal replacement formula diet. By A. NOROUZY, A.R. LEEDS and P.W. EMERY, *Department of Nutrition and Dietetics, King's College London, 150 Stamford Street, London SE1 9NN*

Liquid formula meal replacements have been used for successful, long-term weight control and improvement of blood lipid and glucose concentrations in obese and obese-diabetic subjects (Ditschuneit *et al*. 1999; Flechtner-Mors *et al*. 2000). Meal replacement formulas are high-sugar products, and there is concern that such products may cause undesirably large postprandial increases in blood glucose and insulin concentrations. The glycaemic index has been established as a useful predictor of the effects of diet on blood glucose, insulin and lipid concentrations (Liu *et al.* 2000; Pelkman, 2001). Hence the aim of this study was to measure the glycaemic index of one widely consumed meal replacement product.

Sixteen subjects (eight male and eight female, BMI 18.5–25, age 18–50 years) were recruited, after excluding those who were anaemic, pregnant, or had abnormally high fasting blood glucose or insulin concentrations. All subjects received one reference meal consisting of glucose and one test meal consisting of Slim-Fast Ready-To-Drink (RTD) Chocolate Royale formula (Sun Nutritional) in random order, with a 1-week wash out period between the two tests. Individuals fasted for at least 10 h overnight, and in the morning a cannula was inserted into an arm vein and a fasting blood sample was obtained. The subject then consumed 50 g glucose in 400 ml water or 1.4 cans of Slim-Fast (each can contains 325 ml), which contained 50 g available carbohydrate (48 g sugars) in 455 ml of formula. Serial blood sampling for glucose was done at 0, 15, 30, 60, 90 and 120 min. The area under the curve (AUC) was calculated from mean plasma glucose values and a paired *t*-test was used to determine differences in plasma glucose concentration at each time point.



Plasma glucose curves for the two test meals are presented in the Figure. Plasma glucose peaked 30 min after the reference meal compared with 15 min after Slim-Fast. Differences in mean plasma glucose between the groups were significant at 15, 30, 60 and 90 min ($P \leq 0.05$). The mean AUC values were 126.6 for glucose and 24 for Slim-Fast RTD, leading to a calculated glycaemic index (GI) of 19. This is in the same range as legumes, which are considered to be model low GI foods. It is also considerably lower than the values reported for Slim-Fast in the USA and Australia, which are 40 and 42 This may reflect the fact that Slim-Fast in the USA and Australia contains 27% less protein (10 g protein compared with 13.5 g protein in 100 ml in the UK formula). We concluded that Slim-Fast RTD formula diet is a low GI food. Its use as part of a weight management programme is unlikely to lead to adverse changes in blood glucose and insulin profiles or lipid levels.

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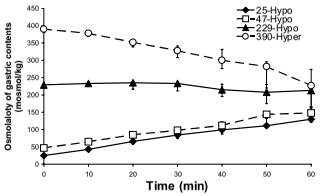
Ditschuneit HH, Flechtner-Mors M & Johnson TD (1999) *American Journal of Clinical Nutrition* **69**, 198–204.
Flechtner-Mors M, Ditschuneit HH, Johnson TD, Suchard MA & Adler G (2000) *Obesity Research* **8**, 399–402.
Liu S, Willett WC, Stampfer MJ, Franz M & Sampson L (2000) *American Journal of Clinical Nutrition* **71**, 1455–1461.
Pelkman CL (2001) *Current Atherosclerosis Report* **3**, 456–461.

Effect of osmolality on gastric emptying of carbohydrate solutions. By C.H. SIMPSON, S.M. SHIRREFFS, J.B. LEIPER and R.J. MAUGHAN, *Biomedical Sciences, University Medical School, Foresterhill, Aberdeen AB25 2ZD*

Prior to absorption in the intestine, ingested nutrients must empty from the stomach. Therefore, the rate of nutrient uptake into the body is influenced by the speed of gastric emptying (GE). Previously, we reported that a 6.4% carbohydrate (CHO) solution emptied more slowly than both a 2% CHO solution and water, suggesting that energy density is important in the control of gastric emptying (Simpson *et al*. 2001). However, osmolality also tends to increase in proportion to energy density and thus may confound the relationship between energy density and gastric emptying. Therefore, the purpose of this study was to ascertain whether solution osmolality affects gastric emptying rate independently of energy density.

Following Ethics Committee approval, eight healthy male volunteers (mean age 22 (SD 7) years, height 182 (SD 6) cm, body mass 79.5 (SD 13.5) kg) were recruited into the study. Subjects were familiarized with the study protocol before undertaking four experimental trials. On each experimental trial GE was measured for 60 min using a gastric aspiration technique with subjects at seated rest (Beckers *et al.* 1988). Trial order was randomized and trials were separated by at least 7 d. All trials commenced after a fast of at least 6 h and on each occasion 500 ml of the test solution at 19 (SD 1) ° was infused into the stomach. All test solutions contained 6.4% CHO as glucose monomers and/or a mixture of glucose polymers, resulting in solutions with the following median osmolalities: 25 mosmol kg^{-1} (25-Hypo (SD 3)), 47 mosmol kg^{-1} (47-Hypo (SD 2)), 229 mosmol kg^{-1} (229-Hypo (SD 5)), 390 mosmol kg^{-1} (390-Hyper (SD 13)). Statistical difference ($P<0.05$) was determined using Kruskal-Wallis and Mann-Whitney tests where appropriate.

	T½ (min)	
	Median	Range
25-Hypo	14.6	12.8–28.5
47-Hypo	16.4	12.0–20.4
229-Hypo	13.6	10.4–21.2
390-Hyper	15.5	9.7–29.8



There were no differences between trials for volume of test solution emptied from the stomach ($P=0.920$) or for half emptying times (T½; see Table) ($P=0.979$). The osmolality of gastric contents were different between trials 25-Hypo and 47-Hypo up to 30 min after ingestion (see Figure). Gastric osmolality was different between all other trials for the duration of the study except at 60 min when there were no differences between 229-Hypo and both 47-Hypo and 390-Hyper.

The present study demonstrates that solutions with an energy density of 1.09 MJ/l empty at a rate that is not affected by their osmolality over the range tested. After the 40 min measurement point, gastric fluid contents were composed mainly of swallowed saliva and gastric secretions. This study confirms the findings of Brouns *et al.* (1995) that gastric osmolality has little effect on the gastric emptying rate of dilute CHO solutions.

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Beckers EJ, Rehrer NJ, Brouns F, Ten Hoor F & Saris WHM (1988) *Gut* **29**, 1725–1729.
Brouns F, Sender J, Beckers EJ & Saris WHM (1995) *Journal of Parenteral and Enteral Nutrition* **19**, 403–406.
Simpson CH, Shirreffs SM & Leiper JB (2001) *Proceedings of the Nutrition Society* **60**, 191A.

Higher intakes of zinc are positively associated with indices of bone health in perimenopausal and early postmenopausal Scottish women. By H.M. MACDONALD[1,3], S.A. NEW[2], W.D. FRASER[4] and D.M. REID[1,3], [1]*Osteoporosis Research Unit, University of Aberdeen, Woolmanhill Hospital, Aberdeen AB25 1LD,* [2]*Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH,* [3]*Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen AB25 2ZD and* [4]*Department of Clinical Chemistry, Royal Liverpool University Hospital, Liverpool L69 3GA*

We have previously shown that increased intakes of zinc (Zn) are associated with greater bone mineral density (BMD) in late premenopausal women (mean age 47.5 years) (*n* 1064) (New *et al.* 1997). The women were a subset of the Aberdeen Prospective Osteoporosis Screening Study (APOSS) of over 5000 women who had a BMD scan in 1990–3 and again in 1997–2000, with 3883 women returning for the second visit (75.9% overall response rate). We now have dietary information on 3239 of these women.

Dietary intake was assessed using the same food frequency questionnaire (FFQ) as used in the baseline assessment (validated using 7 d weighed intake records and markers of antioxidant status). BMD was measured by dual x-ray absorptiometry (Norland XR-26) at the lumbar spine (LS) and femoral neck (FN) sites. Also, 2935 women provided a urine sample for measurement of bone resorption markers by HPLC. Free pyridinoline (fPYD) and deoxypyridinoline (fDPD) cross-links were expressed relative to creatinine (Cr). Bisphosphonate users and women with thyroid disease were excluded prior to statistical analysis.

For women who were still menstruating, energy-adjusted Zn intake was associated with greater BMD and reduced bone resorption markers at the follow-up visit. This remained significant after adjusting for confounding variables (see Table). However, for the remaining women (present HRT users, past HRT users and postmenopausal never used HRT) no associations between Zn and markers of bone health were observed, with the exception of the bone resorption markers measured at the follow-up visit, and change in FN BMD, for the subgroup of postmenopausal women who had never used HRT.

	Pearson correlations[a]								
	Pre/Perimenopausal			All Postmenopausal (inc HRT users)			Postmenopausal (no HRT)		
Marker of Bone Health[b]	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*
LS BMD baseline	321	0.106	0.063	2702	0.003	0.870	962	0.002	0.962
LS BMD follow-up	320	0.121	0.034*	2699	0.009	0.643	962	0.014	0.662
FN BMD baseline	321	0.064	0.267	2700	0.027	0.174	962	–0.013	0.688
FN BMD follow-up	320	0.128	0.024*	2696	0.028	0.159	960	0.043	0.192
Change in LS BMD	320	0.055	0.334	2699	0.005	0.806	962	0.026	0.429
Change in FN BMD	320	0.140	0.014*	2696	–0.003	0.871	960	0.102	0.002**
fPYD/Cr	288	–0.173	0.004**	2456	–0.071	0.001**	863	–0.102	0.003**
fDPD/Cr	288	–0.206	0.001**	2456	–0.064	0.002**	863	–0.059	0.091

[a]Adjusting for age, weight, height, smoking and socio-economic status (and HRT status for postmenopausal women).
[b]Some BMD measurements missing (e.g. hip replacements).
*$P<0.05$; **$P<0.010$.

We previously reported that fruit and vegetables reduced bone loss in women from APOSS (Macdonald *et al.*, 2002). Since meat products provide 40% of zinc in the British diet (MAFF, 1994) these data suggest that a 'balanced' diet (including dairy produce, fruit and vegetables, and some meat) may help maintain healthy bones prior to the menopause, and assist in reducing bone loss after the menopause.

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Macdonald HM, Downie FH, New SA, Moore F, Grubb DA & Reid DM (2002) *Proceedings of the Nutrition Society* **60,** 202A.
Ministry of Agriculture Fisheries and Food (MAFF) (1994) The Dietary and Nutritional Survey of British Adults: Further Analysis. London: HMSO.
New SA, Bolton-Smith C, Grubb DA & Reid DM (1997) *American Journal of Clinical Nutrition* **65**, 1831–1839.

Alcohol intake: an example of how to handle missing dietary questionnaire information. By U.A. NUR[1], D.C.GREENWOOD[1,2], J.E. CADE[2] and N.T. LONGFORD[3], [1]*Medical Statistics Unit, School of Medicine, University of Leeds, Leeds LS2 9LN,* [2]*Nuffield Institute for Health, University of Leeds, 71–75 Clarendon Road, Leeds LS2 9PL and* [3]*Department of Medical Statistics, De Montfort University, The Gateway, Leicester LE1 9BH*

Missing values are a problem in large-scale surveys with extensive questionnaires. Incompleteness raises two kinds of issues. First, having collected less data than anticipated, we have less information than planned. Second, the subjects who fail to respond (to an item or a block of items) may tend to differ (systematically) from subjects whose records are complete. Therefore, the analysis of the complete records may yield inferences substantially different from those that would have been obtained had no data been missing. Developments in the analysis of incomplete data and its applications have been described by Dempster *et al.* (1977), Little & Rubin (1987) and Rubin (1987).

The ultimate goal of the researcher is to make inferences on the population rather than the subset of the smaller, and possibly different, population who were capable of producing responses to all relevant variables in the analysis. Missing data can therefore lead not only to incorrect *P* values but also to incorrect estimates of relationships such as that between diet and cancers.

This study aims to explore multiple imputations developed by Dempster *et al.* (1977) and Rubin (1987), applied to alcohol consumption reported by the UK Women Cohort Study. The UKWCS collected information on 35 372 subjects; information on alcohol intake was collected in two ways: on a food frequency questionnaire and as a separate additional question. For the purpose of illustrating the effect of missing data, we focus on these additional questions recording exact frequencies, the response rate was 48% for beer, 82% for wine, 48% for sherry and 78% for spirits. To calculate alcohol consumption from the additional questions only 12 571 (36%) had complete data. We compare three approaches to handling missing dietary information.

	Complete case analysis			Imputing zero			Multiple imputation		
Alcohol (g/d)	Observed	Mean	SE	Observed	Mean	SE	Observed	Mean	SE
Wine	28 937	6.72	0.047	35 367	5.50	0.041	35 259	7.09	0.041
Beer	16 877	3.11	0.055	35 367	1.481	0.028	35 108	4.30	0.032
Spirits	27 620	1.45	0.019	35 367	1.13	0.015	35 251	1.70	0.016
Sherry	17 122	1.02	0.015	35 367	0.49	0.008	35 112	1.34	0.009
Total intake	12 571	7.75	0.098	35 367	8.60	0.056	35 058	14.41	0.064

Comparing the three shows that ignoring missing values by only analysing complete cases produces bias (lower means). Imputing extreme values, for example zero, can also lead to biased results, as this not only assumes that one knows exactly what the missing values would have been if they have been observed, but also incorrectly increases the apparent precision of estimation (i.e. inappropriately small standard errors). By using multiple imputations all the information in the incomplete records can be used. The advantage of multiple imputations is that the algorithm intended for analysing the complete data is applied several times, without any alterations. Ignoring missing dietary information, or handling it inappropriately, can lead to incorrect conclusions, and where this is due to numbers missing, multiple imputation should be considered.

Dempster AP, Laird NM & Rubin DB (1977) *Journal of the Royal Statistical Society B* **39**, 1–38.
Little RJA & Rubin DB (1987) *Statistical Analysis with Missing Data.* New York: Wiley.
Rubin DB (1987) Multiple Imputation for Non-response in Surveys. New York: Wiley.

Missing data in standard food composition tables. By C. WHITHAM[1], M. STANDING[1], J. POLLARD[2], D. GREENWOOD[2] and J. THOMAS[2], [1]*Leeds Metropolitan University, Calverley Street, Leeds LS1 3HE and* [2]*Nutrition Epidemiology Group, Nuffield Institute for Health, 71-75 Clarendon Road, Leeds LS2 9PL*

Food composition tables are basic tools in nutritional epidemiology, providing the necessary data to calculate the nutrient content of the diet. There are numerous tables in use throughout the world. In the UK the most commonly used food tables are found in '*McCance and Widdowson's The Composition of Foods*' (Holland *et al*. 1991 and supplements). However these tables do not contain complete nutrient content information for many of the foods listed. The letter 'N' appearing in the table indicates that the nutrient is present in significant quantities but that there is no reliable information on the amount. In theory, when using the tables, a 'guesstimated' value should be substituted for the 'N' value, although in practice this rarely happens and therefore a value of zero is used (Cowin & Emmett, 1999). The present study investigated the degree of underestimation of antioxidant nutrients due to missing data in the food tables. The nutrients under investigation were copper, manganese, selenium, vitamin C and vitamin E.

The sample for study was drawn from the UK Women's Cohort Study (UKWCS), and consisted of fifty-four women living in the Leeds, Bradford, Wakefield and Huddersfield areas. 24-h dietary recalls were collected and analysed using the COMP-eat nutrient analysis package (Carlson Bengston Consultants, 2001). These were then re-analysed using a new dietary assessment package (DANTE) which incorporated 'guesstimates' for any 'N' values identified within the diet. 'Guesstimates' were obtained from either the USDA database, when available, or by matching them to comparable foods from the McCance and Widdowson food tables. Altogether 435 different foods were identified from the 24-h recall data. Within these foods 298 'N' values were found for copper, manganese, selenium, vitamin C and vitamin E, and 'guesstimates' added. A paired sample *t*-test was then employed to establish whether there was a significant difference between the two analyses.

Nutrient	Mean intake using COMP-eat	Mean intake using DANTE	% underestimate by COMP-eat	95% CI	*P* value
Copper (mg)	0.98	1.00	–2	–4 to 0	0.09
Manganese (mg)	3.19	3.96	–19	–24 to –14	<0.01
Selenium (µg)	42.07	57.63	–27	–34 to –19	<0.01
Vitamin C (mg)	74.10	75.26	–2	–3 to 0	0.07
Vitamin E (mg)	5.16	7.30	–29	–38 to –20	<0.01

Vitamin C and copper had the fewest missing values, with only seven foods affected by the addition of 'guesstimates'. Selenium had the highest number of missing values, with 132 foods identified as having 'N' values. The results clearly highlight that the mean intakes of manganese, selenium and vitamin E were significantly different when 'guesstimates' were added. Vitamin C and copper showed no significant differences between intakes before and after the addition of missing values.

This study supports the addition of 'guesstimates' in place of the 'N' values when conducting dietary assessments. Without using new data in place of 'N' values, studies investigating antioxidants could be seriously underestimating intakes, particularly of vitamin E. However, it is acknowledged that gathering this information is a time-consuming task and thus is unlikely to become common practice in dietary assessment methodology. It is suggested that 'guesstimated' values should be added to food composition tables at source, rather than leaving this to individual researchers. The use of European specific data is also required particularly for nutrient such as selenium levels which differ greatly between Europe and USA.

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Carlson Bengston Consultants (2001) *COMP-EAT* [5.7]. London: Carlson Bengston Consultants.
Cowin I & Emmett P (1999) *European Journal of Clinical Nutrition* **53**, 891–894.
Holland B, Welch AA, Unwin ID, Buss DH, Paul AA & Southgate DAT (1991) *McCance and Widdowson's The Composition of Foods*, 5th edn. Cambridge: Royal Society of Chemistry.

The prevalence of overweight and obesity in children aged 4–12 years in Gibraltar, using the International Obesity Taskforce standard definition. By S.F.L. KIRK[1] and M. McLEOD[2], [1]*Nutrition Epidemiology Group, Nuffield Institute for Health, 71–75 Clarendon Road, Leeds LS2 9PL and* [2]*Dietetic Department, St. Bernard's Hospital, Gibraltar*

Childhood obesity is now a well-recognized problem in Europe (Livingstone, 2001) although data on prevalence are limited due to a lack of an agreed definition of overweight and obesity in childhood. Using the standard definition proposed for the International Obesity Taskforce (Cole *et al*. 2000), this study analysed height and weight data available on 2994 children in Gibraltar to determine overweight and obesity prevalence. Data collection took place over 3 months from January to May 1998. The same height stand and scales were used throughout the study and measurements were made by the same person (M. McLeod). Weight was measured using SECA battery-operated, self-calibrating scales. Height and weight were converted to BMI and *Z* scores were calculated using the British 1990 growth reference for height, weight, BMI and head circumference (Child Growth Foundation, 1990). Prevalence of overweight and obesity was based on average centiles estimated to pass through BMI 25 and 30 kg/m^2, respectively, at age 18 years. Thus, a *Z* score of 1.30 for males and 1.19 for females was taken as an indicator of overweight, while a *Z* score of 2.37 for males and 2.25 for females was taken as an indicator of obesity. Statistical analyses were performed using SPSS version 9.0 (SPSS). Prevalence of overweight and obesity was calculated for each age group and over the whole sample population.

	Age group (years)									
	4	5	6	7	8	9	10	11	12	Total
Boys	*n 88*	*n 208*	*n 225*	*n 200*	*n 194*	*n 182*	*n 185*	*n 170*	*n 88*	*n 1540*
Overweight	14	39	33	40	34	37	43	40	24	304
n (%)	(15.9)	(18.8)	(14.7)	(20)	(17.5)	(20.3)	(23.2)	(23.5)	(27.3)	
Obese	2	9	23	23	23	29	27	20	10	166
n (%)	(2.3)	(4.3)	(10.2)	(11.5)	(11.9)	(15.9)	(14.6)	(11.8)	(11.4)	
Girls	*n 88*	*n 193*	*n 215*	*n 179*	*n 169*	*n 174*	*n 174*	*n 167*	*n 95*	*n 1454*
Overweight	20	29	41	35	34	45	45	40	22	311
n (%)	(22.7)	(15.0)	(19.1)	(19.6)	(20.1)	(25.9)	(25.9)	(24.0)	(23.2)	
Obese	9	16	24	16	25	16	19	21	8	154
n (%)	(10.2)	(8.3)	(11.2)	(8.9)	(14.8)	(9.2)	(10.9)	(12.6)	(8.4)	

Proportion of children who were overweight or obese according to age.

The results showed a prevalence of overweight in boys of 19.7%, while obesity prevalence was 10.8%. For the girls, overweight prevalence was 21.4%, while obesity prevalence was 10.6%. There were no significant differences in the proportion of overweight or obesity between boys and girls. This was the first time that overweight and obesity prevalence has been estimated in children from Gibraltar. These data provide further information on prevalence rates of overweight and obesity, using defined cut-offs for comparison with data from other countries.

Child Growth Foundation (1990) *British 1990 Growth Reference for Height, Weight, BMI and Head Circumference*. London: Child Growth Foundation.
Cole TJ, Belizzi MC, Flegal KM & Dietz WH (2000) *British Medical Journal* **320**, 1–6.
Livingstone M (2001) *Public Health Nutrition* **4**, 109–116.

Frequency of confectionery consumption from vending machines and other purchase providers: associations with dietary and lifestyle habits in British schoolchildren. By S.A. NEW[1] and M.B.E. LIVINGSTONE[2], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH and* [2]*Northern Ireland Centre for Diet and Health, University of Ulster, Coleraine, Co. Londonderry, BT52 1SA*

There is increasing concern that a high consumption of ready-to-eat foods, such as confectionery, may be associated with poor dietary quality and an increase in susceptibility to obesity, particularly in children. However, the evidence linking confectionery consumption with adverse effects on dietary quality is equivocal. While fat and sugar intakes are high in snacks purchased from school tuck shops (Wildey *et al.* 2000), high snack food consumption does not appear to have detrimental effects on micronutrient intake (Gibson, 1996). The aim of this study was to investigate the association between frequency of confectionery consumption purchased from vending machines, as well as from other sources, with dietary and lifestyle habits in adolescent boys and girls.

A total of 504 subjects were studied (age range 12–15 years) from three schools in southern and northern England. A lifestyle questionnaire was developed to determine the general patterns of physical activity, dietary intake and lifestyle habits during a typical school week. The frequency of confectionery consumption (CC) from all sources (AS) and vending machines (VM) was recorded. Subjects were categorized into non-consumers (N), low (L), medium (M) and high (H) consumers, using the following criteria: N=0 times/week; L=1–5 times/week; M=6–9 times/week; H=10 or more times/week.

No differences were found in the frequency of confectionery consumption (from either AS or VM) between those who ate breakfast and lunch and those who did not. No differences were found in the frequency of fruit and vegetable intake in H VM CC *v.* N VM CC groups. Confectionery consumption (AS) was found to be lower in subjects who stated that they did not eat five portions of fruit per day (mean rank using Npar 256 *v.* 207 respectively, $P<0.05$). No differences were seen between the four AS CC and VM CC groups for milk consumption, frequency of consumption of lunch, lunch content, breakfast content, food preferences or for the reasons stated why fruit was not eaten everyday. Confectionery consumption from AS was found to be higher in subjects who were physically active on the journey to school ($P<0.01$) but also higher in those who spent more time watching television and playing computer games ($P<0.01$). No associations were found with smoking habits or alcohol consumption and the CC groups.

There are several limitations to this study. The design was cross-sectional in nature and thus only associations rather than relationships can be explored. We deliberately chose schools with vending machines and thus work is required to see if their absence has any effect on snack purchase. Furthermore, we do not have good indications of what children were eating for the rest of the day and hence no definite conclusions can be made concerning overall quality of the diet.

These results do not provide support of a link between confectionery consumption purchased from vending machines and 'poor' dietary practice or 'undesirable' lifestyle habits. Findings for total confectionery consumption showed some interesting trends, but the results were not consistent, either for a negative or positive effect.

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Gibson SA (1996) *Proceedings of the Nutrition Society* **55**, 121A.
Wildey MB, Pampalone SZ, Pelletier RL, Zive MM, Elder JP & Sallis JF (2000) *Journal of the American Dietetic Association* **100**, 319–322.

Waist : Height ratios in British children aged 5–16 years: a suggestion for a simple public health message – 'keep your waist circumference to less than half your height'. By H.D. McCARTHY[1] and M. ASHWELL[2], [1]*School of Health and Sports Science, University of North London, Holloway Rd, London N7 8DB and* [2]*Ashwell Associates, Ashwell Street, Ashwell, Herts, SG75P*

The ratio of waist circumference to height (WHTR) in adults has been proposed as a simple, practical indicator of risk for diseases related to internal obesity and for monitoring risk reduction (Ashwell *et al.* 1996). The Ashwell Shape Chart is a consumer-friendly tool based on WHTR where a ratio of 0.5 or greater indicates increased risk of morbidity in adults (Ashwell, 1998). Savva *et al.* (2000) have suggested that WHTR is better than BMI for predicting health risks in children in Cyprus. However, WHTR has not been examined in any British juvenile population and hence it is unknown how growth, age or gender will impact upon this ratio throughout childhood. Waist circumference – a marker of upper body fat accumulation, has been shown to be related to blood lipid and insulin concentrations in children (Freedman *et al.* 1999) and age-related waist circumference percentile charts have now been developed for use in the British paediatric population (McCarthy *et al.* 2001). This study examined WHTR and the influence of gender and age throughout childhood.

The data used were collected cross-sectionally by the HUMAG Research Group, Loughborough University. Subjects were aged between 5.0 and 16.9 years (British Standards Institute, 1990). The sample (*n* 8079; 3502 boys, 4577 girls) was representative, as far as possible, of children in Great Britain at the time of data collection (1978 for boys, 1988 for girls). Waist circumference was measured midway between the 10th rib and the iliac crest. Height and weight were measured using standard procedures. WHTR and BMI were calculated and statistically analysed by age and gender.

In the whole cohort, WHTR ranged between 0.32 and 0.66. Mean WHTR ranged from 0.468 at age 5.0 years to 0.418 at age 16.0 years in boys and from 0.460 to 0.407 in girls between the same ages. WHTR decreased slightly with age in both boys and girls (boys r^2 –0.331, $P<0.01$, girls r^2 –0.459, $P<0.01$). At all ages, mean WHTR was slightly greater in boys than in girls (P ranging between 0.01 and 0.001) except at ages 7, 8 and 16 years, P=NS). 4.7% of 5–10-year-old boys and 4.4% of 11–16-year-old boys had a WHTR greater than 0.50 and in girls the corresponding figures were 3.6% and 1.1%.

These findings show that both linear growth in childhood and gender influence the WHTR. In spite of the tremendous advances in producing age-standardized BMI values to define obesity in children (Cole *et al.* 2000), assessment by this method is time-consuming and still based on a proxy for total fat. We suggest that the simple cut-off of WHTR=0.50 might be a quick assessment for increased health risk in children which relates to excessive accumulation of upper body fat and possibly internal fat. It might slightly overestimate the number of 'at risk' younger, compared with the older, children and slightly overestimate the number of 'at risk' boys compared with girls. Information from other data sets will help to confirm or refute this trend. However, there is certainly benefit to be gained from a simple public health message that is the same for adults and children of both sexes and all ages. It could be stated simply as 'keep your waist circumference to less than half your height'.

Ashwell MA (1998) *International Journal of Obesity* **22** (Supplement 3), S213.
Ashwell MA, LeJeune SRE & McPherson K (1996) *British Medical Journal* **312**, 377.
British Standards Institute (1990) *Body Measurements of Boys and Girls from Birth up to 16.9 Years, BS7321.* London: British Standards Institute.
Cole TJ, Bellizzi C, Flegal KM & Dietz WH (2000) *British Medical Journal* **320**, 1240–1253.
Freedman DS, Serdula MK, Srinivasan SR & Berensson GS (1999) *American Journal of Clinical Nutrition* **69**, 308–317.
McCarthy HD, Jarrett KV & Crawley HF (2001) *European Journal of Clinical Nutrition* **55**, 902–907.
Savva SC, Tornaritis M*et al.* (2000) *International Journal of Obesity* **24**, 1453–1458.

Role of cheese in the diet of children aged 4–18 years: further analysis of the National Dietary and Nutritional Survey. By T.A.B. SANDERS and A. PARKE, *Nutrition, Food and Health Research Centre, King's College London, Franklin-Wilkins Building, Stamford Street, London SE1 9NN*

Cheese is an important dietary source of calcium and vitamin A but is also relatively high in saturated fat. This study involved analysis of the National Diet and Nutrition Survey (NDNS) in 1702 young people aged 4–18 years (Gregory *et al*. 2000) to compare nutrient intakes, prevalence of overweight and obesity, and plasma cholesterol concentrations according to cheese intake. In contrast to the NDNS survey, the total daily amount of cheese consumed included cheese on pizza. The amount and type of cheese on pizza was taken as 17 g cheddar cheese per 100 g pizza. Estimates of obesity and overweight were determined using the cut-off points for BMI (Cole *et al*. 2000). Age-specific quintiles of cheese intake were estimated. The average intake of cheese from all sources was 12 g/d, which is less than half the reported intake in UK adults, and 18.9% of the sample reported avoiding cheese. Children who avoided cheese had poorer diets than those who included cheese in their diets: intakes of calcium, riboflavin and vitamin A were significantly lower than in consumers and they were less likely to meet the RNI for most nutrients. About 30% of children from families receiving family credit or income support were non-consumers compared with 15% in those from socio-economic classes 1 and 2. There was no association between cheese intake and the number of children who were obese but there was a significant trend ($P<0.0001$) between quintile of intake and the proportion of children who were overweight. The table below shows the contribution made by cheese to intakes of calcium, vitamin A and saturated fatty acids according to quintile of cheese intake.

Mean cheese intake (g/d)		Total cheese intake quintiles by age group					
		0	3.8	9.2	16	31.7	
		n 343	*n* 337	*n* 338	*n* 343	*n* 340	
Calcium (mg/d)	Mean	0	29.8 ± 2.6	60.6 ± 2.6	104 ± 2.6	199.5 ± 2.6	$P<0.005$
Vitamin A (r.e./d)	GM	0	10.6 ± 0.9	28.2 ± 2.5	50.4 ± 4.4	94.3 ± 8.3	$P<0.005$
Saturated fatty acids (%energy)	Mean	0	0.43 ± 0.01	0.99 ± 0.02	1.69 ± 0.02	3.0 ± 07	$P<0.005$

Mean or geometric means (GM) with SEM.

Although cheese intake was related to saturated fatty acid intake, there was no significant relationship between cheese intake and plasma cholesterol concentration. The mean intake of cheese was 17.1 g/d in the 178 children (10.5% of the total sample) meeting the RNI for thiamin, riboflavin, niacin, folate, vitamins A, B_{12} and C, and calcium, iron and zinc. If a saturated fatty acid intake of less than 11% of the energy intake was factored into this model then only four boys (cheese intake 15.7 g/day) and one girl (non-consumer of cheese) met the criteria (0.3% of the total sample). The most salient finding was the contribution made by cheese to calcium intake. The higher intake of calcium in the top quintile of calcium intake is almost entirely explained by calcium intake supplied by cheese.

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Cole TJ, Bellizzi MC, Flegal KM & Dietz WH (2000) *BMJ* **320**, 1–6.
Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LJ, Smithers G, Wenlock R & Farron M (2000) *National Diet and Nutrition Survey: Young People Aged 4 to 18 Years. Volume 1: Report of the Diet and Nutrition Survey*. London: TSO.

Ability of the UK Women's Cohort Study food frequency questionnaire to rank dietary intakes: a preliminary validation study. By M. SPENCE, J.E. CADE, V.J. BURLEY and D.C. GREENWOOD, *Nutrition Epidemiology Group, Nuffield Institute for Health, 71–75 Clarendon Road, Leeds LS2 9PL*

Food frequency questionnaires (FFQs) are increasingly being used to assess food intake; therefore it is important that they are a true reflection of dietary practice. They have become the method of choice in most large epidemiological studies, as, amongst other advantages, they are easy to administer and code. The FFQ in the UKWCS (35 000 women, aged 35–69 years) allowed subjects to specify their daily, weekly or monthly intake of 217 foods over the past 12 months (adapted from the FFQ used by the EPIC Cohort Study). The objective of this paper was to assess aspects of the validity of the baseline FFQ. Three years after the baseline FFQ had been completed, a subgroup of the cohort (303 women) completed a second FFQ, a 4 d diary and gave a fasting blood sample. The FFQs and diary were coded and analysed for nutrient content. Plasma nutrient levels for iron, zinc, calcium and vitamins A, C, E and B_{12} were measured.

Correlation coefficients were corrected for imprecision in the 4 d weighed diary (Nelson, 1997). All Pearson correlation coefficients between nutrient intakes assessed by the diary and both FFQs were found to be highly significant ($P<0.01$). A stronger correlation (with the exception of vitamins A and E) was seen between the second FFQ and the diary compared to the baseline FFQ. Most of the correlation coefficients for the baseline FFQ improved or gave similar results when women who had changed their diet since baseline were excluded from the analysis. Correlation coefficients between plasma nutrient levels and both the baseline and second FFQ were shown to be statistically significant ($P<0.01$) for vitamins B_{12} and C. The correlation was also significant at the 0.01 level for calcium between the second FFQ and diary.

	Correlation (corrected for diary imprecision)		
	Baseline FFQ and diary	Second FFQ and diary)	Baseline FFQ and diary (diet unchanged)
Nutrient	(*n* 298)	(*n* 283)	(*n* 187)
Carbohydrate	0.35	0.39	0.34
Protein	0.27	0.33	0.14
Fat	0.29	0.35	0.29
Calcium	0.36	0.43	0.32
Iron	0.35	0.42	0.35
Vitamin A	0.50	0.40	0.60
Vitamin C	0.49	0.62	0.54

Correlations between the two dietary assessment methods were comparable to those found in other studies (Cade *et al*. 2002). The diary and second FFQ were carried out at the same time and this may explain their higher correlation in comparison with the baseline. Food diaries may underestimate intake whilst the FFQ tends to overestimate intake. It is clear that both tools are measuring different aspects of diet. To take this into account all the women in the cohort have been asked to provide a 4 d food diary in addition to the FFQ. It will be important to adjust future relative risk estimates to remove the bias caused by non-differential errors in exposure measurement using a combination of results from these two approaches.

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Cade JE, Thompson RL, Burley VJ & Warm DL (2002) *Public Health Nutrition* (in press).
Nelson M (1997) In *Design Concepts in Nutritional Epidemiology*, 2nd edn, pp. 241–272 [BM Margetts and M Nelson, editors]. Oxford: Oxford University Press.

Association between food variety and selected socio-demographic and lifestyle characteristics in adults in the West of Scotland. By M. EBRAHIMI-MAMEGHANI[1], J.A. SCOTT[1] and G. DER[2], [1]*Department of Human Nutrition, Glasgow University, Glasgow Royal Infirmary, Glasgow G31 2ER and* [2]*MRC Social and Public Health Sciences Unit, University of Glasgow, 4 Lilybank Gardens, Glasgow G12 8RZ*

The link between diet, health maintenance and the development of chronic diseases has become increasingly evident in recent years. The nutrients essential to meet nutritional requirement are not found in a single food item, but come from a diet composed of a variety of foods. Diverse diets have been shown to protect against chronic disease such as cancer (La Vecchia *et al*. 1997), as well as being associated with prolonged longevity (Kant *et al*. 1995) and improved health status (Hodgson *et al*. 1994). A food variety score based on the weekly consumption of biologically different foods has been shown to be predictive of nutritional adequacy (Hodgson *et al*. 1994).

This study aimed to investigate the overall eating pattern, using food variety as an indicator of adequacy, of a population of Scottish adults. The data reported here were collected as part of The West of Scotland Twenty-07 Study, a longitudinal study of three age cohorts designed to explore social patterning in relation to health. A food frequency questionnaire (FFQ) was completed by 777 subjects in the 35-year age cohort and a food variety score (FVS) was calculated based on the weekly consumption of twenty-six biologically different food groups represented in the FFQ.

The median of the FVS was 20 (mean 19.79±3.12). Subjects with a FVS <20 were categorized as 'less varied eaters' and subjects with a FVS ≥20 were categorized as 'varied eaters'. Logistic regression analysis (adjusting for gender, marital status, head of household's occupational class, physical activity level and drinking and smoking habits) revealed that men and alcohol users were significantly more likely to consume less varied diets compared with women and those who had never drunk alcohol.

Factors associated with eating a less varied diet, after adjustment for potential confounders

Variable	Unadjusted Odds ratio (95% CI)	*P*	Adjusted[a] Odds ratio (95% CI)	*P*
Gender				
Female	1.00	0.0596	1.00	0.0233
Male	1.34(0.99, 1.82)		1.44(1.05, 1.98)	
Alcohol use				
Never	1.00	0.0219	1.00	0.0364
Ex-drinker	0.73(0.21, 2.51)		0.86(0.24, 3.10)	
Current drinker	2.02(1.04, 3.93)		2.10(1.04, 4.26)	

[a]Adjusted odds ratios by marital status, head of household's occupational class, physical activity level and smoking habits (with 95% CI).

The indices of food variety in this study were lower than for healthy Australian adults (mean 24.9±0.8) (Wahlqvist *et al*. 1989) and may explain some of the differences in morbidity patterns between the two countries. Future analysis will investigate whether food variety is predictive of health outcomes in this West of Scotland population.

Hodgson JM, Hsu-Hage BH-H & Wahlqvist ML (1994) *Ecology of Food and Nutrition* **32**, 137–148.
Kant AK, Schatzkin A & Ziegler RG (1995) *Journal of the American College of Nutrition* **14**, 233–238.
La Vecchia CL, Munoz SE, Braga C, Fernandez E & Decarli A (1997) *International Journal of Cancer* **72**, 255–257.
Wahlqvist ML, Lo CS & Myers KA (1989) *Journal of the American College of Nutrition* **8**, 515–523.

Impact of whole grain breakfast consumption on daily nutrient intakes. By A.T. SMITH[1], S. KUZNESOF[2], D.P. RICHARDSON[3] and C.J. SEAL[1], [1]*Human Nutrition Research Centre, Dept. of Biological and Nutritional Sciences,* [2]*Dept. of Agricultural Economics and Food Marketing, University of Newcastle, Newcastle upon Tyne NE1 7RU and* [3]*Nestlé UK Ltd, St George's House, Croydon CR9 1NR*

Consumption of diets rich in whole grains has been associated with decreased risk of several chronic diseases including coronary heart disease, some cancers and type 2 diabetes (Richardson, 2000). At least one serving of whole grain per day has been shown to reduce the risk of total mortality and CHD mortality significantly, even after adjustment for other potentially confounding lifestyle variables (Jacobs *et al*. 1999). In the UK health professionals recommend plenty of starchy carbohydrates and whole grain options of these foods whenever possible. Although data on consumption levels are limited in the UK, initial findings indicate that population intakes are extremely low (Lang *et al*. 2001). For those who do consume whole grain foods, bread and breakfast cereals are the major sources (Richardson, 2000).

Twenty healthy, non-dieting habitual breakfast eaters (ten male, ten female) aged 18–26 years participated in a breakfast study investigating the impact of whole grain breakfasts on nutrient intakes, satiety and acceptance ratings. During the study, participants were provided breakfast on two alternate mornings each week for 4 weeks. The breakfasts were of two types based on either cereals (Shredded Wheat [whole grain] or corn flakes [refined]) or toast (wholemeal toast [whole grain] or white toast [refined]). Breakfasts were supplemented according to participant preferences with orange juice, tea/coffee, butter, jam and marmalade. Quantities consumed were self-selected at the first sitting for each breakfast type and then replicated for each subsequent sitting. Participants received each breakfast twice over the study period in a completely randomized order. Dietary intake on each study day was monitored with a self-completed food diary and compared with baseline 3 d food diaries completed before the main study period. Nutrient intakes were calculated using WinDiets (Univation, Aberdeen) and data were analysed by ANOVA.

Daily total energy intake was not different between baseline and study days. There were small differences in nutrient intakes for both cereal and toast-based breakfasts, mainly reflecting compositional differences in the breakfasts provided. There were no significant differences in mean daily energy or macronutrient intakes between whole and refined grain breakfast types for cereal or toast. There were, however, some differences in micronutrient intakes as outlined in the Table (mean daily intakes with SD). The results suggest that differences in micronutrient intakes observed are attributable to differences in fortification of the refined breakfast cereal (* in Table) and the naturally higher micronutrient content of the whole grain foods.

Daily intake	Cereal					Toast				
	Refined† *n*=20		Whole grain *n*=20		*P*	Refined *n*=20		Whole grain *n*=20		*P*
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
NSP (g)	13	4.6	19	6.7	<0.001	16	6.0	19	6.7	0.069
Chloride (g)	5.79	1.91	4.88	1.93	0.032	5.65	1.76	6.18	2.12	0.054
Iron (mg)*	14.2	4.7	13.9	2.9	0.739	13.8	6.3	14.5	5.1	0.590
Magnesium (mg)	312	98.8	399	108.0	<0.001	322	94.4	383	123.9	0.010
Manganese (mg)	3.0	1.13	3.3	1.23	0.338	3.9	1.53	5.1	1.49	<0.001
Niacin (mg)*	28	12.2	23	9.1	0.009	25	12.5	26	8.7	0.699
Riboflavin (mg)*	2.5	0.86	1.9	0.53	0.002	1.4	0.40	1.4	0.61	0.625
Sodium (g)	3.82	1.27	3.08	1.17	0.007	3.61	1.12	4.13	1.43	0.117
Thiamin (mg)*	3.4	6.37	2.5	4.14	0.074	1.6	0.46	1.8	0.56	0.041
Total folate (μg)*	414	138.9	286	90.2	<0.001	294	87.1	289	84.4	0.815
Vitamin B6 (mg)*	3.2	1.10	2.2	0.73	<0.001	2.3	0.81	2.3	0.82	0.669
Zinc (mg)	8.8	3.27	10.1	2.77	0.010	8.4	3.00	10.2	4.50	0.009

Differences in nutrient intake subsequent to breakfast meals

Nutrient	Baseline *n*=20	Cereal *n*=40	Toast *n*=40	Overall *P* value	Probability level Contrast 1	Contrast 2
NMES	86.1 ± 36.3	97.4 ± 32.1	111 ± 39	0.035	0.047	0.092
Riboflavin	2.05 ± 1.62	2.16 ± 0.77	1.39 ± 0.51	0.001	0.247	0.000
Total folate	286 ± 104	350 ± 133	292 ± 85	0.032	0.211	0.020
Vitamin C	109 ± 73.	172 ± 75	158 ± 65	0.006	0.002	0.377
Manganese	3.56 ± 1.47	3.16 ± 1.17	4.48 ± 1.61	0.000	0.463	0.000
Selenium	77.7 ± 32.8	67.8 ± 26.0	107 ± 43	0.000	0.274	0.000
Iodine	94.0 ± 40.9	125 ± 42	95.6 ± 60.9	0.018	0.200	0.011
Vitamin B_6	2.45 ± 0.96	2.71 ± 1.04	2.29 ± 0.81	0.133	0.839	0.046
Phenylalanine	1947 ± 916	2533 ± 866	2018 ± 710	0.007	0.111	0.006

Following breakfast, nutrient intakes through the remainder of the day were not different between breakfast types. The differences observed in the total daily nutrient intakes were resultant from the composition of the particular breakfasts consumed. Hence, the daily NSP intake was significantly higher with the whole grain breakfast types and the total daily sodium intake was influenced by the composition of the breakfast cereal consumed. The results emphasise the contribution of breakfast towards a healthy diet.

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Jacobs DR Jr, Meyer KA, Kushi LH & Folsom AR (1999) *American Journal of Public Health* **89**, 322–329.
Lang R, Thane CW, Bolton-Smith C & Jebb SA (2001) *Proceedings of the Nutrition Society* **60**, (OCB) 218A.
Richardson DP (2000) *Nutrition Bulletin* **25**, 353–360.

Predictors of plasma selenium in participants of the UK Women's Cohort Study. By G.R. McART, J.E. CADE, V.J. BURLEY and D.C. GREENWOOD, *Nutrition Epidemiology Group, Nuffield Institute For Health, 71–75 Clarendon Road, University of Leeds, Leeds LS2 9PL*

The 1997 Total Diet Study estimated the average selenium intake in the UK population to be 39 µg/d. This intake is at the lower end of the reference range for adults recommended by COMA and is well below the UK RNI of 75 µg/d and 60 µg/d for adult men and women, respectively. Selenium is one of the essential trace elements and an integral part of the antioxidant enzymes that protect cells against the effects of free radicals produced during oxygen metabolism. In humans, whole blood selenium can vary significantly between different populations depending on dietary intake (Ihnat & Aaseth, 1989). Plasma and serum contain about 75% of the selenium found in whole blood. Levels in these fractions reflect recent dietary intakes (Lombeck *et al*. 1977).

The objective of this study was to identify predictors of plasma selenium from participants in the UK Women's Cohort Study. We therefore took fasting blood samples from 274 women from the cohort living within easy reach of Leeds. Each participant also completed a 4 d food diary. The mean plasma selenium was 125 µg/l. Plasma selenium was split into tertiles according to the overall range: low, medium and high. An analysis of variance was carried out to show the dietary factors corresponding to each plasma selenium tertile.

	Plasma selenium (µg/l)		
	Low level	Medium level	High level
Mean (SD)	(81–115.9 µg/l)	(116–132.9 µg/l)	(133–270 µg/l)
Total selenium intake	43 (20)	59 (28)	73 (49)
Dietary selenium intake	43 (19)	56 (27)	60 (32)
Supplement selenium intake	0.3 (2.7)	3 (12.5)	12 (41)
Dietary intake:			
Energy (MJ)	7.5 (1.6)	8 (1.7)	8.3 (3.7)
Protein (g)	60 (14)	68 (19)	73 (28)
Calcium (mg)	795 (236)	889 (254)	1038 (673)
Magnesium (mg)	300 (75)	317 (114)	350 (116)
Zinc (mg)	7.3 (2)	8 (2.3)	9 (4)
Riboflavin (mg)	1.7 (0.6)	1.8 (0.6)	2 (1.1)
Nicotinic acid (mg)	21 (9)	25 (12)	27 (13)
Total vitamin B_{12} intake (µg)	3 (2)	4 (3)	10 (20)
Plasma levels:			
Vitamin C (mg/l)	13 (4)	14 (5)	15 (4)
Vitamin A (mg/l)	2 (0.4)	2.3 (0.4)	2.4 (0.5)
Cholesterol (mmol/l)	5.2 (1.2)	5.6 (1.1)	5.6 (1.1)

Plasma selenium levels increased significantly with increasing energy intake. However, adjustment for energy suggested that total selenium intake was still a strongly significant predictor of plasma selenium levels. In a multiple linear regression model, 12% of the variance in plasma selenium was explained by total selenium intake, with 7% of that due to selenium supplementation. In fact, subjects taking selenium supplements had significantly higher plasma selenium levels (146 µg/l) than subjects not taking them (126 µg/l) (difference = 20 µg/l; 95% C.I: 9–30; $P<0.001$). Selenium supplementation does increase plasma selenium levels. This may be a useful strategy to increase plasma levels in subgroups of the population whose diet is inadequate for selenium.

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Ihnat M & Aaseth J (1989) *Occurrence and Distribution of Selenium*. CRC Press, Boca Raton, FL, pp.169–212.
Lombeck I, Kasparek K, Harbisch HD, *et al*. (1977) *European Journal of Paediatrics* **125**, 81–89.

Effect of maternal nutrient restriction during late-gestation on growth hormone (GH) and prolactin (PRL) receptor abundance in neonatal lambs. By M. HYATT, J. BISPHAM, J. DANDREA, D. WALKER, M.E. SYMONDS and T. STEPHENSON, *Academic Division of Child Health, School of Human Development, University Hospital, Nottingham NG9 2UH*

Maternal nutrition during late-gestation may influence neonatal growth by altering the hypothalamo-pituitary-adrenal axis maturation (Edwards & McMillen, 2001). The onset of GH-dependent growth is thought to occur over the first month of life, coincident with peak GH plasma levels (Symonds *et al*. 1989), functional hepatic GH receptor abundance (Bauer *et al*. 1995) and binding (Klempt *et al*. 1993). The extent to which the hypothalamo-pituitary-adrenal axis and the onset of GH-dependent growth are nutritionally regulated remains unknown. The present study aimed to determine whether maternal nutrient restriction during late-gestation has an effect on GH and PRL receptor mRNA abundance after birth.

Fourteen primiparous twin bearing Border Leicester × Swaledale ewes were entered into the study. Six ewes were allocated to the control group. These were fed and consumed 100% of total metabolizable energy (ME) requirements (for both ewe maintenance and growth of the conceptus in order to produce a 4.5 kg lamb at term) for that stage of gestation. The remaining eight ewes were nutrient-restricted (NR) and consumed 60% of total ME requirements between 110 d – term (term is 147 d). The diet comprised chopped hay and concentrate and was fed in a 3:1 weight ratio. All ewes lambed normally at term, and one twin of each pair was euthanased with an overdose of barbiturate (100 mg kg^{-1} pentobarbital sodium: Euthatal) within 4 h of birth to enable liver tissue sampling. The remaining twins were reared with their ewes until 30 d after birth, when liver tissue was sampled after euthanasia.

Total RNA was extracted from liver tissue, reverse transcribed and abundance of GH, and PRL receptors were measured by polymerase chain reaction (PCR) of reverse-transcribed (RT) product using oligonucleotide primers specific to ovine GH receptor (forward 5′-ATGAACCCATCTGCATGTGA-3′ and reverse 5′-TTCAGTCTTCTCATCAGGGTCA-3′) and ovine PRL receptor (forward 5′-CTGACTTACCGCAAGGGAGG-3′ and reverse 5′-CCACTGCCCAGACCATAATC-3′). Genomic DNA contamination was prevented, as all PCR products spanned at least one intron–exon boundary.

Results are given as means with their standard errors as a ratio of 18S rRNA and are expressed as a percentage of a reference sample present on all gels. Differences in nutritional treatments were analysed using a Mann-Whitney *U*-test.

Sampling age		Lamb weight (kg)		Liver weight (g)		GH receptor mRNA (a.u)		PRL receptor mRNA (a.u)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
4 h	C (n=6)	4.1	0.3	85.5	8.0	104.8	13.1	52.0	1.3
	NR (n=8)	3.6	0.2	74.0	3.0	25.4	1.6***	6.7	0.9**
30 d	C (n=6)	16.4	1.4	251.1	44.5	102.7	5.5	41.1	5.8
	NR (n=8)	14.3	1.2	258.7	49.0	45.5	0.2**	17.4	6.2*

Significantly different from control at *P<0.05, **P<0.01, ***P<0.001, as measured by Mann-Whitney *U*-test.

Maternal nutrient restriction over the final month of gestation resulted in significantly lower hepatic GH and PRL receptor transcript levels at both 4 h and 30 d after birth when compared with control lambs. This cytokine receptor downregulation occurred in the absence of any significant difference in lamb or liver weights, but may contribute to significant alterations in later growth following the onset of GH-dependent growth.

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Bauer MK, Breier BH, Harding JE, Veldhuis JD & Gluckman PD (1995) *Endocrinology* **136**, 1250–1257.
Edwards LJ & McMillen IC (2001) *Journal of Physiology* **533**, 561–570.
Klempt M, Bingham B, Breier BH, Baumbach WR & Gluckman PD (1993) *Endocrinology* **132**, 1071–1077.
Symonds ME, Andrews DC & Johnson P (1989) *Journal of Endocrinology* **123**, 295-302.

Maternal nutrient restriction during early to mid-gestation and programming of kidney development of juvenile offspring in sheep. By G. GOPALAKRISHNAN, I.W. SEETHO, G. ROBINSON, A. MOSTYN, S. PEARCE, J. DANDREA, V. WILSON, M.M. RAMSAY, T. STEPHENSON and M.E. SYMONDS, *School of Human Development, University of Nottingham NG7 2UH*

Maternal nutrient restriction over the period of rapid placental growth (i.e. 30–80 d), followed by adequate nutrition up to term, results in a longer fetus with a disproportionately larger placenta (Heasman *et al*. 1998). At term, these lambs have larger kidneys which exhibit an increased abundance of glucocorticoid receptor mRNA (Whorwood *et al*. 2001). At 6 months of age, lambs born to previously nutrient-restricted (NR) ewes exhibit lower resting blood pressure, but show an increased pressor response to noradrenaline (Gopalakrishnan *et al*. 2001). The aim of this study was to determine the consequences of maternal nutrient restriction between early- to mid-gestation on kidney size, mitochondrial function and vascular reactivity in juvenile sheep.

Twelve Welsh Mountain ewes of similar body weight and fat distribution were individually housed from 28 d gestation. Six ewes were NR, these consumed 3.5 MJ of metabolizable energy (ME) per day (≅60% of ME requirements for maintenance and growth of the conceptus) until 80 d gestation, with six controls (C) consuming 6.8–7.5 MJ/d. After 80 d gestation until term (147 d), all animals consumed 6.8–7.5 MJ/d, sufficient to fully meet their ME requirements. Lambs were delivered spontaneously and each ewe raised a single lamb that was weaned at 6 weeks of age. At 6 months of age all lambs were euthanased to enable tissue sampling. The renal artery was immediately dissected and used to assess its contractile response to noradrenaline by use of wire myography under normothermic conditions (i.e. 39º). Following normalization, vessel contraction was assessed in response to increased doses of noradrenaline (0.4–2.2 µg $\log^{10}$). The kidneys were also weighed, measured and representative samples taken for subsequent mitochondrial preparation and immunofluorescence microscopy in order to determine the abundance and localization of cytochrome *c*, respectively. Results are expressed as mean values and standard errors. Statistically significant differences between groups were assessed using a Mann-Whitney test.

There was no statistically significant differences in kidney dimensions or weight (e.g. C 49.4 (SEM 2.1); NR 45.3 (SEM 1.9) g) but cytochrome *c* abundance was significantly increased in mitochondria from kidneys of lambs born to NR ewes (C 86 (SEM 5); NR 118 (SEM 14) arbitrary units (P<0.05)). The location of cytochrome *c* in the kidney was established to be in both the renal medullary tubules and in discrete areas of the tubules within the renal cortex. Maternal NR resulted in a lower contractile response to a low dose of noradrenaline (0.4-0.7 µg) within the renal artery (C 40 (SEM 9); NR 21 (SEM 4) % of maximal response (P<0.05)) a difference that was reversed at higher doses of noradrenaline (e.g. 1.7 µg – C 53 (SEM 11); NR 78 (SEM 9) % of maximal response).

In conclusion, the higher abundance of cytochrome *c* in kidney mitochondria from juvenile lambs born to ewes NR between early- to mid-gestation may be indicative of increased mitochondrial activity within either the medullary tubules or renal cortex. This adaptation, in conjunction with an altered contractile response to noradrenaline, may contribute in part to the raised blood pressure sensitivity previously described in these offspring (Gopalakrishnan *et al*. 2001). Our findings further demonstrate that alterations of maternal nutrition at specific stages of gestation may contribute to hypertension in later life through concomitant physiological and vascular changes.

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Gopalakrishnan G, Pearce S, Dandrea J, Mostyn A, Walker RM, Ramsey MM, Stephenson T & Symonds ME (2001) *Proceedings of the Nutrition Society* **60**, 197A.
Heasman L, Clarke L, Firth K, Stephenson T & Symonds ME (1998) *Pediatric Research* **44**, 546–551.
Whorwood CB, Firth KM, Budge H & Symonds ME (2001) *Endocrinology* **142**, 2854–2864.

Supplementing a maternal low protein diet with folic acid in the rat corrects the high blood pressure effect in the offspring. By R.L. DUNN, G. BURDGE and A.A. JACKSON, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

Programmed metabolic effects can be achieved in the offspring following modest changes to the maternal diet during pregnancy. Intrauterine exposure to a maternal low protein (MLP) diet programmes high blood pressure which may be attributed to changes in the setting of the HPA axis, altered arterial or renal structure or altered function of the renin-angiotensin system (Langley-Evans, 2001). The high blood pressure is not seen when the MLP diet is supplemented with 3% glycine, an effect not found with supplemental alanine or urea (AA Jackson, RL Dunn, MC Marchand and SC Langley-Evans, unpublished results). Glycine is conditionally essential for growth and endogenous formation depends upon folate status. We hypothesized that the addition of folic acid would have a similar effect to supplemental glycine, by facilitating an increase in the endogenous formation of glycine. We examined the effect of supplementing the MLP diet with folic acid on the blood pressure of the resulting offspring.

Fifteen virgin female Wistar rats (200–225 g) were mated. From conception to delivery they received one of three experimental diets; 18% casein (Control) *n* 5; 9% casein (MLP) *n* 5; 9% casein+folic acid (MLPF) *n* 5. There were 115 offspring, which were weighed within 12 h of birth and each litter was culled to eight pups. The mother was placed on a standard laboratory chow diet. The pups were weaned at 3 weeks and their weight and blood pressure determined at 4 weeks, using the indirect tail-cuff method (Pfeffer *et al*. 1971). Statistical differences between treatments were by one-way ANOVA using Tukey's *post hoc* correction.

Maternal diet	Body weight at 4 weeks (g)						Systolic blood pressure at 4 weeks (mmHg)					
	Males			Females			Males			Females		
	n	Mean	SE	*n*	Mean	SE	Mean	*n*	SE	Mean	*n*	SE
Control	20	84.49	1.86	16	78.91	1.43	106.3	18	2.5	109.4	17	2.7
MLP	22	74.92***	1.62	18	67.57***	1.17	117.2*	22	3.4	124.1**	18	3.2
MLPF	23	76.93**	1.62	16	71.77**	1.91	101.4†	21	2.9	102.3†	17	3.8

Significant difference from control group: *P<0.05, **P<0.01, ***P<0.001.
Significant difference from MLP group: †P<0.001.

There was no difference in birth weight amongst the three groups. At 4 weeks of age, blood pressure was significantly higher in the MLP pups than both the controls and the MLPF pups, with no difference between the controls and MLPF pups. The MLP and MLPF pups were significantly lighter than the control group, but not different from each other.

Folic acid was added to the diet at five times the amount normally present. The effect of the MLP diet on blood pressure of the offspring was lost when folic acid was added. That supplemental glycine and folic acid both reverse the effect of MLP on blood pressure provides strong evidence that the underlying mechanism either relates directly to the availability of glycine, or to a pathway in intermediary metabolism closely related to glycine formation, such as the availability of 1C units to metabolism.

Langley-Evans SC (2001) *Proceedings of the Nutrition Society* **60**, 505–513.
Pfeffer JM, Pfeffer MA & Frohlich ED (1971) *Journal of Laboratory and Clinical Medicine* **78**, 957–962.

Maternal nutrient restriction between early- to mid-gestation has differential effects on hepatic class 1 cytokine receptor mRNA abundance in mid- and late-gestation ovine fetuses. By M. HYATT, J. BISPHAM, J. DANDREA, D. WALKER, T. STEPHENSON and M.E. SYMONDS, *Academic Division of Child Health, School of Human Development, University Hospital, Nottingham NG9 2UH*

Maternal nutrition during pregnancy determines fetal tissue development in the absence of any effect on fetal or tissue weight (Whorwood *et al*. 2001). The abundance of class 1 cytokine receptors for growth hormone (GH) (Bauer *et al*. 1995) and prolactin (PRL) (Phillips *et al*. 1997) normally peak near to term but the extent to which this development is nutritionally regulated remains to be established. The present study aimed to determine whether maternal nutrient restriction during early–mid-gestation has an effect on hepatic GH and/or PRL receptor mRNA abundance.

Eighteen singleton-bearing ewes of similar body weight and parity were randomly allocated to one of two feeding groups from 28 d gestation as described by Dandrea *et al.* (2002). Nine ewes were provided with 60% of their total energy requirements for body weight and pregnancy in order to produce a 4.5 kg lamb at term, from 28 to 80 d (i.e. nutrient-restricted (NR)) gestation, whilst the remainder continued to receive *ad libitum* feeding and consumed 150% of requirements. After 80 d gestation, four of the nutrient-restricted ewes and five control ewes were provided with 150% of their total energy requirements for body weight and pregnancy until term (i.e. nutrient restricted–well fed (NR-WF), or well fed–well fed (WF-WF) groups, respectively). All ewes were humanely euthanased with an overdose of barbiturate (100 mg kg^{-1} pentobarbital sodium: Euthanal) at either 80 or 140 d gestation (term = 147 d), to enable fetal and liver tissue sampling. Total RNA was extracted from liver tissue, reverse-transcribed and abundance of GH and PRL receptors were measured by polymerase chain reaction (PCR) of reverse-transcribed (RT) product using oligonucleotide primers specific to ovine GH receptor (forward 5′-ATGAACCCATCTGCATGTGA-3′ and reverse 5′-TTCAGTCTTCTCATCAGGGTCA-3′) and ovine PRL receptor (forward 5′-CTGACTTACCGCAAGGGAGG-3′ and reverse 5′-CCACTGCCCAGACCATAATC-3′). Genomic DNA contamination was prevented, as all PCR products spanned at least one intron–exon boundary.

Results are given as means with their standard errors as a ratio of 18S rRNA and are expressed as a percentage of a reference sample present on all gels. Differences in nutritional treatments were analysed using a Mann-Whitney *U*-test.

Fetal group	Lamb birth weight (g)		Liver weight (g)		GH Receptor mRNA (a.u)		PRL Receptor mRNA (a.u)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
80 d gestation								
WF (*n* 4)	214.6	7.0	15.0	1.7	61.8	21.1	8.7	1.9
NR (*n* 5)	235.0	10.4	14.1	0.5	18.3	1.9	21.8	6.0*
140 d gestation								
WF-WF (*n* 5)	4783.1	219.0	103.4	13.4	95.1	12.1	67.0	1.9
NR-WF (*n* 4)	4801.0	256.7	115.8	10.2	51.1	22.2*	19.9	7.6*

Significantly different from WF controls: * $P<0.05$, as measured by Mann-Whitney *U*-test.

Maternal nutrient restriction between 28–80 d gestation initially resulted in upregulation of hepatic PRL receptor mRNA; but following 60 d of nutritional rehabilitation the normal gestational rise in both PRL and GH receptor mRNA did not occur. No nutritional effect on fetal or hepatic weight was observed.

In conclusion, abundance of both PRL and GH receptor are downregulated by early maternal nutrient restriction. This had no effect on liver size but may contribute to altered hepatic function in later life, possibly by affecting gluconeogenesis (Gopalakrishnan *et al*. 2002).

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Bauer MK, Breier BH, Harding JE, Veldhuis JD & Gluckman PD (1995) *Endocrinology* **136**, 1250–1257.
Dandrea J, Cooper S, Ramsey MM, Keller-Woods M, Broughton-Pipkin F, Symonds ME & Stephenson T (2002) *Experimental Physiology* **87**, 353–359.
Gopalakrishnan G, Pearce S, Dandrea J, Mostyn A, Walker RM, Ramsey MM, Stephenson T & Symonds ME (2002) *Early Human Development* (In the Press).
Phillips ID, Anthony RV, Butler TG, Ross JT & McMillen IC (1997) *Endocrinology* **138**, 1351–1354.
Whorwood CB, Firth KM, Budge H & Symonds ME (2001) *Endocrinology* **142**, 2854–2864.

Fetal exposure to a maternal low-protein diet and the immune system. By S.C. LANGLEY-EVANS[1], P.J. BUTTERY[1] and D. WAKELIN[2], [1]*School of Biosciences, University of Nottingham, Loughborough LE12 5RD and* [2]*School of Life and Environmental Sciences, University of Nottingham, Nottingham NG7 2RD*

Epidemiological studies which demonstrate nutritional programming influences in fetal life, upon adult physiology and disease risk (Barker, 1998), are supported by experimental studies of animal models (Langley-Evans *et al.* 1996). There is a growing body of evidence which suggests that the developing fetal immune system is subject to programming in pregnancies associated with undernutrition. Impairment of the development of the thymus (McDade *et al*. 2001) may result in either a predisposition towards atopic disease, or a reduction in immunocompetence and reduction of resistance to infectious disease (Moore, 1998). The impact of undernutrition in early- to mid-pregnancy upon the development of the immune organs, was assessed in an established rat model of nutritional programming.

Fourteen virgin female Sprague Dawley rats were mated and singly housed with free access to food and water. Six animals were fed a control diet containing 180 g casein/kg as the protein source. The remaining animals were fed an isoenergetic low-protein diet containing 90 g casein/kg (Langley-Evans *et al.* 1996). Half of these were fed low=protein diet for the first 7 d of pregnancy only and the rest over the period day 8 to day 14 of pregnancy. Following delivery on day 22, litters were culled to a maximum of eight pups and all rats were then fed a standard laboratory rat diet. At 4 weeks of age the pups were weaned and then killed for the collection of blood and organs.

Maternal diet had no effect upon the weight of the pups at birth or before weaning. Body weight at 4 weeks of age and the size of the liver, kidney, heart and lungs were unaffected by maternal dietary manipulation. The Table shows the weights of the thymus, spleen and mesenteric node relative to body weight. Data from male and female offspring are combined as there was no significant effect of sex on the size of these organs. Analysis of variance indicated a significant influence of maternal diet upon the thymus ($P=0.006$) which was relatively smaller in the low-protein d0–d7 group compared with both the other groups.

	Maternal diet								
	Control			Low-protein d0–d7			Low-protein d8–d14		
	Mean	*n*	SE	Mean	*n*	SE	Mean	*n*	SE
Body weight (g)	94	24	2	92	16	1	92	16	3
Thymus (% body weight)	0.46	24	0.01	0.40*	16	0.01	0.45†	16	0.01
Spleen (% body weight)	0.37	24	0.01	0.35	16	0.01	0.37	16	0.01
Mesenteric node (% body wt)	0.18	24	0.01	0.20	16	0.01	0.17†	16	0.01

*Indicates significantly different from control ($P<0.05$). †Indicates significantly different from low protein d0–d7 ($P<0.05$).

There were no significant differences in the total blood leucocyte count, or numbers of lymphocytes and neutrophils between the groups. The size of the thymus relative to body weight was significantly related to circulating numbers of neutrophils ($r=0.39$, $P=0.003$).

These data are consistent with the hypothesis that prenatal undernutrition has a programming effect upon the immune system of the developing fetus. Differences in thymic weight may reflect qualitative or quantitative changes to T-cell populations, which may influence later immune responses. The findings parallel observations in humans which suggest that the environment encountered in fetal life and early infancy may determine long-term thymic function and later immunocompetence (McDade *et al*. 2001).

Barker DJP (1998) *Clinical Science* **95**, 115–128.
Langley-Evans SC, Welham SJM, Sherman RC & Jackson AA (1996) *Clinical Science* **91**, 607–615.
McDade TW, Beck MA, Kuzawa CW & Adair LS (2001) *Journal of Nutrition*,**131**, 1225–1231.
Moore SE (1998) *Proceedings of the Nutrition Society* **57**, 241–247.

Assessment of biological variability in plasma metabolite and insulin concentrations after an overnight fast in humans. By B.A. FIELDING[1], F. KARPE[1], V. ILIC[1], S.M. COPPACK[2], V. LAWRENCE[2] and K.N. FRAYN[1], [1]*Oxford Lipid Metabolism Group, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE and* [2]*Royal London Hospital, Whitechapel, London E1 1BB*

Nutritional studies typically require the measurement of plasma variables in subjects after an overnight fast. However, within-subject biological variability can be marked, even under carefully controlled conditions. It has recently been shown that the day-to-day coefficient of variation (CV) in plasma non-esterified fatty acid concentrations (NEFA) was 45% when measured in twelve subjects over a 12-d period (Widjaja *et al.* 1999). In this study, we aimed to accurately quantify within-subject biological variability after an overnight fast for plasma glucose, NEFA, glycerol, triacylglycerol (TAG) and insulin in normal subjects with a wide range of BMI.

Eight normal subjects (four male, four female), with mean age of 42 years (range 2–61 years) and mean BMI 29.5 (range 19.7–50.8) participated in the study, having fasted from 7 p.m. the previous evening. Subjects were instructed to consume a low-fat meal the evening before the study, to abstain from consuming alcoholic beverages and caffeine-containing drinks, and to refrain from strenuous exercise. Subjects lay on a bed in a quiet temperature-controlled room (25°) with subdued lighting. A cannula was inserted retrogradely into a distal forearm vein and kept patent by a continuous slow infusion of saline (NaCl 9 g/l). The lower part of the forearm was heated to provide arterialized blood samples. After a resting period of 1 h, blood samples were taken at 2 min intervals for 60 min into heparinized syringes. Plasma glucose was measured the same day on samples stored at 4° using an enzymatic method. Plasma non-esterified fatty acids (NEFA) and TAG were measured enzymatically on samples stored at –20°. Plasma insulin was measured by radioimmunoassay (Pharmacia and Upjohn, Milton Keynes, UK).

Analytical variation (CV_A) in plasma measurements was calculated from duplicates at each time point and sample-to-sample variation (CV_S) was calculated from the mean of samples taken every 2 min. Within-subject biological variability (CV_W) was calculated as the square root of $((CV_S^2) - (CV_A^2))$ for eight subjects (five subjects for plasma glucose) in 28–31 replicate samples.

	CV_S		CV_A		CV_W	
	Mean	SE	Mean	SE	Mean	SE
NEFA	9.08	1.39	2.39	0.43	8.76	1.47
Glucose	2.40	0.55	2.28	0.54	0.74	0.35
TAG	2.99	0.32	2.37	0.33	1.81	0.49
Insulin	19.56	1.96	13.39	1.89	14.26	1.82

In contrast to plasma insulin and NEFA, the biological variability of glucose and TAG was less than 2%. Biological variability of plasma insulin and NEFA includes pulsatility which is well established for insulin and has recently been reported in NEFA (Getty *et al.* 2000). In addition, plasma NEFA concentrations may change with prolonged fasting and stress. High analytical and biological variability of fasting insulin concentrations should be considered when calculating insulin sensitivity from fasting parameters such as HOMA. Particular consideration should be given to monitoring plasma variables in studies where achievement of near steady-state conditions is required.

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Getty L, Panteleon AE, Mittelman SD, Dea MK & Bergman RN (2000) *Journal of Clinical Investigation* **106**, 421–430.
Widjaja A, Morris RJ, Levy JC, Frayn KN, Manley SE & Turner RC (1999) *Clinical Chemistry* **45**, 561–566.

Effect of two methods of hypohydration on rehydration and exercise performance: a comparison with the euhydrated state. By D.T. ARCHER and S.M. SHIRREFFS, *Biomedical Sciences, University Medical School, Foresterhill, Aberdeen AB25 2ZD*

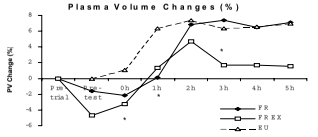
Exercise in the heat, fluid restriction, or a combination of the two, are commonly used by athletes in weight-categorized sports to make the required weight (Oppliger *et al.* 1993). The aim of the present study was to examine the effect of the two methods of dehydration on body fluids, rehydration and exercise performance and to compare this to a state of normal hydration.

With local ethics committee approval, five healthy male subjects (age 24 (SD 4) years, height 176 (SD 7) cm, body mass 77.3 (SD 10.0) kg) attempted to dehydrate by approximately 2% body mass by two methods. On one trial, they refrained from ingesting fluids for a 24 h period (FR). In the other trial they arrived in the laboratory in the evening, performed intermittent cycle exercise to lose 1% body mass in a warm (34°), humid (60–70% rh), environment, had a meal and returned to the laboratory 13 h later during which they refrained from drinking (FREX). In another trial they arrived in the laboratory in their normal euhydrated state (EU). On each trial, subjects gave a blood and urine sample and then performed an exercise capacity test on a cycle ergometer at 95% VO_2max following a 5-min warm-up at 70% VO_2max. Beginning 30 min after this exercise test (0h), they drank a sports drink in a volume corresponding to 150% of their body mass loss (or equivalent on EU). Urine and blood samples were obtained before exercise, 30 min after exercise and every hour until 5 h post-exercise (5h). Blood samples were taken after 15 min of supine rest and plasma volume changes were calculated from haemoglobin and packed cell volume values (Dill & Costill, 1974). Data were analysed by repeated measures ANOVA, paired *t*-tests and one-way ANOVA and *post hoc* Tukey tests.

Subjects experienced a 2.3 (SD 0.3) % and 1.9 (SD 0.3) % body mass loss after the exercise test on FR and FREX, respectively ($P>0.05$). Time to exhaustion was 285 (SD 75) s, 249 (SD 84) s and 223 (SD 75) s on EU, FR and FREX, respectively. When both FR and FREX are combined, exercise capacity was reduced ($P<0.05$). Net fluid balance (NFB) is equal to fluid gains (drinking) minus fluid losses (dehydrating exercise and urination) at each time point. The volume of drink ingested in each trial was the same ($P>0.05$) and was sufficient to return subjects to the same hydration state at the end of each trial ($P>0.05$) (see Table). Drink retention was 3 (SD 8) %, 49 (SD 28) % and 45 (SD 10) %, in EU, FR and FREX, respectively. The change in plasma volume was significantly higher on FR than FREX ($P<0.05$) (see Figure). It did not differ between EU and FR ($P>0.05$) but was markedly lower in FREX than EU ($P<0.05$).

NFB (ml)	EU	SD	FR	SD	FREX	SD
Pre-trial	0	0	0	0	0	0
Pre-test	0	0	–1624	437	–1334	272
0h	–278	104	–1820*	328	–1448*	480
1h	2056	387	868*	235	715*	163
2h	998	414	369*	284	159*	144
3h	247	360	–181	563	–281*	209
5h	–203	189	–558	749	–460	237

*Denotes significantly different from EU.



*FREX significantly different from EU: $P<0.05$.

In summary, dehydration of approximately 2% body mass can cause a reduction in high-intensity exercise capacity and the method of dehydration may have a significant effect on plasma volume recovery during post-exercise rehydration.

Dill DB & Costill DL (1974) *Journal of Applied Physiology* **37**, 247–248.
Oppliger RA, Landry GL, Foster SW & Lambrecht AC (1993) *Paediatrics* **91**, 826–831.

Does the dietary *n*-6 : *n*-3 polyunsaturated fatty acid ratio modulate the hypolipidaemic effects of fish oil supplementation in British Sikhs? By L.M. BRADY, S. LESAUVAGE, S. LOVEGROVE, A.M. MINIHANE, C.M. WILLIAMS and J.A. LOVEGROVE, *Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading, Reading RG6 6AP*

Indian Asians living in the UK have a higher rate of cardiovascular disease (CVD) than the native Caucasian population. This increased risk cannot be attributed to traditional risk factors but may be influenced by dietary intake, particularly an imbalance in the intake of polyunsaturated fatty acids (PUFA). British Sikhs have previously been reported to have a reduced intake of the cardioprotective long chain (LC) *n*-3 PUFA and an increased intake of *n*-6 PUFA compared with Caucasians (Lesauvage *et al*. 2001). The present study tested the hypothesis that a high background dietary *n*-6 : *n*-3 PUFA ratio attenuates the blood lipid response to fish oil supplementation. Twenty-nine British Sikhs (mean age 48.1 (SD 1.6) years) were recruited to participate in a 12-week dietary intervention trial. Volunteers were randomized to receive either a moderate or a high *n*-6 : *n*-3 PUFA diet over a 6-week run-in period (moderate *n*-6 : *n*-3 PUFA = 9; high *n*-6 : *n*-3 PUFA = 16), using modified oils and spreads. Both groups were supplemented with fish oil for a further 6 weeks (2.0 g/d of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) in combination with the dietary treatment. Volunteers participated in a postprandial study at the end of the run-in period with either the moderate or high *n*-6 : *n*-3 PUFA diet and again after the 6-week fish oil supplementation period.

	Moderate *n*-6 : *n*-3 PUFA diet (*n* 15) % change	Within group change *P* value	High *n*-6 : *n*-3 PUFA diet (*n* 14) % change	Within group change *P* value	Between group comparison *P* value
Fasting TAG	–18 (7)	0.01	–22 (5)	0.00	0.61
TAG AUC	–9 (7)	0.08	–21 (7)	0.01	0.26
TAG IAUC	4 (11)	0.52	–14 (12)	0.03	0.16
Fasting NEFA	–17 (9)	0.17	–5 (9)	0.17	0.35
NEFA suppression*	–8 (7)	0.31	–10 (7)	0.08	0.89
LDL3	3 (12)	0.83	–22 (4)	0.00	0.12
HDL	–4 (3)	0.16	1 (4)	0.16	0.89
LDL	6 (3)	0.15	6 (4)	0.96	0.89

TAG, triacylglycerol; AUC, area under the curve; IAUC, incremental area under the curve; NEFA, non-esterified fatty acids; LDL3, small dense low-density lipoprotein; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *Suppression measured at 90 min. Values are presented as means (SEM).

There were no significant differences in the percentage change in blood lipid measurements between the high and moderate *n*-6 : *n*-3 dietary PUFA groups. Both dietary groups showed a significant reduction in fasting plasma triacylglycerol (TAG) after the 6-week fish oil supplementation period. In addition, the high *n*-6 : *n*-3 dietary PUFA group showed a significant reduction in TAG AUC and TAG IAUC. The percentage of LDL3 was significantly reduced in the high *n*-6 : *n*-3 PUFA group. However, due to the significantly higher LDL3 value prior to fish oil supplementation in the high *n*-6 : *n*-3 PUFA group, this response may represent regression towards the mean. In conclusion, the expected reductions in fasting plasma TAG subsequent to fish oil were observed in both dietary groups and there were no significant differences between the groups. The background dietary *n*-6 : *n*-3 PUFA ratio did not modulate the effect of fish oil supplementation on lipid measures associated with insulin resistance in this ethnic group.

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Lesauvage SVM, Lovegrove JA, Minihane AM & Williams CM (2001) *Annals of Nutrition and Metabolism* **45,** 86.

***n*-3 PUFA supplementation improves insulin sensitivity in a group of overweight women with an inflammatory phenotype.** By L.M. BROWNING, J.D. KREBS, M.A. O'CONNELL, G.D. MISHRA, and S.A. JEBB, *MRC Human Nutrition Research, Fulbourn Road, Cambridge CB1 9NL*

Epidemiological evidence shows an inverse relationship between oily fish consumption and risk of diabetes, but short- to medium-term long chain (LC) *n*-3 PUFA intervention studies have shown inconsistent effects on insulin resistance in humans. The mechanism of this effect is unclear but obese subjects have raised concentrations of inflammatory markers and this is a predictor of the risk of type 2 diabetes, even after adjustment for BMI (Schmidt *et al.* 1999).

This study tests the impact of LC *n*-3 PUFA on insulin sensitivity in individuals with an inflammatory phenotype relative to those who exhibit low background inflammation. Overweight, non-diabetic, pre-menopausal women attended for a screening blood test to determine serum sialic acid concentration, a stable marker of inflammatory status (Browning *et al*. 2001). Women with a serum sialic acid concentration <2.00 or >2.20 mmol/l were recruited to a randomized crossover study. Thirty-two subjects aged 26–51 years received capsules containing either LC *n*-3 PUFA (1.3 g EPA and 2.9 g DHA) or a placebo (2.8 g linoleic and 1.4 g oleic acid) per day for 12 weeks each, with a 4-week washout between treatments. At baseline and after 12 weeks of each intervention period, blood samples for insulin and glucose were collected at 0, 30, 60, 90 and 120 min after a 75 g oral glucose load. Area under the curve (AUC) for insulin and glucose were calculated to provide a dynamic measure of insulin sensitivity and a more appropriate index of whole body, insulin-mediated glucose disposal than a static fasting index.

At baseline, using Mann-Whitney tests (median (interquartile range)), the raised inflammatory status group had significantly higher sialic acid, (2.19 (0.25) *v.* 1.82 (0.15) mmol/l; $P<0.001$), BMI (32.0 (6.7) *v.* 28.0 (5.7) kg/m^2; $P<0.05$) and AUC insulin (35 663 (30 784) *v.* 22 796 (10 091) pmol·min/l; $P<0.01$), but no significant difference in fasting insulin, glucose, HOMA or AUC glucose.

	Raised inflammatory status				**Control**			
	LC *n*-3 PUFA		Placebo		LC *n*-3 PUFA		Placebo	
	Mean diff	SD	Mean diff	SD	Mean diff	SD	Mean diff	SD
Sialic acid (mmol/l)	0.02	0.19	–0.002	0.18	–0.06	0.15	0.02	0.15
Weight (kg)	1.21	1.94	0.74	3.30	–0.66	2.35	0.31	2.27
Fasting glucose (mmol/l)	0.0	0.4	0.0	0.3	0.0	0.2	0.0	0.3
Fasting insulin (pmol/l)	–6.9	29.4	4.5	54.2	1.9	17.3	4.0	19.3
HOMA (μU/mmol/l)	–0.3	1.1	0.2	1.8	0.0	0.6	0.1	0.6
AUC glucose (mmol·min/l)	–24.8	120.1	3.2	141.3	0.0	93.3	–10.8	97.5
AUC insulin (pmol·min/l)	–7871**	3421	–3682	3376	–562	2554	188	2393

** $P<0.01$. *P* values calculated using paired *t*-tests of the mean difference (final – baseline).

The Table shows the change in metabolic parameters in each group following treatment with LC *n*-3 PUFA or placebo. There was no significant difference in sialic acid weight, fasting or AUC glucose or insulin during either the LC *n*-3 PUFA or placebo treatment in the group as a whole. For individuals with an inflammatory phenotype, LC *n*-3 PUFA supplementation was associated with a significant improvement in AUC insulin ($P<0.01$), but there was no change with placebo treatment.

This inter-individual responsiveness to dietary intervention may explain some of the inconsistent findings of previous studies of LC *n*-3 PUFA and insulin sensitivity. This study suggests that overweight individuals with raised levels of background inflammation derive significant metabolic benefits from LC *n*-3 PUFA supplementation.

Browning LM, O'Connell MA & Jebb SA (2001) *Annals of Nutrition and Metabolism* **45,** Suppl. 1, 290.
Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP & Heiss G (1999) *Lancet* **353**, 16049–16052.

Differences in intestinal glucose absorption, and insulin and GIP secretion in volunteers following consumption of isogenic onions differing in flavonoid contents. By K.L. JOHNSTON[1], M.N. CLIFFORD[1], E.W.G. SCHYLEN[2], C KIK[2], A.G. BOVY[2] and L.M. MORGAN[1], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH and* [2]*Plant Research International, PO Box 16, 6700 AA Wageningen, The Netherlands*

There is convincing evidence that the absorption and bioavailability of important dietary flavonoids, such as the quercetin glucosides of onion, is partly dependent on small intestinal uptake, possibly via the Na^+-dependent glucose transporter (SGLT1) (Hollman *et al*. 1996, 1997). To investigate possible interactions of these flavonols with this intestinal transporter, we used a randomized, single-blind, within-subject experimental design measuring differences in glucose tolerance and circulating gastrointestinal (GI) hormone profiles as biomarkers of exposure to and effect of such flavonoids.

The study used isogenic onion lines with high or low flavonoid contents that had been obtained after extensive conventional breeding. Yellow onions contained 28 times more flavonoids than white onions (83.4 mg *v.* 3.0 mg/100 g fresh weight, respectively) and complete nutrient analysis revealed only minor differences in total reducing sugars and crude fibre content between the two types. Eight lean healthy volunteers (three females, five males; age 24–34; BMI <25 kg/m^2) were studied on two occasions (one for each onion type). Fasted subjects consumed a meal of either cooked white or yellow onions, consisting of 368 g of onions, corresponding to 25 g of glucose. Maltodextrin (5.5 g) was added to each white onion meal to compensate for the difference in total glucose equivalents between the onion types. Serial venous blood samples were taken over 6 h and plasma was analysed for glucose, insulin, and glucose-dependent insulinotropic polypeptide (GIP).

Two-way repeated measures ANOVA showed a trend towards lower plasma glucose concentrations (P<0.10) after consumption of the yellow onions compared with the white onions, suggesting a flavonoid-mediated inhibition of glucose uptake in the small intestine. Experiments using an *in vitro* simulated digestion technique were performed to determine whether between-onion differences in the rates of starch hydrolysis might contribute to the differences observed. There was no difference in the rate of release of glucose between the two types of onions. This inhibition of glucose uptake is also supported by differences observed in the GI hormone profiles. There was a significant treatment × time interaction in plasma insulin profiles (P<0.005) with reduced concentrations after consumption of the yellow onions compared with the white onions. Analysis of plasma GIP results showed that there was a significant treatment effect (P<0.01), again with reduced concentrations after consumption of yellow onions compared with white onions.

These data suggest that quercetin glucosides at concentrations present in onions interact with the intestinal transporter SGLT1, with consequential effects on glucose uptake, as reflected in plasma glucose, insulin and GIP profiles in human volunteers.

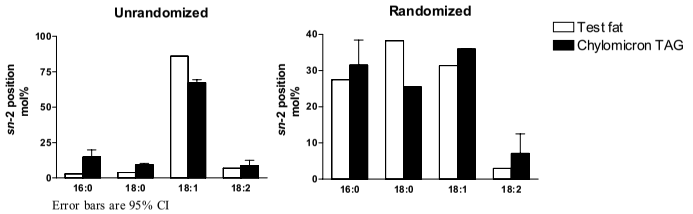
Hollman PC, van der Gaag MS, Mengelers MJ, van Trijp JM, de Vries JH & Katan MB (1996) *Free Radical in Biology and Medicine* **21**, 703–707.
Hollman PC, van Trijp JM, Buysman MN, van der Gaag MS, Mengelers MJ, de Vries JH & Katan MB (1997) *FEBS Letters* **418**, 152–156.

Influence of the structure of stearic acid rich triacylglycerols on postprandial chylomicron triacylglycerol composition and structure. By T.A.B. SANDERS and S.E.E. BERRY, *Nutrition, Food and Health Research Centre, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN*

Randomization is a technique being widely adopted by the food industry as an alternative to the partial hydrogenation of fats, because it increases the melting point of fats without leading to the generation of *trans*-fatty acids. Cocoa-butter, which is rich in stearate, consists mainly of symmetrical triacylglycerol (TAG) with almost all of the stearic acid being present as either 1,3-di-stearyl-oleyl glycerol (SOS) or 1-stearyl,2-oleyl,3-palmitoleyl glycerol (SOP). Consequently randomization of cocoa-butter leads to the generation of TAG with saturated fatty acids in the *sn*-2 position. The digestibility of saturated fatty acids (SFA) in position *sn*-2 of TAG is believed to be greater than when in *sn*-1 and *sn*-3. However, we reported that randomized cocoa-butter, which contained a high proportion of SFA in the *sn*-2 position, led to 41% lower postprandial lipaemia than a meal containing a similar amount (50 g) of unrandomized cocoa-butter (Sanders *et al.* 2001). We have subsequently undertaken further analysis of the chylomicrons isolated in that study; the results are shown below.

	Chylomicron TAG (mol %)		Test fat
	Unrandomized cocoa-butter	Randomized cocoa-butter	(mol %)
16:0	33.2 (1.3)	35.7 (4.1)*	29.0
18:0	28.8 (4.2)	18.9 (4.3)**	35.8
18:1	32.7 (2.6)	36.5 (4.3)**	32.8
18:2	5.2 (2.1)	8.9 (2.3)**	2.4

Mean values (SD), n=17, *P<0.05 and **P<0.01 compared with unrandomized.



Error bars are 95% CI

The proportion of stearic acid in the chylomicron TAG at 3 h was 34% lower after the randomized cocoa-butter in comparison with the unrandomized cocoa-butter. The proportion of fatty acids in the *sn*-2 position of the chylomicron TAG reflected that of the dietary fat. However, following randomization, the proportion of stearic acid in the *sn*-2 position of the chylomicron TAG tended to be lower than that in the test fat. Summers *et al.* (1999), using 60 g SOO or OSO TAG, found no difference in the extent of postprandial lipaemia, but in common with the present study found that TAG structure was preserved in chylomicron TAG. However, their results suggested that the absorption of OSO was delayed compared with SOO. Our observations suggest that TAG containing stearate in the *sn*-2 position are less well absorbed than when in the *sn*-1 or *sn*-3 position.

SandersTA, Berry SE & Miller GJ (2001) *Proceedings of the Nutrition Society* **60**, 216A.
Summers LK, Fielding BA, Herd SL, *et al.* (1999) *Journal of Lipid Research* **40**, 1890–1898.

Lack of association between measures of adiposity and fasting triacylglycerol levels in British Sikhs. By J.A. LOVEGROVE[1], S.V.M. LESAUVAGE[1], S.S. LOVEGROVE[1], B.A. GRIFFIN[2], A.M. MINIHANE[1] and C.M. WILLIAMS[1], [1]*School of Food Biosciences, University of Reading, Whiteknights, Reading RG6 6A and* [2]*School of Biological Science, University of Surrey, Guildford GU2 5XH*

British Indians have a higher rate of coronary heart disease than other ethnic groups living in the UK. This has been attributed to a high prevalence of insulin resistance, central adiposity and associated lipid abnormalities indicative of an atherogenic lipoprotein phenotype (ALP). This study investigated whether the positive association between central fat and plasma TAG found in other populations is observed in Sikh men. Caucasian (n=55) and Sikh (n=55) men were matched for age (mean (SD): 49 (11) *v.* 48 (10) years, respectively) and BMI (26.1 (2.8) *v.* 26.3 (3.2) kg/m^2, respectively). Anthropometric measurements and fasted blood samples were collected for the analysis of plasma insulin (I) and glucose (G) and blood lipids. The homeostasis model assessment (HOMA) (G (mmol/l) × I (μU/ml) / 22.5) was calculated as a surrogate measure of insulin resistance. The Sikh men had significantly higher HOMA (P=0.05), plasma TAG (P=0.04), LDL3 (P=0.0001), Ssk (sum of four skinfold thicknesses, a measure of subcutaneous fat mass) (P=0.009) and significantly lower plasma HDL cholesterol (P=0.007) concentrations compared with the Caucasian men (see Table).

	Caucasian (n=55)		Sikh (n=55)	
	Mean	SD	Mean	SD
HOMA	1.85	1.46	2.04*	1.13
TAG (mmol/l)	1.3	0.5	1.7*	0.8
NEFA (μmol/l)	340	164	415	156
Total cholesterol (mmol/l)	5.4	0.8	5.2	0.9
LDL cholesterol (mmol/l)	3.4	0.8	3.2	0.7
LDL3 (%)	16	19	37**	24
HDL cholesterol (mmol/l)	1.3	0.4	1.2§	0.3
Waist (cm)	95	9	94	9
BMI (kg/m^2)	26.0	2.8	26.3	3.2
Ssk (cm)	56.4	18.4	67.0§	22.7

NEFA (non-esterified fatty acids), Ssk (sum of four skinfold thicknesses).
*P<0.05, ** P<0.0001 Student's t-test, §P<0.01 Mann-Whitney.

Statistical associations between measures of adiposity, insulin resistance and TAG concentrations were determined in the two ethnic groups. After controlling for age, the expected positive associations were observed between measures of adiposity and insulin resistance (HOMA) for both groups: waist (Caucasian r=0.64, P=0.0001; Sikh r=0.48, P=0.0001) and BMI (Caucasian r=0.57, P=0.0001; Sikh r=0.48, P=0.0001). The Caucasian men also showed the expected significant positive associations between measures of adiposity and plasma TAG concentrations (waist r=0.38, P=0.005; BMI r=0.38, P=0.005). However, the Sikh men showed an unexpected lack of association between measures of adiposity and TAG (waist r=0.04, NS; BMI r=0.19, NS). Recent work by our group has shown a similar lack of association between measures of central adiposity and TAG in Sikh men (Lesauvage *et al*. 2001) and in Caucasian men with an ALP (Minihane *et al*. 2000). The present study supports the view that factors other than total body fat and its distribution may impact on circulating TAG in Sikh men.

This study was funded by the FSA.

Lesauvage SVM, Lovegrove SS, Lovegrove JA & Williams CM (2001) *Annals of Nutrition and Metabolism* **45**, 86.
Minihane AM, Khan S, Talmud PJ, Williams DL, Wright JW, Murphy MC, Griffin BA & Williams CM (2000) *International Journal of Obesity* **24**, 1097–1106.

Estimation of the mean intakes of fourteen classes of dietary phenolics in a population of young British women aged 20–30 years. By S.L. GOSNAY, J.A. BISHOP, S.A. NEW, J. CATTERICK and M.N. CLIFFORD, *Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH*

Epidemiological studies have suggested health benefits from the consumption of fruit and vegetables (WCRF, 1997). Some investigators have attributed these benefits to phenolic compounds, related to their antioxidant properties. Thus, quantification of intakes of dietary phenols is of critical importance. The aim of this study was therefore to characterize the habitual intakes of phenols in a population group of young British women.

Data on the content of phenols in foods were gathered from the literature, particularly the NEODIET reviews. Subcategories included Hydroxybenzoic acid derivatives, Cinnamates (including chlorogenic acids), Flavonols, Flavanols, Flavones, Flavanones, Dihydrochalcones, Anthocyanins, Isoflavones, Proanthocyanidins, Ellagitannins, Stilbenes, Derived Polyphenols (characteristic of traditional commodities such as black tea and matured red wine, but absent from fresh green tea leaf and grape) and Lignans.

The dietary diaries from a total of 103 British women aged 20–30 years were used to establish habitual intake of phenols. The subjects were randomly selected from general practice registers in Surrey as part of a nutrition and bone health study (Porteous *et al*. 2002).

The mean population phenols intake (n=103) was 0.75 g/d (range 0.05–4.11 g/d). The mean intakes of vegetarian subjects (n=4) produced a subpopulation mean of 1.26 g/d; comparison of this with the non-vegetarian (n=99) and population (n=103) means showed a difference of borderline significance (P=0.07). Subjects were characterized by their intake of phenols as either having an intake below the mean (lower intakes) or equal to and above the mean (higher intakes). The mean lower intake was 0.38 g/d, substantially different from the mean higher intake of 1.17 g/d.

	Mean of lower intakes (g/d)	Mean of higher intakes (g/d)	Population mean (g/d)
Phenols intakes	0.38	1.17	0.75
Standard deviation	0.20	0.66	0.40

Comparison of the characteristics of subjects with intakes of phenols above and below the population mean showed that those with higher intakes consumed more coffee and black tea, brassica vegetables and yellow onions. Some foods containing phenols were high in fat and sugar; the mean intakes of these foods (g/d) were also greater in subjects with higher phenols intakes. The cinnamates and derived polyphenols (components of coffee and black tea, respectively) made the greatest contribution to the mean total intake of phenols (357 mg/d and 170 mg/d, respectively).

This project has given the first estimates of the mean intakes of phenols by 20–30-year-old women in Britain. It has also identified coffee and black tea as the major dietary contributors to intakes of phenols. The greater consumption of coffee and high-fat and high-sugar foods in the subjects with intakes of phenols above the population mean is notable, since these components of the diet have been implicated in cancer and cardiovascular disease risk (WRCF, 1997; DoH, 1991). It is also important to note that the major sources of phenols, black tea and coffee, do not supply significant vitamin C, which may be required to clear phenoxyl radicals. Conversely, the consumption of fruit and vegetables incorporates antioxidant vitamins and other beneficial compounds (e.g. glucosinolates) into the diet.

Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the UK*. London: HMSO.
Porteous L, Catterick J, Bishop JA, Crossley LE, Pattison DJ, Woolf AD, Taylor P, Cooper C & New SA (2002) *Proceedings of the Nutrition Society* **60**, 225A.
World Cancer Research Fund (1997) *Food, Nutrition and the Prevention of Cancer: A Global Perspective*. Washington DC: World Cancer Research Fund/American Institute for Cancer Research.

Randomized controlled trial of stanol ester-fortified milk on plasma lipids. By T.A.B SANDERS, K. GLEASON, N. JONES and H. THEOBALD, *Nutrition Food and Health Research Centre, King's College London, Franklin-Wilkins Building, Stamford Street, London SE1 9NN*

Plant sterols and stanols are naturally occurring plant-derived compounds, which act physiologically as lipid lowering agents. Their hypothesized mechanism of action is through the inhibition of dietary and biliary cholesterol absorption, either by displacing cholesterol in mixed micelles or competitively binding to cholesterol transport receptors in gut enterocytes. The safety and efficacy of plant sterol- and stanol-enriched margarines have been documented (Law, 2000). This study aimed to demonstrate the beneficial effects on plasma total and LDL cholesterol of plant stanol esters incorporated into semi-skimmed milk. A randomized double-blind placebo controlled crossover trial was conducted in nineteen free-living healthy middle-aged subjects. Subjects were required to consume 500 ml of semi-skimmed milk daily for two consecutive 3-week periods. On days 19 and 21 of both treatment periods, fasting blood samples were collected for determination of serum lipids and plasma lathosterol, a biomarker of cholesterol synthesis. The results are shown in the Table.

Serum lipids (mmol/l)	Control		Stanol		Statistical significance
	Mean	SD	Mean	SD	
Total cholesterol	5.54	0.72	5.30	0.59	P=0.0016
LDL cholesterol	3.43	0.71	3.23	0.65	P=0.0266
HDL cholesterol	1.38	0.45	1.38	0.45	P=0.9022
Triacylglycerol	1.65	1.00	1.51	0.76	P=0.7687

Both milks were well tolerated. Consumption of the milk enriched with 2 g plant stanol as esters significantly reduced plasma total and LDL cholesterol by 4.3% (0.24 mmol/l) and 5.8% (0.20 mmol/l), respectively, compared with the control milk. Plasma lathosterol concentration, an *in vivo* indicator of cholesterol biosynthesis, was significantly greater (P=0.043) following the stanol-containing milk (mean 35.7 µmol/l compared with 28.1 µmol/l). There were no significant changes in cholesterol adjusted fat-soluble vitamin concentrations. The addition of plant stanol esters to semi-skimmed milk results in a significant reduction in serum cholesterol concentration.

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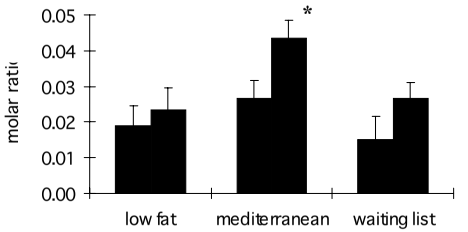
Law M (2000) *BMJ* 2**320**, 861–864.

Changes in phospholipid profiles in response to dietary advice to consume a low-fat or Mediterranean-type diet. By E.K. LUND[1], M.A. TAYLOR[2], L. RAPOPORT[3], I.T. JOHNSON[1] and J. WARDLE[3], [1]*Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA,* [2]*School of Biological Sciences, University of Nottingham, Nottingham NG7 2UH and* [3]*Department of Epidemiology and Public Health, University College London, London WC1E 6BT*

The standard treatment for patients with moderately elevated cholesterol levels is to give dietary advice designed to reduce total fat intake. It is, however, recognized that high cholesterol levels tend to be associated with a northern European diet. An alternative method to reduce plasma cholesterol may therefore be to suggest a Mediterranean-style diet. Such dietary advice might not only impact on cholesterol levels and cardiovascular disease. The importance of the fatty acid content of the diet is becoming increasingly well recognized with respect to other diseases such as diabetes, cancer, arthritis, autism and affective disorders. It is therefore important to establish how such dietary advice affects the types of circulating fatty acids.

We analysed the phospholipid fraction of erythrocytes taken from a subgroup of patients used in a larger study (Wardle, 2000) for changes in fatty acid profile in response to advice designed to reduce total fat intake (n=20) or to encourage a more Mediterranean-style diet (n=19). Patients allocated to the Mediterranean diet group were strongly encouraged to eat oil-rich fish (high in C20:5 and C22:6 n-3 PUFAs) and were given oil and fat spreads high in C18:1. In contrast those on the 'low-fat' diet were encouraged to eat white fish and were given oil and spreads high in C18:2. A control group of patients was taken from those waiting to receive dietary advice (n=27). Blood samples were taken at 0 and 12 weeks, and erythrocytes separated from the plasma by centrifugation, prior to isolation of phospholipids, methylation and analysis of fatty acid methyl esters by gas chromatography. Cholesterol measurements were made on the plasma fraction.



The n-3 fatty acid content of erythrocyte phospholipids before (diagonal lines) and after (grey shading) 12 weeks dietary intervention (*P=0.005)

Total and LDL cholesterol levels were significantly reduced only in those patients recommended a Mediterranean-style diet. This was associated with a significant increase in n-3 fatty acids, particularly C20:5 eicosapentaenoic acid, in the erythrocyte phospholipids of these patients (see Figure). The Mediterranean diet caused no increase in C18:1 but did lead to a decrease in C18:2. This result confirms previous findings that erythrocyte C18:1 is not a good marker of oleic acid intake (Vicario *et al*. 1998). However, dietary advice designed to encourage the consumption of oil-rich fish effectively raised n-3 fatty acid intake and led to an approximately 40% increase in the molar ratio of n-3 fatty acids in the erythrocyte membrane phospholipid pool.

Funded by the Biotechnology and Biological Sciences Research Council UK. Technical assistance provided by Paul Banoob.

Vicario IM, Malkova D, Lund E K & Johnson IT (1998) *Annals of Nutrition and Metabolism* **42**, 160–169.
Wardle J, Rogers P, Judd P, Taylor MA, Rapoport L, Green M & Nicholson Perry K (2000) *American Journal of Medicine* **108**, 547–553.

Effect of barley β-glucan on plasma cholesterol may depend on apolipoprotein E4 genotype – results from a pilot study. By G. LIETZ, J.C. MATHERS and C.J. SEAL, *Human Nutrition Research Centre, Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne NE1 7RU*

Animal and human studies have shown beneficial effects of ingested oat and barley products on blood lipid levels, and a meta-analysis showed that daily consumption of 3g β-glucan significantly reduced blood cholesterol concentrations (Ripsin *et al*. 1992). However, conflicting results have been found in free living subjects after incorporating β-glucan into the diet with both reduction (Bartram *et al*. 1992) and no effects (Lovegrove *et al*. 2000) on blood cholesterol concentration. The apolipoprotein E4 polymporphism is associated with increased cholesterol concentrations (Mahley & Rall, 2000) and effects of β-glucan on plasma lipid levels could be expected to differ between ApoE genotype status. This experiment was designed to investigate the effect of two levels of intake of barley β-glucan on blood cholesterol concentrations and included a comparison of response according to ApoE genotype. 17 healthy free-living students (13 female, 4 male; age range 19-27 years; average BMI 23.2 kg/m^2) took part in this pilot study. Subjects were randomly allocated to consume either a 50g high fibre (6g β-glucan) or low fibre (3g β-glucan) breakfast cereal/day for 3 weeks. The breakfast cereal was prepared from barley products and presented in the form of a porridge in pre-weighed packages for re-constitution with milk or water depending on individual preference. Fasting blood samples were collected pre- and post-intervention.

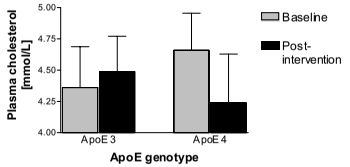
No significant differences in fasting plasma total cholesterol concentrations were observed between the low and high fibre groups (Table 1), although plasma triacylglycerol concentrations were significantly lower in the low fibre group at post-intervention. However, plasma total cholesterol concentrations fell by 9% in subjects with the ApoE4 variant averaged across both treatments (Figure 1), whereas no change was observed for subjects with the ApoE3 genotype.

Table 1: Effect of barley β-glucan on plasma total cholesterol and triacylglycerides

mmol/L	*High fibre diet (n=9)*		*Low fibre diet (n=7)*	
	Mean	SD	Mean	SD
Baseline				
Total cholesterol	4.59	0.91	4.50	1.41
Triacylglycerols	1.23	0.52	1.22	0.45
Post-intervention				
Total cholesterol	4.63	0.95	4.18	0.73
Triacylglycerols	1.25*	0.31	1.06*	0.37

* significantly different at p<0.05 (Mann-Whitney U test)

Figure 1: Effect of barley β-glucan intake on plasma cholesterol depending on genotype



Whilst some studies have found substantial reductions in blood cholesterol concentrations after consumption of β-glucan, the present study did not show a significant effect. This may be due to the low dose of β-glucan used in this study compared with other intervention trials (Truswell, 2002). Another reason for the lack of response in our study may be the small number of subjects, who also had low initial plasma cholesterol concentrations, since greater responses are reported in hypercholesterolemic subjects (Ripsin *et al.* 1992). The ApoE4 allele has been associated with a greater response of plasma LDL-cholesterol to dietary modification (Lopez-Mirinda *et al.* 1994). The results of this pilot study indicate that the cholesterol-lowering effects of β-glucan may be influenced by an individual's ApoE genotype. Further studies with larger numbers of subjects are needed to investigate this interaction.

Cereal products and funding for this project were provided by HO Short & Sons, Berwick

Ripsin CM, Keenan JM, Jacobs DR *et al*. (1992) *JAMA* **267**, 3317–3325.
Bartram P, Gerlach S, Scheppach W, Keller F & Kasper H (1992) *Journal of Parenteral and Enteral Nutrition* **16**, 533–537.
Mahley RW & Rall SC (2000) *Annual Reviews in Genomics and Human Genetic* **1**, 507–537.
Lovegrove JA, Clohessy A, Milon H & Williams CM (2000) *American Journal of Clinical Nutrition* **72**, 49–55.
Truswell AS (2002) *European Journal of Clinical Nutrition* **56**, 1–14.
Lopez-Miranda J, Ordovas JM, Mata P, Lichtenstein AH, Clevidence B, Judd JT & Schaefer EJ (1994) *Journal of Lipid Research* **35**, 1965–1975.

Influence of dietary α-linolenic acid and fish-oil on markers of cardiovascular risk in subjects with an atherogenic lipoprotein phenotype. By B.A. GRIFFIN[1], P.A. WILKINSON[1], C. LEACH[1], N. HOSEIN[1], G. MILLER[2] and D.J. MILLWARD[1], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH and* [2]*MRC Epidemiology and Medical Care Unit, Wolfson Institute of Preventive Medicine, London EC1M 6BQ*

Dietary long chain *n*-3 PUFAs have proven efficacy in reducing the risk associated with an atherogenic lipoprotein phenotype (ALP) and in reducing CHD mortality, but the acquisition of these health benefits is seriously limited by a low habitual intake of oily fish in the UK diet. Since dietary α-linolenic (α-LA) acid can be converted *in vivo* to its longer and more unsaturated counterparts, a study was designed to examine whether a diet enriched with α-LA can promote fish-oil-like reductions in risk factors for cardiovascular disease. Normal, healthy non-smoking males (*n*=57), aged 35–60 years, with moderately raised plasma TAG (>1.5 mmol/l), a low HDL cholesterol (<1 mmol/) and a predominance of small, dense LDL (ALP) were recruited for a 12-week dietary intervention and randomly assigned to one of three single-step diets. These included a high-α-LA diet (*n*=21) based on flaxseed-oil (*n*-6 : *n*-3 ratio <1), a 'control' diet (*n*=17) (high linoleic acid (LA), *n*-6 : *n*-3 ratio = 30), and a high LC *n*-3 PUFA diet (*n*=19) (control diet + 6 g fish-oil capsules (containing 3 g EPA+DHA/d, *n*-6 : *n*-3 ratio = 5). Flaxseed oil (kindly donated by Savant Distribution, Leeds), and sunflower oils were provided in laminated foil sachets (17 g oil). Subjects were instructed to incorporate two sachets per day into their diet (flaxseed-oil ~15 g α-LA). Subjects were also provided with either rapeseed or sunflower cooking oils, and rapeseed-oil-based margarines (St. Ivel's standard 'Mono' and a specially formulated rapeseed-enriched 'Mono'). Target intakes of dietary α-LA and LC *n*-3 PUFA were achieved, as indicated by the analysis of 7 d food diaries and changes in red blood cell membrane fatty acid composition: (0→12 weeks: fish-oil diet EPA, 2.5→5.6% (P<0.001); DHA 4.7→7.5% (P<0.001); flaxseed-oil diet: α-LA 0.4→1.3% (P<0.001), EPA 1.5→3.8% (P<0.005), DHA 4.0→4.3%, NS). Total plasma cholesterol decreased significantly within all three diets (flaxseed-oil diet – 0.74 mmol/l (P<0.001), fish-oil –0.49 mmol/l (P<0.02), control –0.43 mmol/l (P<0.05). Plasma TAG decreased significantly after the fish-oil diet (–0.59 mmol/l, P<0.001). There was no overall effect on plasma lipids or HDL or LDL cholesterol between the three diets, as indicated by ANOVA. Fish-oil decreased the ratio of total to HDL cholesterol (–10%, P<0.005) and redistributed LDL subclasses towards larger, less dense particles (small, dense LDL-3, P<0.001). The percentage of DHA in RBC membrane phospholipids correlated with the changes in plasma TAG (r –0.69, P=0.001) and LDL subclasses on fish-oil at 12 weeks. Moreover, the percentage of DHA in RBC membranes was positively correlated with LDL-cholesterol and the ratio of total cholesterol to HDL cholesterol (r 0.63, P<0.001) at baseline. There were no dietary effects on haemostatic variables including plasma Factor VIIc, PAI-1 activity and fibrinogen or endothelial function as measured by flow-mediated dilatation. In conclusion, while the fish-oil diet produced predictable responses in blood lipids and lipoproteins, the high α-LA diet (flaxseed-oil) did not increase DHA or reproduce the fish-oil-like effects, even with a low intake of *n*-6 PUFA. The decrease in total and LDL cholesterol on the control and fish-oil diets are consistent with the effects of a high LA diet, and suggest that a high intake of α-LA in flaxseed-oil exerts a similar effect on plasma cholesterol to this *n*-6 fatty acid. The relatively poor level of enrichment of DHA in RBC membranes after the flaxseed-oil diet, together with the evidence of inter-relationships between DHA and plasma lipids and lipoproteins suggests that this fatty acid is a more important determinant of lipid-mediated risk than EPA.

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Relative rates of long chain conversion of ^{13}C α-linolenic acid in adult men fed high α-linolenic acid or high linoleic acid diets. By N.M. HUSSEIN[1], P.A. WILKINSON[1], C. LEACH[1], G.C. BURDGE[2], S.A. WOOTTON[2], B.A. GRIFFIN[1]and J. D. MILLWARD[1]. *[1]Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH and [2]Institute of Human Nutrition, Southampton General Hospital, Tremona Road, Southampton SO16 6Y1*

The *n*-3 polyunsaturated fatty acids, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, are important regulators of cell function and derive from either preformed sources, of which fish oil is the richest, or from long chain conversion of their precursor α-linolenic acid (α-LA) in plant oils. The current studies were designed to explore the conversion of α-LA to EPA and especially DHA in the context of a low *n*6 : *n*3 diet providing generous amounts of α-LA from flaxseed oil, the richest source.

A group of normal, healthy non-smoking males (a subset of the study described by Griffin *et al.* 2002), were fed either a high-α-LA diet (*n*-6 : *n*:3 ratio <1, *n*=6), or a high *n*-6 diet (*n*-6 : *n*-3 ratio = 30, *n*=5), for 12 weeks. At breakfast, after an overnight fast, they were given a single oral dose of 400 mg of uniformly [^{13}C]-labelled α-LA in a milkshake (Burdge *et al.* 2001). ALNA conversion was assessed from ^{13}C enrichments in EPA, DPA and DHA in phosphatidylcholine fractions in plasma (PPC) and erythrocytes (EPC) at 1, 2, 3, 7 and 14 d measured by GC-combustion-IRMS (Burdge *et al.* 2001). ^{13}C labelling was calculated as ^{13}C atoms% excess (APE) or total ^{13}C (APE × % total membrane fatty acids), reported here as the area under the enrichment–14 d time course curve (AUC).

There was marked between-subject variation in enrichment. Although conversion rates cannot be precisely calculated from the current blood pool sampling, substantial conversion to EPA and DPA occurred, but with a much slower conversion to DHA. In this case very low, but significant ^{13}C labelling was observed by 14 d in all subjects on both diets, at least in the PC fractions. The 14-d ^{13}C distribution between α-LA, EPA, DPA and DHA was 22%, 53%, 21% and 4%, respectively (mean values for PPC and EPC on both diets) with no differences between the two dietary groups. The higher proportion of label in EPA than in α-LA (theoretically impossible for a unidirectional, true precursor–product relationship) may partly reflect an underestimation of the α-LA labelling if peak labelling of α-LA occurred before the 24-h blood sample, or may indicate differential exchange rates between plasma α-LA and EPA and intra-hepatic pools. To examine the effect of the background diets on ^{13}C α-LA conversion rates, the ^{13}C AUC values for EPA, DPA and DHA for each subject were standardized to the same assumed precursor enrichment. This was necessary since the ^{13}C APE AUC of α-LA was higher after the high *n*-6 compared with the α-LA diet, due in part to the higher α-LA pool size.

Dietary group	Lipid fractions	α-LA			EPA			DPA			DHA		
		AUC ^{13}C APE			AUC (standardized) total ^{13}C*								
		Mean	SD	*p*	Mean	SD	*p*	Mean	SD	*p*	Mean	SD	*p*
High *n*-6 diet	RBC PC	285	25		141	167		27	12		7	5	
	Plasma PC	270	77		133	28		28	9		6	1	
Flax oil	RBC PC	88	43	<0.0	277	211	ns	55	29	ns	4	5	ns
	Plasma PC	17	2	<0.0	454	79	<0.05	54	15	<0.05	12	5	<0.05

*^{13}C total AUC (standardized) = ^{13}C total AUC (measured)/^{13}C precursor APE AUC (measured) × ^{13}C precursor APE AUC (mean, *n*=13), with α-LA, EPA and DPA assumed as precursors for EPA, DPA and DHA, respectively.

After standardization, mean values for ^{13}C EPA were higher after the flax diet, significantly so for the plasma PC fraction. In conclusion, in adults α-LA is extensively converted to EPA, especially on a high ALNA low *n*-6 PUFA diet, consistent with the elevated membrane EPA concentrations after flax-oil diets, but is only very slowly converted to DHA, at least as indicated by sampling of blood-lipid pools. It may be that this low rate of DHA synthesis is adequate to provide for metabolic demands in these subjects.

We gratefully acknowledge support from the Food Standard Agency, Grant No. N02008 and Savant Ltd, Pronovo Biocare and St Ivel for the supply of oils and fats.

Burdge GC, Jones AE, Wright P, Ware L & Wootton SA (2001) *Proceedings of the Nutrition Society* **60**, 22A.
Griffin BA, Wilkinson PA, Leach C, Hussein N, Miller G & Millward DJ (2002) *Proceedings of the Nutrition Society* (In the Press).

The effect of short-term weight loss on the systemic acute markers of inflammation in the overweight Turkish male. By H.T. BESLER, *Department of Nutrition and Dietetics, School of Health Technology, Hacettepe University, 06100 Ankara, Turkey*

Obesity is associated with an increased risk of atherosclerotic cardiovascular disease, which represents a major cause of mortality (Lean, 2000). A number of factors contribute to accelerated atherogenesis in obese or overweight individuals, including hypertension, diabetes and dyslipidaemia (Noakes & Clifton, 2000). C-reactive protein and fibrinogen levels, the extremely sensitive systemic markers of inflammation, are strongly associated with adiposity, body mass index (BMI) and some other features of obesity. Therefore the purpose of the present study was to examine the short-term effects of an energy-reduced diet on the systemic acute markers of inflammation as measured by serum C-reactive protein, fibrinogen, serum amyloid A (SAA) and albumin, in a small sample of overweight Turkish men.

The study included 102 healthy adult males aged 21–52 years with a BMI ≥25 kg/m^2 who were not taking any medication and who were willing to lose weight. The amount of energy to be provided during the study was adjusted depending upon the weight loss, being limited to not to more than 1.0 kg/week. To achieve this, the diet was adjusted to maintain a 2.51 MJ/d deficit over a 2-month period. Energy intake was calculated for each individual using a multiple of Schofield basal metabolic rate and estimation of physical activity using a questionnaire. Fasting venous blood samples were obtained after a 12-h fast (no food after 9.00 pm). Serum or plasma levels of blood lipids, glucose, insulin, C-reactive protein, fibrinogen, SAA and albumin were determined. All the measurements were repeated at the end of week 8.

After 8 weeks of energy-reduced dieting, 9% of the participants had experienced no weight change; the remaining 91% had weight loss goals of up to 9.6 (±0.38) kg (10.9%) throughout the study period. The body fat percentage was decreased by 19% (P<0.001) whereas the mean lean body mass was increased by approximately 8% (P<.001) at week 8 compared with the baseline values. After 8 weeks of energy-reduced dieting, total cholesterol (–14%), LDL-C (–15%) and insulin (–12%) concentrations were significantly reduced (P<0.05). The insulin to glucose molar ratio was also decreased by 14% (P<0.05).

	Week 0		Week 8		*P**
	Mean	SD	Mean	SD	
C-reactive protein (mg/l)	2.05	1.29	1.65	1.06	0.000
SAA (mg/l)	20.4	26.0	17.6	23.3	0.005
Fibrinogen (mg/l)	2.97	0.97	2.79	1.00	0.077
Albumin (mg/l)	42.0	2.83	41.9	2.18	0.514

*P** Paired-sample *t*-test; differences between week 0 and week 8.

The present study demonstrated that multiple cardiovascular risk factors associated with obesity, including the systemic acute markers of inflammation, can be reduced by a short-term, but simple individualized energy-reduced diet alone.

Lean MEJ (2000) *Proceedings of the Nutrition Society* **59**, 331–336.
Noakes M & Clifton PM (2000) *American Journal of Clinical Nutrition* **71**, 706–712.

Gastrointestinal handling of vitamin A in cystic fibrosis patients. By P.R. AFOLABI[1], A.L. CAWOOD[1], J.M. JACKSON[1], J.L. MURPHY[1], M. CARROLL[2], G. CONNETT[3] and S.A. WOOTTON[1], [1]*Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD,* [2]*Department of Respiratory Medicine, Southampton University Hospitals NHS Trust, SO16 6YD and* [3]*Department of Paediatrics, Southampton University Hospitals NHS Trust, SO16 6YD*

Supplements of retinyl palmitate (RP) are routinely prescribed to patients with cystic fibrosis (CF) in response to low circulating plasma retinol, in the belief that the availability of dietary RP is compromised in association with the maldigestion and malabsorption of dietary lipid. The extent to which the gastrointestinal handling of exogenous RP compromises vitamin A status has not been systematically investigated. The aim of the present study was to determine the availability of exogenous retinyl palmitate by characterizing faecal losses of vitamin A and lipid and postprandial changes in plasma RP and lipid following a test meal.

Six healthy male adults, median age 22.5 years (range 22–23), and six patients with CF, median age 21 years (range 17–39 years; 5M, 1F) participated in the study. None of the subjects were taking vitamin supplements for the period of the study. The study had the approval of the local ethical committee. All subjects received an oral dose of [10,19,19,19] ^{2}H-retinyl palmitate (300 µg/kg body weight) within an emulsion along with a test breakfast. Baseline and postprandial bloods were drawn at 2, 5 and 10 h. Subjects only drank water between blood samples, and after 5 h subjects received a test lunch low in vitamin A. All faeces passed over 3 d were collected and frozen immediately. Vitamin A, as retinol and retinyl palmitate, was determined by HPLC. [$^{2}H_4$]Retinol was determined by gas chromatography mass spectrometry following fractionation by HPLC and derivitization. Total faecal lipid was measured by the method of Folch *et al.* (1957). Plasma TAG concentrations were analysed by an enzymatic method.

	Total faecal Vitamin A loss (µmol/3 d)		Total faecal labelled Vitamin A loss as % administered dose (%dose/3 d)		Total faecal lipid loss (g/d)	
	Median	Range	Median	Range	Median	Range
Cystic fibrosis (CF)	1.87*	0–9.82	6.51*	0–29.5	11.90	2.5–27.2
Healthy adults (HA)	0.02	0–0.19	0	0–5.18	6.8	5.0–7.4

*Significantly different from healthy adults: $P<0.05$ (Mann-Whitney *U*).

There was a marked variability in faecal losses of both vitamin A and lipid. Whilst the CF patients excreted relatively more total vitamin A, [$^{2}H_4$] retinol and [$^{2}H_4$] retinyl palmitate in their faeces than that observed in the healthy adults, these patients were still able to absorb most of the administered dose (>70%). There was no simple association between faecal vitamin A losses and faecal lipid. Postprandial excursions in plasma RP were not different between groups (AUC over 10 h: CF 1.54 (0.42–2.98) *v.* HA 2.26 (1.28–4.62) µmol/l per 10 h; $P>0.05$). Similarly, there were no differences between groups in postprandial excursions of plasma TAG (AUC over 5 h: CF 0.09 (0.00-–1.35) *v.* HA 0.44 (0.00–1.22) mmol/l per 5 h; $P>0.05$). There was no simple association between the magnitude of the postprandial plasma RP and either postprandial plasma TAG response, faecal RP losses or faecal lipid.

These results do not support the view that retinyl palmitate availability is limited by impaired gastrointestinal function in CF subjects and it is unlikely to be the primary cause of the low plasma retinol concentrations often seen in this group of patients. Moreover, the additional increase in vitamin A intake necessary to cover these modest faecal losses is considerably less than that routinely prescribed in clinical practice and raises concerns over the possibility of excessively high intakes. Further attention needs to be directed towards examining the subsequent metabolism of retinol and, in particular, the extent to which the liver is able to mobilize retinol to the peripheral tissues.

Folch J, Lees M & Sloan Stanley GM (1957) *Journal of Biological Chemistry* **226**, 497–509.

Diet in the first and third trimesters of pregnancy in relation to babies' weight, ponderal index and head circumference at birth. By S.C. LANGLEY-EVANS[1] and A.J. LANGLEY-EVANS[2], [1]*School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD and* [2]*Human Nutrition and Metabolism Group, Division of Health and Life Sciences, University College Northampton, Boughton Green Road, Northampton NN2 7AL*

Epidemiological associations between low weight or disproportion at birth and later disease have been attributed to maternal nutritional influences (Barker, 1998) Whilst these associations seem robust and are reproducible in many populations, the evidence implicating maternal undernutrition in the fetal programming of disease is relatively weak. In animals, programming influences of maternal diet on the physiology of the developing fetus are readily demonstrable (Langley-Evans, 2001). In well-nourished British populations, weight at birth has been reported to be related to maternal macronutrient intakes (Godfrey *et al.* 1996) but these findings were not replicated in a later study by Mathews *et al.* (1999).

A group of 301 pregnant women in the first trimester of pregnancy were recruited to the Northampton Diet and Pregnancy Study. The mean age of the women at recruitment was 27.9 years (range 16–42 years) and the mean gestation was 10.5 weeks (range 7–15 weeks). All women completed a simple questionnaire concerning social class indicators and the use of nutritional supplements. The women were provided with a 5 d estimated food record for completion at home, at first trimester recruitment and in the third trimester (approximately 32 weeks gestation). A total of 223 diaries recording intakes in the first trimester and 172 recording intake in the third trimester were returned and analysed using Comp-Eat version 5. At delivery the weights of all the babies for whom dietary information was available were recorded and measurements were made of head circumference and crown-heel length. Placental weight was recorded for 113 of the pregnancies.

The data were analysed using linear regression, considering the contribution of nutrient intakes in the first or third trimesters adjusted for gestational age, social class, maternal smoking habit, maternal height and maternal weight at recruitment. Placental weight was not related to intakes of any nutrients at either time point. In the first trimester, high intakes of selenium were associated with lower birth weight, whilst in the third trimester high iodine intake was related to lower birth weight. The fetal : placental ratio was increased by higher intakes of PUFA ($P=0.05$) in the first trimester and lower intakes of alcohol in the third ($P=0.037$). Whilst placental and birth weights were largely unrelated to maternal nutrient intakes, body proportions were found to be associated with the intakes of several nutrients. Ponderal index was related to third trimester intakes of copper ($P=0.016$). Head circumference at birth was related to first trimester intakes of vitamin E ($P=0.036$), iron ($P=0.016$) and folate ($P=0.031$) and to third trimester intakes of vitamin E ($P=0.046$) and alcohol ($P=0.042$).

Tertiles of intake in first trimester related to head circumference at birth								
Iron (mg/d)	HC (cm)	SD	Vitamin E (mg/d)	HC (cm)	SD	Folate (µg/d)	HC (cm)	SD
<9.92	34.50	1.14	<4.79	34.46	1.21	<548	34.27	1.44
9.92–13.69	34.65	1.41	4.79–7.84	34.50	1.39	548–674	34.57	1.41
>13.69	34.80	1.39	>7.84	35.04	1.28	>674	34.87	1.43

The data are consistent with the findings of Mathews *et al.* (1999) in that birth weight was not strongly related to maternal nutritional status. However, the observations that micronutrient intakes may be associated with body proportions at birth are consistent with the assertions of Barker (1998) and in general support the hypothesis that nutritional programming effects upon fetal development may manifest as readily measurable markers at birth.

Barker DJP (1998) *Mothers, Babies and Health in Later Life.* London: Churchill Livingstone.
Godfrey KM, Robinson S, Barker DJP, Osmond C & Cox V (1996) *British Medical Journal* **312**, 410–414.
Langley-Evans SC (2001) *Proceedings of the Nutrition Society* **60**, 505–513.
Mathews F, Yudkin P & Neil A (1999) *British Medical Journal* **319**, 339–343.

Diet and pregnancy outcomes in vegetarian and non-vegetarian women. By S.C. LANGLEY-EVANS[1] and A.J. LANGLEY-EVANS[2], [1]*School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD and* [2]*Human Nutrition and Metabolism Group, Division of Health and Life Sciences, University College Northampton, Boughton Green Road, Northampton NN2 7AL*

The number of vegetarians in the United Kingdom is rising steadily. Vegetarianism is more common among women than in men, and those aged 16–24 years are more likely to avoid meat than other groups in the population. Given that there is a preponderance of women of child-bearing age in the vegetarian population, it is of interest to determine whether a vegetarian lifestyle significantly impacts upon nutrient intakes in pregnancy and upon pregnancy outcomes. There is some evidence that a vegetarian diet improves dietary intakes of folate and other micronutrients (Koebnick *et al*. 2001), but this is matched by concerns that the associated high phytoestrogen intake may be associated with fetal abnormalities (North & Golding, 2000).

A group of 301 pregnant women in the first trimester of pregnancy, attending an antenatal ultrasound clinic, were recruited to the Northampton Diet and Pregnancy Study. The mean age of the women at recruitment was 27.9 years (range 16–42 years) and the mean gestation was 10.5 weeks (range 7–15 weeks). All women completed a simple questionnaire concerning social class indicators and the use of nutritional supplements. The women were provided with a 5 d estimated food record for completion at home, on two separate occasions. A total of 223 diaries recording intakes in the first trimester and 172 recording intake in the third trimester were returned and analysed using Comp-Eat version 5. A total of nineteen women (6%) were following a diet which contained no meat. For the purposes of this study the lactovegetarian (n=3), lacto-ovo-vegetarian (n=15) and vegan (n=1) women were grouped together and compared to meat-eating women. The nutrient intakes of vegetarian and non-vegetarian women differed markedly in the first trimester (see Table), with vegetarians consuming more carbohydrate, calcium, magnesium, iron, copper, B vitamins, vitamin C, vitamin E and vitamin D. Differences in third trimester nutrient intakes were less marked. The main difference between the two groups of women lay in supplement consumption. In the first trimester, 47% of the vegetarian women consumed a multivitamin and/or mineral supplement compared with 18% of non-vegetarians (P=0.003). In the third trimester consumption of nutritional supplements was similar in the two groups.

	First trimester					Third trimester				
	Vegetarians		Non-vegetarians			Vegetarians		Non-vegetarians		
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Energy intake (MJ/d)	9.00	1.96	8.59	1.82		8.46	1.61	8.49	1.52	
Energy from protein (%)	11.8	1.8	13.9	2.0	*	12.9	1.9	14.4	3.9	
Energy from fat (%)	35.8	5.3	37.2	5.4		36.9	5.2	36.6	5.0	
Energy from carbohydrate (%)	53.6	8.6	48.8	5.4	*	49.4	4.7	48.9	5.6	
Energy from alcohol (%)	0.4	0.8	0.3	0.6		0.7	1.0	0.3	0.6	*

*Indicates significant difference between vegetarians and non-vegetarians (P<0.05).

The vegetarian women (47%) reported less nausea and vomiting in the first trimester of pregnancy than non-vegetarians (69%). They were more likely to develop gestational diabetes (P=0.002) and tended to have a greater frequency of caesarian delivery (21%) than non-vegetarians (15%). Birthweights and body proportions were similar in the babies delivered to the two groups of women, but among the vegetarians the fetal : placental ratio was reduced (P=0.02).

The data indicate that there is no evidence of low nutrient intakes associated with a vegetarian diet in pregnancy. This is mainly due to the high use of micronutrient supplements by vegetarian women. There was some evidence to suggest that a vegetarian diet impacts upon pregnancy outcomes.

Koebnick C, Heins UA, Hoffman I, Dagnelie PC & Leitzmann C (2001) *Journal of Nutrition* **131**, 733–739.
North K & Golding J. (2000) *British Journal of Urology International* **85**, 107–113.

Nutrient intake in early pregnancy. By B. AL-RASASI[1], J. COAD[3] and J. MORGAN[2], [1]*European Institute of Health and Medical Sciences, University of Surrey, Guildford GU2 7TE,* [2]*School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7GX and* [3]*Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11222, Palmerston North, New Zealand*

Nausea and vomiting in pregnancy (NVP) is a common symptom of early pregnancy affecting 50–90% of all pregnant women (Broussard & Richter, 1998). NVP may have an effect on the amount and type of diet consumed by women during early pregnancy. The aim of this study was to assess the effect of NVP on nutritional intake in early pregnancy. Fifty-two women (both with and without NVP) were recruited in Guildford, Surrey, to take part in a prospective study. Women were interviewed to gain background information and were asked to keep a 1 week food diary during early pregnancy. Symptoms of NVP were recorded in a symptom diary in order to assess severity of NVP. Dietary data were analysed using WinDiets, and statistical analysis was carried out using SPSS (version 10.0). In the diaries, 78% of the women reported suffering from NVP. The nutrient intake of women with and without NVP are shown in the table below.

	No NVP (n 10)		NVP (n 33)		Significance
	Mean	SD	Mean	SD	
Energy (kJ)	7936	1479.60	7094	1682.23	0.163
Fat (g)	77.78	19.4	65.94	21.65	0.129
Protein (g)	68.03	11.32	61.55	13.17	0.168
Carbohydrate (g)	241.44	49.10	224.53	55.26	0.930
Vitamin B_6 (mg)	2.04	0.46	1.74	0.46	0.075
Riboflavin (mg)	1.53	0.44	1.25	0.37	0.050*
Calcium (mg)	858.10	112.72	678.76	222.40	0.002*
Magnesium (mg)	262.50	41.83	211.06	54.19	0.009*
Potassium (mg)	2794.40	337.78	2310.11	581.72	0.017*
Zinc (mg)	7.37	1.13	6.27	1.52	0.042*
Copper (mg)	1.10	0.22	0.90	0.21	0.014*

ANOVA was used to find whether there was a difference in intake between the three groups. Although it was observed that energy and macronutrient intakes fell as the degree of nausea experienced rose, these observations failed to reach significance. As for micronutrient intake, it was found that women with NVP consumed significantly less riboflavin (P= 0.050), calcium (P=0.002), magnesium (P= 0.009) and potassium (P=0.017) compared to women without NVP. Zinc and copper intake was also lower in women with NVP compared to those without NVP (P=0.042) and (P=0.014) respectively. It was found that women with aversions also had reduced intakes of riboflavin (P=0.011), calcium (P=0.063), zinc (P=0.052) and copper (P=0.067). These women commonly avoided meat, vegetables and chocolates, which are good sources of calcium, magnesium, potassium, copper and zinc. It has been shown that although NVP may not have an effect on the amount of intake, NVP does seem to have an effect on the quality of the diet consumed during early pregnancy. In a previous study carried out by the same authors it was found that NVP was highly related to aversions in pregnancy. The effect on the diet may be due to aversions felt by women with NVP. Larger studies are needed to assess the true effect of NVP on nutritional intake in early pregnancy.

Al-Rasasi B, Siegler R, Nichols J, Coad J & Morgan J (2001) Dietary cravings and aversions in pregnancy. *Proceedings of the Nutrition Society* **60,** 136A
Broussard CN & Richter JE (1998) *Gastroenterology Clinics of North America* **27**, 123–151.

Evidence of increased age-related renal injury in rats exposed to maternal low protein diets *in utero*. By M.C. MARCHAND[1], R. DUNN[2], A. AIHIE SAYER[3,4], S.C. LANGLEY-EVANS[5] and C. COOPER[4], [1]*Division of Health and Life Sciences, University College Northampton, Boughton Green Road, Northampton NN2 7AL, Departments of* [2]*Human Nutrition,* [3]*Geriatric Medicine,* [4]*MRC Environmental Epidemiology Unit, University of Southampton, Southampton General Hospital, Tremona Road, Southampton SO16 6YD and* [5]*School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD*

The beneficial effects of post-weaning diet restriction on ageing include prolongation of lifespan, reduced age-related disease and attenuation of changes in physiological function (Merry, 1991). Prenatal dietary restrictions appear to have the opposite effect upon the ageing process and exposure to nutritional manipulations in fetal life are known to permanently alter organ structures and activities of hormone axes and promote chronic conditions such as hypertension and diabetes (Langley-Evans, 2001). However, the effects of nutritional manipulations during fetal life upon the ageing process are, as yet, not well characterized. Intrauterine exposure to a maternal low-protein diet has been shown to significantly increase systolic blood pressure and shorten the lifespan of the rat (Aihie Sayer *et al*. 2001). Studies of young rats exposed to maternal low-protein diets *in utero* have shown that nephrogenesis is impaired in fetal life, leading to postnatal functional deficits. The aim of this study is to assess the longer-term effects of fetal undernutrition upon renal structure.

Ten virgin female Wistar rats (200–225 g) were mated and on the day of conception were fed either a 180 g casein/kg (control n=5) or a 90 g casein/kg (low-protein n=5) diet. Feeding of these diets was maintained throughout pregnancy. At littering, the rat dams were transferred to a non-purified laboratory rat diet (189 g protein/kg) and this same diet was used to wean the offspring at 4 weeks of age. The rats received minimal handling thereafter and were allowed to die without intervention unless they exhibited evidence of pain, distress or discomfort. Lifespan ranged from 43 to 130 weeks and was significantly shorter in female, but not male, rats exposed to prenatal undernutrition (Aihie Sayer *et al.* 2001). At death, kidneys were rapidly removed and fixed in formalin before being embedded in paraffin wax, cut into 10-µm sections and stained with haematoxylin and eosin. A semi-quantitative scoring method was employed to determine glomerular condition (100 random glomeruli per slide) under a light microscope. Glomeruli were characterized as histologically normal, or showing evidence of mild glomerulosclerosis, severe glomerulosclerosis, or hypertrophy.

	Maternal diet											
	Control						Low-protein					
	Male			Female			Male			Female		
	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE
Normal (%)	17†	7	6	59	4	13	8†	7	2	66	4	4
Mild injury (%)	39†	7	4	23	4	8	53†*	7	2	21	4	6
Severe injury (%)	34†	7	4	18	4	5	34†	7	3	13	4	2
Hypertrophy(%)	10†	7	2	1	4	1	6†	7	2	0	4	0

Data show mean percentage of glomeruli within kidney sections exhibiting abnormal or normal structures.
*P<0.05 compared to control diet. †P<0.001 compared to female of same dietary group.

Within this colony, the kidneys of males from both the control and low-protein groups had a significantly greater proportion of nephrons exhibiting mild or severe glomerular injury and hypertrophy and significantly fewer structurally normal glomeruli, when compared with the females of the same group. A greater proportion of glomeruli exhibited mild glomerulosclerosis in the kidneys of the male low-protein group compared with the equivalent male control. The findings of this study suggest that whilst in male rats, nutritional programming of renal morphology in fetal life appears to promote age-related glomerulosclerosis, female animals are not subject to the same programming influence.

Aihie Sayer A, Dunn RL, Langley-Evans SC & Cooper C (2001) *Gerontology* **47**, 9–14
Langley-Evans SC (2001) *Proceedings of the Nutrition Society* **60**, 505–513.
Merry BJ (1991) Reviews in Clinical Gerontology **1**, 203–213.

Fetal ontogeny of mitochondrial abundance for cytochrome *c* and voltage-dependent anion channel (VDAC) in the kidney, liver and lung in the sheep. By D.P. YAKUBU, I.W. SEETHO, A. MOSTYN, J. DANDREA, V. WILSON, M.E. SYMONDS and T. STEPHENSON, *Academic Division of Child Health, School of Human Development, University of Nottingham NG7 2UH*

Mitochondria determine cellular energy metabolism. This process is regulated in part by specific mitochondrial proteins including VDAC and cytochrome *c*. VDAC is a general diffusion porin present on the outer membrane of the mitochondria (Kirk & Strange, 1998) and is known to be involved in energy metabolism and apoptosis. Cytochrome *c* is located in the mitochondrial inter-membrane space and is important in cellular energy conversion (Lehninger *et al.* 1993). The extent to which these mitochondrial proteins may be related and how their ontogeny may differ between tissues during fetal life remains to be elucidated, and the present study aimed at addressing this.

Kidney, liver and lungs were sampled from 5–7 singleton fetuses of sheep fed *ad libitum* at either mid- (80 d) or late- (140 d) gestation (term = 148 d). Mitochondrial fractions were prepared and abundance of each protein determined by immunoblotting. In the case of VDAC a polyclonal antibody raised to ovine VDAC was utilized. Results (in arbitrary units) are means with their standard errors (SEM). Statistically significant differences between gestational age groups were assessed using a Mann-Whitney test.

	Kidney		Liver		Lung	
	Mean	SEM	Mean	SEM	Mean	SEM
VDAC:						
80 d	110	25	56	4	40	4
140 d	115	15	96	2**	79	18*
Cytochrome *c*:						
80 d	64	8	28	4	6	0.4
140 d	98	27	31	5	17	2**

Significant differences between age groups: *P<0.05, **P<0.01 as measured by Mann-Whitney test.

In both the lung and liver a marked increase in VDAC abundance occurred with gestational age, which was not apparent in the kidney, where as a significant increase in cytochrome *c* abundance with gestation was only observed in the lung. The concentration of VDAC was also greater in the kidney than lung and liver, irrespective of gestational age.

The precocious peak in VDAC abundance in the kidney compared with liver and lung may be indicative of accelerated maturation and the fetal requirement to closely regulate fluid balance. A higher VDAC concentration in the fetal kidney may also relate to a greater metabolic demand in the kidney than in the liver and lung. As a consequence, the fetal kidney may be at greater risk of adverse development compared with other tissues following environmental challenges throughout gestation.

Kirk K & Strange K (1998) *Annual Reviews in Physiology* **60**, 719–739.
Lehninger AL, Nelson DL & Cox MM (1993) *Principles of Biochemistry*, 2nd edn. New York: Worth Publishers, pp. 480–483.

Comparison of adipose tissue deposition between singleton, twin and triplet lambs at one month of age. By S. PEARCE, J. DANDREA, M.E. SYMONDS and T. STEPHENSON, *Academic Division of Child Health, School of Human Development, University of Nottingham, Nottingham NG7 2UH*

Fetal, adipose tissue deposition is influenced by fetal number and maternal nutrition (Mostyn *et al*. 2002). Twins have reduced adipose tissue at birth, but the abundance of the short form of the prolactin receptor is greatly enhanced compared to singletons (Budge *et al*. 2000). Lack of prolactin receptor is associated with reduced adipose tissue in growing mice (Freemark *et al*. 2001). It is not known, however, whether an increase in prolactin receptor abundance after birth may contribute to enhanced adipose tissue deposition. The following study therefore compared adipose tissue and organ weights between single and multiple births, when each was ewe-reared without competition for milk from its sibling.

Twenty-two multiparous Bluefaced Leicester × Swaledale ewes of similar body weight and parity were entered into the study of which twelve were singleton-bearing (S), six twin-bearing (Tw), and four were triplet bearing (Tr). All ewes were offered straw *ad libitum* and a fixed amount of concentrate sufficient to fully meet their total energy requirements with respect to fetal number and stage of gestation. For twins and triplets, after lambing, only one randomly selected lamb was reared with its mother as a singleton, six of the singletons were euthanased on day 1 and the other six remained with their mother until day 30. At 30 d of postnatal life all lambs were humanely euthanased and tissues were dissected and weighed. Results were analysed by Mann-Whitney *U*-test and are presented as means and standard errors (SEM).

Mean birth weight was significantly ($P<0.05$) greater in the singletons compared to the twins and triplets, although at 30 d of age triplets were significantly ($P<0.05$) larger than singletons or twins. At 1 month of age twins possessed significantly ($P<0.05$) less total adipose tissue, while triplets possessed more total adipose tissue when compared to the singleton lambs.

	Weight at birth (kg)		Weight at 1 month of age (kg)		Total adipose tissue weight (g)	
	Mean	SEM	Mean	SEM	Mean	SEM
Singleton lambs	6.4	0.18[a]	19.1	0.55	311.0	17.3
Twin lamb raised as a singleton	5.4	0.28	17.3	0.8	274.2	32.9[a]
Triplet lamb raised as a singleton	5.4	0.3	21.4	0.6[a]	362.8	17.7

[a]Significant difference between groups at $P<0.05$ level, as measured by Mann-Whitney *U*-test.

In conclusion, being from a larger litter had differential effects on subsequent adipose deposition when nutrition is no longer limiting.

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Budge HJ, Dandrea J, Ingleton P, Stephenson T & Symonds ME (2000) *Early Human Development* **58**, 69–76.
Freemark M, Fleenor D, Driscoll P, Binart N & Kelly PA (2001) *Endocrinology* **142**, 532–537.
Mostyn A, Dandrea J, Evens Y, Alves-Guerra MC, Pecqueur C, Miroux B, Stephenson T & Symonds ME (2002) *Journal of Physiology* (*Proceedings*), 539P.

Red cell folate status of nulliparous women in the first trimester of pregnancy. By Z.M. BROOKE, G.A. REES and W. DOYLE, *Institute of Brain Chemistry and Human Nutrition, University of North London, 166–220 Holloway Road, London N7 8DB*

Optimal folate status, both preconceptionally and during early pregnancy, is recognized as being important in the prevention of neural tube defects (MRC Vitamin Study Research Group, 1991; Czeizel *et al.* 1994). Poor dietary folate and low circulating levels of folate have been associated with increased risk of neural tube defects and low birth weight (Mahomed, 2002; Scholl & Johnson, 2000; Daly *et al*. 1995).

This study was undertaken to examine the relationships between maternal nutritional status in the first trimester of pregnancy and pregnancy outcome in women who were expecting their first baby. A group of 100 nulliparous women were recruited from the Homerton Hospital in East London. The woman's current occupation was used as a proxy measure of socio-economic status. The mean gestation at booking was 11 weeks and 3 d (confirmed by ultrasound scan). Red cell folate (RCF) analysis was performed with participants' consent at the Homerton Hospital using blood collected for routine antenatal tests. Folate levels were measured by ion capture technology using an IMx analyser, a fully automated immunoassay analyser which was routinely subjected to quality assurance checks.

Thirty-four participants had folate deficiency in the first trimester of pregnancy; twenty-two women had marginal deficiency (RCF <345 nmol/l) and twelve women had severe deficiency (RCF <230 nmol/l). Unwaged women under 35 years old of non-Caucasian origin were found to be most at risk of folate deficiency, which reflects the low level of folic acid supplementation amongst these women (Brooke & Doyle, 2001). Caucasians had significantly higher mean RCF levels ($P<0.001$) than the non-Caucasian women. Women employed in non-manual occupations had significantly higher mean RCF levels than both the unwaged and manual women ($P<0.001$). Women taking folic acid supplements had significantly higher RCF levels than those who did not take supplements ($P<0.001$). RCF levels correlated with duration of supplementation ($P<0.001$, $r=0.520$). Of the women who reported taking folic acid supplements, the women who started taking supplements prior to conception had the highest mean RCF levels ($P<0.001$).

The magnitude of folate deficiency identified amongst this group of apparently healthy women in the first trimester of pregnancy highlights the need for further study. Future studies should determine the effects of low folate status on maternal and infant health, coupled with effective interventions to improve women's folate status, particularly among the disadvantaged.

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Brooke ZM & Doyle W (2001) *Proceedings of the Nutrition Society* **60**, 224A.
Czeizel AE, Dudás I & Métneki J (1994) *Archives of Gynaecology & Obstetrics* **255**, 131–139.
Daly LE, Peadar NK, Molloy A, Weir DG & Scott JM (1995) *Journal of the American Medical Association* **274**, 1698–1702.
MRC Vitamin Study Research Group (1991) *Lancet* **338**, 131–137.
Mahomed K (2002) *The Cochrane Library*, Issue 1. Oxford: Update Software.
Scholl TO & Johnson WG (2000) *American Journal of Clinical Nutrition* **71**(supplement), 1295s–1303s.

Thiamin status of nulliparous women in the first trimester of pregnancy and the relationship to birth outcomes. By G.A. REES, Z.M. BROOKE and W. DOYLE, *Institute of Brain Chemistry and Human Nutrition, University of North London, 166–220 Holloway Road, London N7 8DB*

Previous work in Hackney, East London, identified strong associations between maternal nutrient intake and birth weight (Doyle *et al*. 1990). Nutrients strongly associated with birth weight included thiamin and other B vitamins. Studies of women post-partum who had delivered a low birth weight baby showed low intakes of many nutrients including thiamin (Doyle *et al*. 1999, 2001).

This study was undertaken to examine the relationship between maternal nutrient status in the first trimester of pregnancy and pregnancy outcome in nulliparous women. A group of 100 participants were recruited during booking at the Homerton Hospital antenatal clinic. Participants provided an extra 5 ml of blood taken during routine phlebotomy. The woman's occupation was used as a proxy for socio-economic status (SES). Mean gestational age was 11 weeks and 3 days (confirmed by ultrasound). Birth outcomes were collected from the medical notes.

Thiamin status was measured in ninety women. Whole blood was spun and packed erythrocytes were frozen at –80º until transported to the MRC HNR, Cambridge, for analysis. Thiamin analysis was performed using the erythrocyte transketolase (ETK) method, based on the initial procedure by Vuilleumier *et al*. (1990) (normal range ETK activation coefficient 1.00–1.14).

Approximately one-third of participants had low thiamin status. Of these 26.5% had a marginal thiamin status and 7.5% were thiamin deficient.

Group		Mean ETKAC (SD)	% low thiamin status	N marginal status (ETKAC 1.15–1.25)	N thiamin deficient (ETKAC >1.25)
Whole group	*n*=90	1.12 (0.080)	34	24	7
Ethnic origin	African *n*=20	1.17 (0.092)	65	10	3
	Asian *n*=8	1.13 (0.056)	25	1	1
	Caucasian *n*=51	1.10 (0.075)	27	11	3
	West Indian *n*=11	1.12 (0.062)	18	2	0
SES	Unwaged *n*=24	1.14 (0.094)	46	9	2
	Manual *n*=26	1.13 (0.072)	38	8	2
	Non-manual *n*=40	1.11 (0.077)	25	7	3

African women had significantly higher mean ETKAC levels than the Asian (P=0.032) and Caucasian (P=0.001) women. There were no statistically significant differences in thiamin status between SES groups. ETKAC levels were negatively correlated with gestation (P<0.001, r –0.407). The mean gestation of babies whose mothers' thiamin status was adequate was significantly longer than mean gestation of babies whose mothers had marginal status (P=0.002) or thiamin deficiency (P=0.008). The mean birth weight of babies whose mothers had adequate thiamin status was 151 g and 281 g more than those babies whose mothers had marginal or deficient thiamin status. This trend was not significant.

These results highlight the widespread poor thiamin status in nulliparous women in the first trimester of pregnancy in an inner city community. Further work is required to determine the exact effect of this on infant health and whether interventions are effective.

The authors gratefully acknowledge financial support from the Kellogg's Company of Great Britain.

Doyle W, Crawford MA, Wynn AHA & Wynn SW (1990) *Journal of Nutritional Medicine* **1**, 1–9.
Doyle W, Crawford MA, Srivastava A & Costeloe (1999) *Journal of Human Nutrition and Dietetics* **12**, 517–527.
Doyle W, Srivastava A, Crawford M, Bhatti R, Brooke Z & Costeloe K (2001) *British Journal of Nutrition* **86**, 81–87.
Vuilleumier JP, Keller HE & Keck E (1990) *International Journal of Vitamin Nutrition Research* **60**, 126–135.

Effects of irregular meal pattern on energy expenditure. By H. FARSHCHI, M.A. TAYLOR and I.A. MACDONALD, *School of Biomedical Sciences, University of Nottingham, Nottingham NG7 2UH*

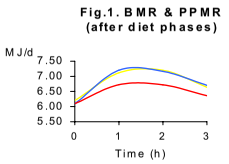
Irregular meal patterns and irregular snacking seem to have become more prevalent in industrial countries during the last decade (Samuelson, 2000). Obesity has increased concurrently. No studies have evaluated the association between irregular meal pattern and obesity. The purpose of this study is to investigate the impact of irregular meal frequency on energy expenditure.

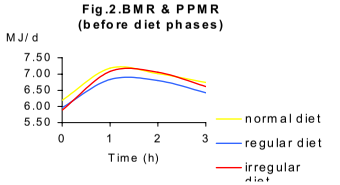
A randomized crossover controlled trial with four phases over 71 d was undertaken for each subject. Subjects attended six measurement visits during the experiment, at the start and end of each phase. In Phase 1 (28 d), subjects were asked to eat their normal diet. In Phase 2 (14 d), they were asked to eat and drink similar things to normal but to either consume them on six occasions per day (regular meal frequency) or follow a variable meal frequency according to a randomized protocol (between three and nine meals per day). In Phase 3 (14 d) subjects continued their normal diet as a wash-out period. In Phase 4 (14 d), subjects followed the opposite meal frequency to that which they had followed in Phase 2.

Nine healthy, non-obese women aged 18–42 years (mean 23.7 (SD 7.4) years) with regular menstruation and no history of dieting or depression (according to standard questionnaires) were recruited from the students of Queen's Medical Centre. Mean body mass index (BMI) was 22.4 (SD 2.4) kg/m².

Basal metabolic rate was measured by an open circuit indirect calorimeter after an overnight fast. Postprandial metabolic rate was measured for 3 h after consumption of a high-carbohydrate milkshake test meal (% total energy, 50% CHO, 15% protein and 35% fat). Repeated-measures ANOVA was used for the statistical comparisons.

Pre-meal basal metabolic rate showed no significant differences over the experiment. Metabolic rate after the test meal increased significantly above basal values on all three occasions. The increases were different between the different meal patterns at 1 (P=0.023), 2 (P=0.012) and 3 (P=0.022) h after the test meal. The overall thermic effect of food over the 3 h period was significantly different between the three meal patterns (P=0.004). The irregular meal pattern had the smallest response (Figure 1 and 2).





In conclusion, irregular meal frequency may have a significant impact on energy expenditure. Irregular meal frequency, compared with regular, would result in more positive energy balance, if intake remained constant.

Samuelson G (2000) *European Journal of Clinical Nutrition* **54,** Suppl. 1, 21–28.

Birth and infant characteristics as indicators of atopy in childhood. By L.J. CARRINGTON and S.C. LANGLEY-EVANS, *School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD*

Diseases such as asthma and eczema are increasing in children. Whilst the underlying cause of the present increase remains unidentified, it has been proposed that atopy can be programmed by less than optimal nutrition in fetal life, resulting in an immature immune system (Langley-Evans, 1997). Fetal programming of the immune system is thought to be a result of brain sparing, whereby undernutrition during critical periods results in adaptations to ensure that the nutrient supply to the brain is maintained at the expense of other organs such as the thymus (Barker, 1998). Large head size and disproportion at birth are thought to be indicators of such programming.

A group of 133 children were recruited around their seventh birthday, to study anthropometric characteristics at birth and through childhood, using historical records from the patient-held record book, and current measurements of weight, height and head size. After exclusion of twenty-one children born prior to 37 weeks gestation, forty-nine males and sixty-three females were included in the study. Informed consent was gained and at interview a family history of allergy was obtained. Simple standardized core questions measured prevalence of asthma (wheeze), allergic rhinitis and atopic eczema using the ISAAC study criteria (Asher, 1998). The questionnaire also contained information about environmental and social factors that could influence development of these diseases. The best of three readings, using the mini-Wright peak flow meter, determined variation in airflow obstruction, as an indicator of asthma. Fifty-two children (46%) answered positively to one or more questions about wheezing, eczema or allergy. Of these, nine had wheezing, eczema and allergy, four had wheezing and allergy, and two had eczema and allergy and overall thirty-two children reported wheeze. Binary logistic regression analysis, correcting for exposure to parental smoking, suggested an association between head circumference at 6–10 weeks and self-reported allergy. There were no associations between weight, head circumference at birth, thinness at birth (low ponderal index) and allergy or atopic disease. After adjustment for familial factors, childhood wheezing and eczema appeared to be related to weight and ponderal index in early infancy (see Table). Peak flow tended to be lower in children reported as suffering from allergies or asthma. After adjusting for the children's current height and weight, peak flow was positively associated with ponderal index at 7–9 months.

Infant characteristics		Wheeze			Eczema		
		Odds ratio relative to baseline	95% CI	*P*	Odds ratio relative to baseline	95% CI	*P*
Weight at 6–10 wks (kg)	<4.47	1.0			1.0		
	4.48–4.94	1.26	0.32–4.98	0.73	0.22	0.05–0.97	0.045
	>4.94	0.66	0.18–2.45	0.54	0.24	0.06–1.01	0.059
Ponderal index (kg/m^3) at 7–9 months	<24.80	1.0			1.0		
	24.81–26.66	0.61	0.17–2.14	0.44	0.57	0.12–2.67	0.478
	>26.67	1.24	0.33–4.58	0.75	0.31	0.07–1.36	0.123

These findings suggest that anthropometric characteristics in infancy rather than at birth predict risk of allergy and atopic disease in later life. For every kilogram greater body weight at 6–10 weeks the risk of eczema at 7 years of age increased by 3.29 (CI 1.1–9.8). This is in contrast to the findings of Barker and colleagues who cite low birth weight and shortness relative to head circumference as predictors of reduced growth of the thymus and therefore the development of atopy. The high prevalence of asthma, eczema and allergy reported here reflects current trends. Further studies will consider the contribution of maternal nutritional status to the development of atopic predisposition *in utero*.

Asher MI, Keil U, Anderson HR, *et al.* (1998) *European Respiratory Journal* **8**, 483–491.
Barker DJP (1998) *Mothers, Babies and Health in Later Life*, 2nd edn. London: Churchill Livingstone.
Langley-Evans SC (1997) *Clinical and Experimental Allergy* **27**, 1377–1379.

Alpha-linolenic acid does not reproduce the effects of the very long chain *n*-3 polyunsaturated fatty acids on fasting blood lipids. By Y.E. FINNEGAN, A.M. MINIHANE, E.C. LEIGH-FIRBANK and C.M. WILLIAMS, *Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, University of Reading, Reading RG6 6AP*

There is evidence that α-linolenic acid (ALNA), the precursor to the very long chain *n*-3 polyunsaturated fatty acids (VLCP), may play a role in secondary prevention in coronary heart disease patients (De Lorgeril *et al.* 1994). These beneficial effects are generally assumed to be due to the conversion of ALNA to the VLCP eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, few studies have made a direct comparison of the effects of an increase in dietary ALNA with an increase in EPA+DHA on lipid risk factors for coronary heart disease in a long-term study.

In a double-blind, placebo-controlled parallel trial, moderately hyperlipidaemic, but otherwise healthy males and females (aged 25–72 years) were randomized to one of four *n*-3 PUFA interventions or to a control intervention for 6 months. The intakes of EPA+DHA in the two VLCP intervention groups were 0.8 and 1.7 g/d (4–9 times the average UK intake). The intakes of ALNA in the two ALNA groups were 4.5 g and 9.5 g/d (2.5–5 times the average UK intake). Target ALNA intakes were calculated using a biological equivalence of 7:1 dietary ALNA to EPA+DHA as estimated in the literature (Emken *et al.* 1994). Supplementation of the diet was achieved by the use of specially formulated margarines (25 g/d), which replaced the subject's normal margarine, in addition to three oil capsules. For 1 month prior to the study, all individuals underwent a run-in period, where all participants followed the control treatment (an *n*-6 PUFA-rich margarine). Fasting blood samples were collected at 0, 2, 4 and 6 months.

Fasting plasma values	Time	Control (*n*=30)		0.8 g EPA+DHA (*n*=30)		1.7 g EPA+DHA (*n*=31)		4.5 g ALNA (*n*=30)		9.5 g ALNA (*n*=29)	
(mmol/l)	(months)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TAG	0	1.69	0.11	1.65	0.14	1.60	0.13	1.66	0.13	1.35	0.08
	6	1.60	0.11	1.63	0.12	1.40	0.11	1.83	0.16	1.47	0.10
	6–0 (%)	–3.5	4.1	1.7	3.7	–7.7[a]	4.99	11.4	5.5	10.9	4.5
Total cholesterol	0	5.80	0.17	5.50	0.16	5.49	0.15	5.62	0.14	5.50	0.17
	6	5.95	0.14	5.76[1]	0.17	5.99[2]	0.18	5.83	0.15	5.77	0.18
	6–0 (%)	3.4	2.0	5.0	1.6	9.7	2.4	4.1	1.6	5.4	1.9
LDL-C	0	3.63	0.16	3.41	0.17	3.42	0.14	3.55	0.13	3.46	0.16
	6	3.84	0.13	3.62	0.18	3.96[3]	0.16	3.71	0.13	3.64	0.17
	6–0 (%)	8.2	3.5	7.0	2.9	17.6	4.4	6.4	3.1	6.2	2.9
HDL-C	0	1.35	0.06	1.37	0.07	1.34	0.07	1.29	0.06	1.43	0.07
	6	1.35	0.05	1.45	0.07	1.40	0.08	1.31	0.06	1.46	0.08
	6–0 (%)	1.8	2.3	7.0	2.8	4.5	2.5	2.8	2.8	2.4	2.6
LDL-C : HDL-C ratio	0	2.9	0.2	2.7	0.2	2.8	0.22	3.0	0.2	2.5	0.2
	6	3.0	0.2	2.7	0.2	3.1	0.23	3.0	0.1	2.7	0.2
	6–0 (%)	7.8	4.3	2.6	4.4	15.5	6.5	7.12	5.2	5.5	3.7
LDL-C : Apo B ratio	6–0 (%)	6.14	4.0	2.2	2.4	11.3[1]	3.0	1.5	3.2	0.6	2.8

One-way ANOVA/Kruskal-Wallis for % change between groups. Repeated measures/Friedman test for within group.
[1–3]Significantly different from baseline; [1]$P<0.05$, [2]$P<0.01$, [3]$P<0.001$; [a]significantly different from 9.5 g/d ALNA group.

There was no significant difference in the percentage change (6–0 months) in fasting triacylglycerol (TAG), total cholesterol (TC), LDL-C, HDL-C, LDL : HDL-C ratio or LDL-C : Apo B ratio between the *n*-3 intervention groups and the control. Within groups, there was no significant difference in mean fasting TAG concentrations at 6 months compared to baseline, although mean concentrations tended to be lower following 1.7 g/d EPA+DHA. The percentage change in fasting TAG following the 1.7 g/d EPA+DHA and the 9.5 g/d ALNA interventions were significantly different ($P<0.05$), suggesting possible divergent effects of the plant and marine *n*-3 PUFA on circulating levels of TAG. Mean fasting TC and LDL-C concentrations tended to increase in all groups but were significantly higher at 6 months compared to baseline following the 1.7g/d EPA+DHA intervention. However, an increase in the LDL: Apo B ratio was also observed suggesting a shift towards the formation of less dense LDL particles. The data suggest that plant- and marine-derived *n*-3 PUFA have different effects on blood lipids with no evidence of a hypotriglyceridemic response following an increase in dietary ALNA.

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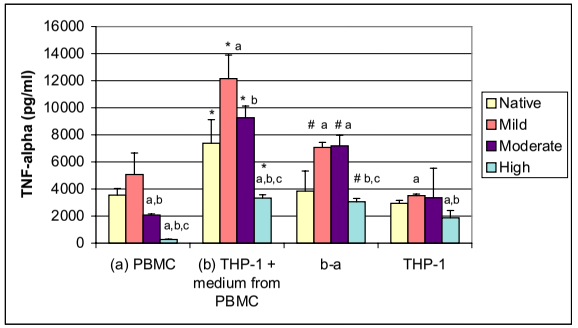
De Lorgeril M, Renaud S, Mamelle N, Salen P, Martin J-L, Monjaud I, *et al.* (1994) *Lancet* **343**, 1454–1445.
Emken EA, Adlof RO & Gulley RM (1994) *Biochimica Biophysica Acta* **1213**, 277–288.

Lymphocytes exposed to oxidized LDL produce mediators which induce an inflammatory response in macrophages. By E. NOVA, M.D. MESA and P. YAQOOB, *School of Food Biosciences, The University of Reading, Whiteknights, Reading RG6 6AP*

There is evidence that pro-inflammatory cytokines, and especially TNF-α, are important mediators in chronic inflammatory responses. Atherosclerosis is a chronic inflammatory disease. However, little is known about the exact role of immune cells and the cytokines secreted by them within atherosclerotic lesions. One hypothesis is that oxidized low-density lipoprotein (oxLDL) within lesions could induce lymphocyte activation, which in turn could activate macrophages to produce pro-inflammatory cytokines.

To test this hypothesis, peripheral blood mononuclear cells (PBMC) (1×10^6 cells/ml) were cultured for 24 h with either native LDL (50 μg/ml) or oxLDL (50 μg/ml) at different degrees of oxidation (mild, moderate and high). The supernatant from these cultures was obtained and one aliquot was frozen while a second was added to PMA-differentiated THP-1 cells (1×10^6 cells) for a further 24 h. As a control, PMA-differentiated THP-1 cells were cultured for 24 h in the presence of native, mild, moderate or highly oxLDL (50 μg/ml). All cultures were performed in triplicate in the presence of 5% lipid depleted serum. TNF-α was analysed in the supernatants obtained from each of the cultures by ELISA.

TNF-α in the culture supernatant of PBMC exposed to oxLDL, THP-1 cells cultured with supernatant from PBMC exposed to oxLDL and THP-1 cells exposed to oxLDL.



*Mean values significantly different from both PBMC and THP-1 exposed to oxLDL (Mann-Whitney test, $P< 0.05$).
#Mean values significantly different from those of THP-1 exposed to oxLDL (Mann-Whitney test, $P<0.05$).
a,b,c Mean values significantly different from native, mild and moderately oxLDL, respectively (Mann-Whitney, $P<0.05$).

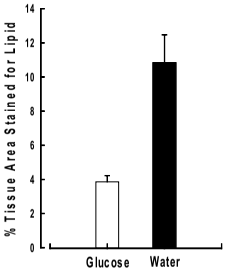
Results showed that THP-1 cells cultured with the supernatant from PBMC exposed to oxLDL produced significantly more TNF-α than THP-1 cells exposed directly to oxLDL (see Figure). In addition, mildly and moderately oxLDL appear to induce TNF-α production to a higher extent than both native or highly oxLDL. In conclusion, it appears that oxLDL is not directly pro-inflammatory towards monocytic cells, but that lymphocytes mediate an inflammatory response initiated by oxLDL. The degree of oxidation of LDL influences this inflammatory response.

The second-meal effect for plasma lipids: observations following oesophago-gastro-duodenoscopy (OGD). By M.D. ROBERTSON[1], M. PARKES[2], B.F. WARREN[3], K.G. JACKSON[4], D.P. JEWELL[2] and K.N. FRAYN[1], [1]*Oxford Centre for Diabetes Endocrinology and Metabolism and* [2]*Nuffield Department of Gastroenterology, University of Oxford OX2 6HE*, [3]*Department of Cellular Pathology, John Radcliffe Hospital, Oxford OX3 9DU and* [4]*Hugh Sinclair Unit for Human Nutrition, School of Food Biosciences, University of Reading, Reading RG6 6AP*

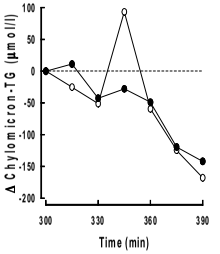
Earlier studies have noted the presence of an early postprandial peak in plasma triacylglycerol (TAG) following successive fat-rich meals. This early peak (45–60 min) in chylomicron-TAG concentration suggests the release into the circulation of pre-formed chylomicrons from the previous meal. The aim of this study has been to investigate the location and release of these lipid particles from within the gastrointestinal tract using this two-meal protocol.

Ten subjects (six female) scheduled to undergo a routine oesophago-gastro-duodenoscopy (OGD) were recruited and allocated randomly into two groups. At 7 a.m. all subjects drank a 50 g liquid fat emulsion (Calogen, SHS) and at 12 p.m. subjects had either a small glass of water (100 ml) or a glucose drink (38 g glucose in 100 ml water). The OGD was performed at 1 p.m. (6 h after the fat load and 1 h after the second meal). Jejunal biopsy samples were collected for histological staining with oil-red-O and for electron microscopy (EM). Oil-red-O staining was quantified for lipid content using a semiautomatic morphometric analysis system and EM. images were graded. As it was not possible to take plasma measurements during the OGD, a subgroup of five subjects (two female) underwent parallel metabolic studies. The 50 g fat load was again followed 5 h later by water or a glucose drink, with subjects undergoing both protocols.

Biopsy samples from the "water" group showed significantly more lipid staining with oil-red-O than did the "glucose" group (P=0.028; see Figure 1). During the metabolic study glucose ingestion following fat ingestion resulted in a peak in the plasma-TAG, chylomicron-TAG (P=0.033; see Figure 2), and apo B48 concentrations.



(1)



(2)

Six h after a high-fat meal there were fat particles visible within the enterocytes and lamina propria of the jejunal mucosa but none visible within the stomach or the lumen of the GI tract. Glucose ingestion following the fat load resulted in less fat visible within this tissue, which coincided with a peak in plasma chylomicron-TAG. We propose that after a fat load, fat is retained within the jejunal mucosa and released into the plasma following glucose ingestion.

Elevated blood cholesterol levels with different measures of body size. By E. ELLIOTT, J.E. CADE, V.J. BURLEY and D.C. GREENWOOD, *Nutrition Epidemiology Group, Nuffield Institute for Health, 71-75 Clarendon Road, Leeds LS2 9PL*

High total cholesterol is associated with angina, hypercholesterolaemia, hypertension and diabetes. It is a well known risk factor for coronary heart disease (CHD), the single most common cause of mortality in western society (Jenkins *et al*. 1997). Elevated total cholesterol (TC) and low-density lipoprotein (LDL) are known to be related to increasing body mass index (BMI). We aimed to investigate whether anthropometric measurements could be a good indicator of elevated blood cholesterol level.

Subjects were drawn from participants in the UK Women's Cohort Study, living within a 30-mile radius of Leeds. A baseline food frequency questionnaire was completed by 303 women, who also completed a second questionnaire and a 4 d food diary. At the same time a fasting blood sample was collected from 273 of these women. Food diaries were analysed using COMPEAT, a dietary analysis program which is based on the UK food composition tables. The results in the Table are Spearman correlations between the various self-reported anthropometric measures and blood lipid values.

	Total cholesterol		LDL cholesterol		HDL cholesterol		Triacylglycerols	
	R	*P* value	*r*	*P* value	*r*	*P*-value	*r*	*P* value
Waist (cm)	0.20	0.001	–0.04	0.52	–0.13	0.06	0.32	<0.001
Hip (cm)	0.27	<0.001	–0.03	0.63	–0.19	0.004	0.26	<0.001
Waist : Hip ratio	–0.01	0.84	–0.11	0.09	–0.14	0.04	0.14	0.04
Height (cm)	–0.17	0.006	–0.19	0.002	–0.13	0.04	–0.18	0.004
BMI	0.30	<0.001	0.08	0.22	–0.10	0.11	0.45	<0.001
Blouse size	0.28	<0.001	0.07	0.29	–0.13	0.03	0.33	<0.001
Skirt size	0.32	<0.001	0.05	0.44	–0.16	0.01	0.35	<0.001

Total cholesterol was strongly correlated with waist and hip measurements, body mass index, blouse and skirt size, but not waist : hip ratio. Stronger associations were found between measures of obesity and triacylglycerols, with waist : hip ratio having a weak but significant positive association. Height was inversely related to all types of cholesterol concentrations, but LDL cholesterol was not correlated with any other anthropometric measurements. HDL was negatively correlated with all anthropometric measures used. Blouse and skirt sizes were as strongly associated as BMI to TC, HDL and TAG. In the absence of BMI, skirt size predicted cholesterol concentrations nearly as well. This stayed true even after adjusting for age.

Analysis of the food diaries indicated that total blood cholesterol was not associated in this population with fat intake or exercise level. The strong correlation of blouse and skirt size with total cholesterol has implications for simplifying CHD risk factor assessment. In fact, no subjects with blouse or skirt size 12 or less had clinically raised total cholesterol. Waist circumference, waist : hip ratio and BMI are known to be associated with increased risk factors for CHD (Molarius *et al*. 2000). Our results show that blouse and skirt size could be added to the list. Primary care staff may find clothes sizes in women a quick and easy way to indicate potentially raised blood cholesterol levels.

The UK Women's Cohort study is funded by the World Cancer Research Fund. Thanks to James Thomas for data management. Blood samples were collected as part of MAFF Funded Study ANO318

Jenkins DJ, Popovich DG, Kendall CW, Vidgen E, Tariq N, Ransom TP, Wolever TM, Vuksan V, Mehling CC, Boctor DL, Bolognesi C, Huang J & Patten R (1997) *Metabolism: Clinical & Experimental* **46**, 530–537.
Molarius A, Seidell JC, Visscher TLS & Hofman A (2000) *Journal of the American Geriatric Society* **48**, 1638–1645.

Influence of the apoC3 -2854T>G polymorphism on plasma lipid levels: effect of gender and age. By A.M. MINIHANE[1], Y.E. FINNEGAN[1], P. TALMUD[2] and C.M. WILLIAMS[1], [1]*The Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading RG6 6AP and* [2]*Department of Medicine, UCL Medical School, London WC1E 6JJ*

Apolipoprotein C3 (apoC3) is a component of most classes of lipoproteins and is a major determinant of the metabolism and clearance of triacylglycerol-rich lipoproteins (TRL) from the circulation. Increased circulating apoC3 levels are associated with an exaggerated lipaemic response in humans. The apoC3 gene forms part of the ApoA1-C3-A4 gene cluster, with the -2854T>G polymorphism lying in the C3-A4 intergenic region. Information on the impact of this genotype on plasma lipid levels is currently lacking. The aim of the study was to investigate the impact of the apoC3 -2854T>G polymorphism on plasma lipids and to determine which physiological factors influence genotype–lipid associations in a group of mildly hyperlipidaemic UK adults.

A total of 150 individuals participated in the study. Each participant provided a fasting blood sample. In addition each individual underwent a postprandial assessment, whereby following a test breakfast (0 min, 50 g fat) and lunch (330 min, 30 g fat), blood samples were collected up to 8 h post-breakfast, in order to assess postprandial triacylglycerol (TAG) and glucose responses. Retrospective genotyping was conducted and individuals were classified as either homozygous wild type (TT, 41%), or heterozygous (TG, 46%) or homozygous (GG, 13%) for the rare allele.

The apoC3 -2854T>G polymorphism had no significant impact on fasting total-cholesterol or LDL-cholesterol. However, there was a significant codominant association with TAG levels (P=0.000); TT individuals having the lowest mean (SE) levels of 1.35 (0.06), GG individuals having the highest 2.09 (0.21), and heterozygous carriers intermediate levels of 1.61 (0.07) mM. The trend of decreasing baseline HDL-C values from TT to GG reached borderline significance (P=0.066).

	All		ApoC3-TT		ApoC3-TG		ApoC3-GG		*P*
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Age (years)	53	1	53	1	53	2	56	2	0.518
BMI (kg/m^2)	26.4	0.3	26.3	0.4	26.9	1.0	26.9	1.0	0.846
TC (mM)	5.53	0.07	5.54	0.13	5.45	0.10	5.78	0.17	0.314
LDL-C (mM)	3.44	0.07	3.47	0.12	3.36	0.10	3.60	0.19	0.699
HDL-C (mM)	1.37	0.03	1.45	0.05	1.32	0.04	1.27	0.09	0.066
TAG (mM)	1.57	0.07	1.35[a]	0.06	1.61[b]	0.07	2.09[b]	0.21	0.000
Glucose (mM)	5.11	0.07	5.01	0.11	5.23	0.11	5.06	0.17	0.309
TAG AUC*	974	36	897	51	1038	52	1020	40	0.192

TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TAG = triacylglycerol, TAG AUC = area under the TAG curve (mM/480min).

The impact of gender, age and BMI on genotype–TAG associations was also determined. On division of the group by gender, a significant association with genotype was only evident in males (P=0.012), with 87% higher TAG levels in the GG relative to the TT group. A genotype–TAG association only remained significant in the younger subgroup (<55 years) when the study population was divided by age.

In conclusion, the apoC3 -2854T>G polymorphism was significantly associated with fasting TAG. Subgroup analysis indicated gender–genotype and age–genotype interactions, with the greatest impact of genotype on fasting TAG in a younger male population. Further work is needed to establish whether the effect on fasting TAG is due to a direct impact of the polymorphism on circulating apoC3 protein levels, or due to a linkage disequilibrium with other functional sequences in the ApoA1-C3-A4 cluster.

This research is funded by a DEFRA-BBSRC Link project with Unilever Research and Hoffman–La Roche Ltd

Low selenium status is associated with the development of the pregnancy disease pre-eclampsia in UK women. By M.P. RAYMAN[1], P. BODE[2] and C.W.G. REDMAN[3], [1]*Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH,* [2]*Delft University of Technology, Interfaculty Reactor Institute, Mekelweg 15, 2629JB Delft, The Netherlands and* [3]*Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, University of Oxford, Oxford OX2 9DU*

Pre-eclampsia is a major complication of pregnancy that is associated with damage to the endothelium and high maternal and perinatal morbidity and mortality. It is one of the major indications for elective premature delivery as there is currently no other cure. Oxidative stress has been implicated in its pathophysiology (Hubel, 1999) and antioxidant vitamins (C and E) have shown a beneficial effect in a high-risk pregnant group (Chappell *et al*. 1999). The trace element, selenium, when incorporated into selenoproteins, behaves as an antioxidant and so might be expected to be able similarly to reduce the risk of pre-eclampsia. Furthermore selenoproteins can scavenge the powerful oxidizing agent peroxynitrite (Arteel *et al.* 1999), which is formed in the placenta and vasculature of pre-eclamptic women (Myatt *et al*. 1996; Roggensack *et al*. 1999).

In this study, we investigated the possible role of selenium status in the aetiology of pre-eclampsia. Toenails were collected from fifty-three pre-eclamptic patients and fifty-three pregnant controls, matched for age, gestation and parity, at the John Radcliffe Hospital, Oxford. This gives a means of determining trace element concentrations before the development of symptoms and even before pregnancy, as toenails are laid down from 3–12 months prior to clipping. The selenium content of the toenails was determined by instrumental neutron activation analysis. The study was approved by Central Oxford Research Ethics Committee.

The frequency distribution of selenium concentrations (not shown) showed that the data were positively skewed, precluding the use of parametric statistical methods. Wilcoxon's signed rank test for non-parametric, paired data was used to compare toenail selenium concentrations in the pre-eclamptic patients and their matched controls. Median toenail selenium concentrations in the pre-eclamptic women (0.56 mg/kg) were significantly lower than in their matched controls (0.62 mg/kg) (P<0.001).

	Median (mg/kg)	Minimum–maximum (mg/kg)	Interquartile range (mg/kg)
Pre-eclampsia	0.56	0.36–0.79	0.51–0.64
Control	0.62	0.45–1.00	0.57–0.69

The results of this study show that in UK pregnant women, low selenium status spanning the period from 3–12 months before diagnosis is significantly associated with the development of pre-eclampsia. Adequate selenium status may reduce the risk of developing this oxidative-stress condition through the action of the antioxidant selenoenzymes, or by the scavenging of peroxynitrite in the plasma by selenoprotein P, an ability unique to this selenoprotein.

Arteel GE, Briviba K & Sies H (1999) *FEBS Letters* **445**, 226–230.
Chappell LC, Seed PT, Briley AL, Kelly FJ, Lee R, Hunt B, Parmar K, Bewley SJ, Shennan AH, Steer PJ & Poston L (1999) *Lancet* **354**, 810–816.
Hubel CA (1999) *Proceedings of the Society of Experimental Biology and Medicine* **222**, 222–235.
Myatt L, Rosenfield RB, Eis AL, Brockman DE, Greer I & Lyall F (1996) *Hypertension* **28**, 488–493.
Roggensack AM, Zhang Y & Davidge ST (1999) *Hypertension* **33**, 83–89.

Estimation of haem iron intake from a food frequency questionnaire. By B.A. BRATLEY, V.J. BURLEY, D.C. GREENWOOD, J. BARRETT and J.E. CADE, *Nutrition Epidemiology Group, Nuffield Institute for Health, 71-75 Clarendon Road, Leeds LS2 9PL*

Density of *bioavailable* iron in the diet, rather than total intake or iron density, is the most useful estimator of nutritional adequacy in the case of iron. Recently, algorithms for calculating absorption and bioavailability of dietary iron have appeared in the literature (Hallberg & Hulthén, 2000; Reddy *et al*. 2000). Such algorithms rely on measuring the factors present in a meal that inhibit or enhance iron absorption. Since other constituents of the meal do not affect absorption of haem iron, it is absorbed at a greater rate than non-haem iron. It is therefore important to have accurate estimates of the haem iron content of the diet. To date, few methods of analysis have been validated to determine the amounts of haem and non-haem iron in foods. For this reason, the haem iron content of meat, fish and poultry (MFP) is generally taken to be 40% of total iron (Monsen *et al*. 1978). Depending on the relative contribution of MFP to the diet, however, this figure may over- or underestimate the haem iron content of the diet. This would introduce bias into bioavailability calculations and misclassify individuals in terms of overall adequacy of the diet. This is a particular problem in populations that consume limited amounts of red meat, and relatively larger amounts of fish, such as the UK Women's Cohort Study (UKWCS) (Greenwood *et al*. 2000).

A literature review was carried out to derive new values for the haem iron content of MFP. These values were used to estimate iron intake from a 217-item food frequency questionnaire, which had been completed by 34 319 participants of the UKWCS. The analysis was repeated, using the Monsen figure to derive two estimates of haem iron intake, which were then compared.

Top 5 contributors to haem intake: Monsen (40%) method	Mean (% total haem)	Top 5 contributors to haem intake: literature review	Mean (% total haem)
Beef dishes	10.2	Beef dishes	13.8
Oily fish	9.7	Quiche	10.8
Quiche	9.5	Shellfish	9.3
Bovril	9.1	Oily fish	7.9
Offal	7.7	Roast beef	6.8

The main sources of haem in the diet generated by the two methods are broadly similar, except that the Monsen method ranks Bovril as the fourth greatest source of haem. This is unlikely to be the case, as the cooking methods involved in its manufacture are likely to destroy any haem iron.

On average, mean intake calculated by the Monsen method is 0.08 mg greater than intake calculated by using figures for MFP derived from a literature review. The limits of agreement (LOA) (Bland & Altman, 1986) of –0.34 mg to 0.19 mg are wide given that median intake is 0.53 mg by the calculated method, and 0.61 mg by the Monsen method. This means that there is the potential for large differences in intake according to which method is used. On average, the Monsen method overestimates by 13%, but for an individual it could as much as double actual intake. Given that UKWCS participants' meat intake reflects modern patterns of consumption, using specific values for the haem content of MFP is more appropriate. The values obtained from the literature review require validation by laboratory analysis.

The UK Women's Cohort Study is funded by the World Cancer Research Fund. The current project is funded by the Food Standards Agency (CSA5404).

Bland JM & Altman DG (1986) *Lancet* **i**, 307–310.
Greenwood DC, Cade JE, Draper A, Barrett JH, Calvert C & Greenhalgh A (2000) *European Journal of Clinical Nutrition* **54**, 314–320.
Hallberg L & Hulthén L (2000) *American Journal of Clinical Nutrition* **71**, 1147–1160.
Monsen E, Hallberg L, Layrisse M, Hegsted DM, Cook JD, Mertz W & Finch CA (1978) *American Journal of Clinical Nutrition* **31**, 134–141.
Reddy MB, Hurrel RF & Cook JD (2000) *American Journal of Clinical Nutrition* **71**, 937–943.

Development of a new cut-off point to distinguish between high-fat and medium-fat consumers using the Dietary Instrument for Nutrition Education questionnaire. By S.L. JACKSON[1], C. GOLDING, D.C. GREENWOOD[2], J.E. CADE[2] and S.E. HIRST[1], [1]*School of Health Sciences, Leeds Metropolitan University, Calverley Street, Leeds LS1 3HE and* [2]*Nutrition Epidemiology Group, Nuffield Institute for Health, 71-75 Clarendon Road, Leeds LS2 9PL*

The Dietary Instrument for Nutrition Education (DINE) questionnaire is a food frequency questionnaire (FFQ) developed to assess dietary intakes of fat and fibre (Roe *et al.* 1994). The DINE lists eleven fat-containing food groups, scored according to frequency of consumption and relative fat content. The scores are added to give a total and respondents classified as below:

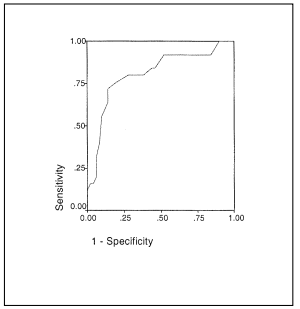
Total score <30 = low-fat consumer,
Total score 30–40 = medium-fat consumer,
Total score >40 = high-fat consumer.

The DINE method of classification was compared to data from the UK Women's Cohort Study (UKWCS), which used a 217-item FFQ to classify subjects into equal tertiles based on their reported absolute intake of fat (UKWCS Steering Group, 1996).

A random sample of twenty-five women was selected from each tertile, and the DINE questionnaire completed over the telephone. The women were placed into high-, medium- and low-fat classifications as indicated by the DINE.

The agreement between the two methods was calculated using kappa (κ) statistics.

A ROC curve was produced, to show all possible cut-off points between high-, medium- and low-fat consumers. This was used to identify the cut-off point that gave the highest values for sensitivity and specificity, between high- and medium-fat consumers.



High-fat consumers, as defined by the new cut-off point of 25, were compared to people in the highest tertile of fat consumption based on the FFQ. The agreement between these methods was tested using κ statistics.

Cut-off point	Sensitivity	Specificity	κ	Agreement
40	0.120	0.100	0.154	Poor
25	0.800	0.720	0.538	Moderate

The low sensitivity and specificity of the original cut-off point could be because of a "healthy cohort effect", whereby the women in the sample are more health-conscious, and eat diets typically containing less fat, than the general population. A large proportion of the women were vegetarians so could be expected to eat diets lower in fat. If men had been included in the sample, a higher cut-off point may have been more appropriate, as men tend to eat diets that are higher in fat (Gregory *et al.* 1990). The agreement between the DINE and the UKWCS tertiles was moderate, and much greater than could be expected by chance alone. This level of agreement with a 217-item FFQ is impressive for an FFQ with only eleven questions.

The UK Women's Cohort Study is funded by the World Cancer Research fund.

Gregory J, Foster K, Tyler H & Wiseman M (1990) *The National Diet and Nutrition Survey of British Adults.* London: HMSO.
Roe L, Strong C, Whiteside C, Neil A & Mant D (1994) *Family Practice* **11**, 375–391.
UKWCS Steering Group (1996) *Proceedings of the Nutrition Society* **56**, 117A.

Postnatal ontogeny of insulin-like growth factor (IGF)-II and the receptors for insulin-like growth factor-I and -II in ovine perirenal adipose tissue. By J. BISPHAM[1], L. CLARKE[2], M.E. SYMONDS[1] and T. STEPHENSON[1], [1]*Academic Division of Child Health, School of Human Development, University Hospital, Nottingham NG7 2UH and* [2]*Huxley School, Imperial College at Wye, University of London, Ashford, Kent, TH25 5AH*

After birth, rapid growth of adipose tissue occurs as the newborn establishes independent feeding (Clarke *et al.* 1997). This occurs in conjunction with a reversal of the relative concentrations of IGF-I and IGF-II in the circulation (Gluckman & Butler, 1983). IGF-I is a mitogen involved in differentiation-related gene expression in brown adipocytes (Lorenzo *et al.* 1993). The following study aimed to determine whether abundance of either mRNA for IGF-II and IGF-I receptor (R) and -IIR increase in adipose tissue over the first week of neonatal life.

Twelve twin-bearing Bluefaced Leicester × Swaledale ewes of known mating date and of similar body weight and parity were entered into the study. All ewes were allowed to give birth normally at term and were randomly assigned to one of the lamb sampling times (i.e. within 1 h of birth, 2, 4 and 7 d post-lambing). Adipose tissue was sampled and weighed before being snap-frozen in liquid nitrogen. All samples were then stored at –80° until analysis. Total RNA was extracted. The expression of IGF-II and the receptors for IGF-I and -II mRNAs were examined by RT-PCR using specific oligonucleotide primers. IGF-II (GenBank M89789: forward 5′-TCA CAG CAG GAA AGT CGA TG-3′ and reverse 5′-GGC ACA GTA AGT CTC CAG CA-3′ product 248 bp), IGFI-R (GenBank X54980: forward 5′-GCC TCC AAC TTT GTC TTT GC-3′ and reverse 5′-GCT GAA ATA CTC CGG GTT CA-3′ product 498 bp) and IGFII-R (GenBank AF327649: forward 5′-ACC GGC ACT TCA ACT ACA CC-3′ and reverse 5′-ACT CAG AAT GAC GGC TTC GT-3′ product 401 bp). Results, in arbitrary units (a.u.; mean plus/minus SEM) are a ratio of an 18S rRNA internal control. Differences between ages were analysed using a Kruskal-Wallis test.

Postnatal age	Weight (g)		IGFII mRNA (a.u.)		IGFII-R mRNA (a.u.)		IGFI-R mRNA (a.u.)	
(d)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0.1	20	2.8	68.5	2.7	5.8	0.5^{a}	0.2	0.1^{a}
2	26	3.7^{a}	70.9	2.2	13.2	3.4	4.7	1.3^{b}
4	41	3.9^{c}	83.7	12.5	19.5	5.6^{b}	3.4	0.5^{b}
7	70	9.1^{d}	79.4	1.3	2.5	1.8^{a}	0.1	0.0^{a}

Significant differences between age groups are represented by different superscripts: $^{ab}P<0.05$, $^{ac/ad/cd}P<0.01$ as measured by Kruskal-Wallis and Mann-Whitney *U* tests.

The large increase in adipose tissue deposition (3.5-fold) up to 7 d of age was preceded by a significant increase in both IGF-IR and IGF-IIR (3.5- and 17-fold, respectively) mRNA expression. In conclusion, this upregulation of both IGF-I and -II receptors may have an important role in promoting nutrient partitioning to adipocytes during their rapid phase of growth during the first week of postnatal life.

Clarke L, Buss DB, Juniper DT, Lomax MA & Symonds ME (1997) *Experimental Physiology* **82**, 1015–1027.
Gluckman PD & Butler JH (1983) *Journal of Endocrinology* **99**, 223–232.
Lorenzo M, Valverde AM, Teruel T & Benito M (1993) *Journal of Cell Biology* **123**, 1567–1575.

Substrate metabolism in the hindlimb of fetal sheep during adverse intrauterine conditions and subsequent hypoxaemia. By D.S. GARDNER, A.L. FOWDEN and D.A. GIUSSANI, *Department of Physiology, University of Cambridge, Cambridge CB2 3EG*

Adverse intrauterine conditions, resulting in a reduction in P_aO_2, require an increase in oxygen extraction in the ovine fetus to maintain tissue oxygenation (Richardson & Bocking, 1998). When investigated in specific vascular beds such as the hindlimb, changes in oxygen extraction serve as a useful index of skeletal muscle metabolism (Boyle *et al*. 1990). No study to date has investigated whether fetal metabolic adaptation to intrauterine adversity of this kind affects subsequent fetal metabolic responses during a further acute stress imposed after 'recovery' from the period of intrauterine compromise. Hence, the present study investigated fetal hindlimb oxygen, glucose and lactate metabolism during acute hypoxaemia after recovery from 3 d of partial compression of the umbilical cord, a challenge commonly faced by fetuses during gestation.

Sixteen sheep fetuses were chronically instrumented under 1–2% halothane (50/50 O_2/N_2O) with vascular catheters and umbilical and femoral Transonic flow probes. Between 125–128 d (term 145 (±2) d), umbilical blood flow was reduced by 30% from baseline in nine of these fetuses (UCC) as described previously (Gardner *et al*. 2001). The remaining fetuses acted as sham-operated controls in which the occluder remained deflated throughout the study period. At 130 (±1) d acute hypoxaemia was induced for 1 h in all fetuses by reducing maternal F_iO_2. Paired hindlimb arterio-venous blood samples were taken at appropriate intervals before, during and after UCC and during the subsequent 1 h episode of acute hypoxaemia for analyses of blood gases and metabolite concentrations. Substrate delivery, uptakes and output were calculated by the Fick principle, using femoral blood flow.

Umbilical cord compression (unilateral umbilical blood flow was reduced from 195 (±21) to 140 (±16) ml min^{-1}) reduced P_aO_2 and precipitated a significant increase in fetal oxygen extraction across the hindlimb (from 19.6 (±2) to 26.2 (±3) %, $P<0.05$). During subsequent acute hypoxaemia (P_aO_2 from 21 (±1) to 12 (±1) mmHg in all fetuses) oxygen uptake fell to similar levels in UCC and control fetuses while glucose uptake and delivery were maintained in both groups (see Table). However, glucose extraction and lactate output significantly increased in sham-control, but not UCC fetuses, despite similar reductions in O_2 delivery to the hindlimb (Table) and similar increases in blood lactate concentrations (to 4.15 (±0.48) and 3.43 (±0.35) mmol l^{-1}, in control and UCC fetuses, respectively).

	NORMOXIA		HYPOXAEMIA		RECOVERY	
HINDLIMB…	CONTROL	UCC	CONTROL	UCC	CONTROL	UCC
O_2 delivery (µmol min^{-1})	105±13	72±19	38±13†	33±11†	94±16	65±19
O_2 uptake (µmol min^{-1})	20.6±2.3*	14.3±2.8*	9.6±2.3*†	9.3±2.2*†	18.8±2.2*	14.7±3.8*
Glucose delivery (µmol min^{-1})	22.6±3.6	17.9±4.8	20.4±5.4	17.8±5.2	28.6±6.4	22.0±5.3
Glucose uptake (µmol min^{-1})	3.7±0.5*	4.2±0.6*	5.7±0.5*	4.7±0.6*	4.6±0.8*	4.0±0.4*
Glucose extraction (%)	17.9±2.9*	28.2±5.5*	34.4±4.3*†	35.6±5.2*	18.3±2.0*	25.5±4.7*
Lactate output (µmol min^{-1})	–1.0±0.5	–1.2±0.8	–2.5±0.7*†	–1.4±0.7	–2.2±1.8	–1.8±1.2

Data are means ± SEM of metabolic data for control (n=7) and UCC (n=7) fetuses. * $P<0.05$ for significant basal uptake or output of substrate; † $P<0.05$ normoxia *v.* hypoxaemia or recovery.

These data show that pre-exposure of the fetus to a temporary period of adverse intrauterine conditions alters the metabolic response of the fetal hindlimb to subsequent hypoxaemia. In addition, the data suggest that circulating blood lactate may be derived from sources other than the fetal hindlimb under these circumstances. The lack of hindlimb lactate output during hypoxaemia in UCC fetuses, despite a significant fall in oxygen delivery to and uptake by the hindlimb, suggests that the fetal hindlimb may not respire anaerobically following exposure to adverse intrauterine conditions.

Boyle DW, Hirst K, Zerbe GO, Meschia G & Wilkening RB (1990) *Pediatric Research* **28**, 94–100.
Gardner DS, Fletcher AJ, Fowden AL & Giussani DA (2001) *Journal of Physiology* **535**, 217–229.
Richardson BS & Bocking AD (1998) *Comparative Biochemistry and Physiology A* **119**, 717–723.

Changes in food intake in free-living adults after changing to different types of vegetarian diets. By R.L. REID, A.F. HACKETT and D. BILLINGTON, *Liverpool John Moores University, I.M. Marsh Campus, Barkhill Road, Aigburth, Liverpool L17 6BD*

Recent studies of free-living vegetarians (Nathan, 1995; Robinson, 1998) and market research show a massive growth in the sales of vegetarian convenience foods, suggesting a new era of vegetarians, who appear to be increasingly dependent on convenience foods. If there is a relationship between vegetarian diets and health it may be possible that current knowledge about the health impact of following a vegetarian diet is outdated as convenience foods replace foods that have previously been a dominant feature of vegetarian diets. A sample of thirty-seven omnivorous volunteers was recruited. Subjects allocated themselves to either a convenience (n=19) (which included the use of vegetarian convenience foods) or wholesome (n=18) (which minimized the use of vegetarian convenience foods) vegetarian diet for 12 weeks. A series of 3 d dietary diaries were completed. Summary measures (Matthews *et al*. 1990), which describe the diet during the vegetarian period, were used to investigate differences in food intake between and within subjects compared with baseline (omnivorous diet) and follow-up (return to omnivorous diet).

Food group	Baseline mean (g/person/d)	Summary mean (g/person/d)	Follow-up mean (g/person/d)	Baseline-summary *P* value	Summary-follow-up *P* value	Baseline-follow-up *P* value
				Within-group changes		
Bread C	126.0	124.9	114.7	0.93	0.20	0.47
Bread W	97.8	118.3	100.4	0.05	0.05	0.82
Milk, Dairy & Eggs C	366.3	319.0	333.0	0.14	0.62	0.51
Milk, Dairy & Eggs W	307.1	339.2	317.6	0.19	0.33	0.74
Fruit & Vegetables C	408.8†	450.5	345.0†	0.26	0.00*	0.20
Fruit & Vegetables W	537.9†	562.9	517.1†	0.64	0.40	0.65
Vegetarian Convenience Foods C	65.8†	155.6†	92.8	0.00*	0.00*	0.03*
Vegetarian Convenience Foods W	33.2†	34.1†	78.0	0.93	0.09	0.10
Fish & Fish Products C	23.3	39.2	35.0	0.16	0.69	0.39
Fish & Fish Products W	18.7	59.0	18.1	0.00*	0.00*	0.92

Significant between group difference †$P<0.05$. Significant within group difference *$P<0.05$.

Significant between-group differences were only apparent for intakes of fruit and vegetables at baseline (P=0.02) and follow-up (P=0.01), and vegetarian convenience foods at baseline (P=0.03) and during the vegetarian period (P=0.00). The change to a vegetarian diet resulted in a significant increase in the amount of vegetarian convenience foods consumed within the convenience group (P=0.00), whilst the intake of fish and fish products significantly increased in the wholesome group (P=0.00). No significant changes in the intake of fruit and vegetables or milk, dairy products and eggs were observed within either group. A number of foods appear to have been displaced by vegetarian convenience foods. If many of the health benefits of vegetarian diets are due to the high intake of fruit and vegetables then the implications of the present findings in terms of long-term health benefits are uncertain.

Matthews JNS, Altman DG, Campbell MJ & Royston P (1990) *British Medical Journal* **300**, 230–235.
Nathan I (1995) *The dietary intake and growth of vegetarian children (aged 7–11) compared with matched omnivores in NW England*. PhD Thesis, LJMU, Liverpool.
Robinson F (1998) *Changing to a self selected vegetarian diet: two studies of diet and selected physical and lifestyle parameters*. PhD Thesis, LJMU, Liverpool.

An evaluation of a community dental health education project to promote healthy eating. By A.F. HACKETT[1], M. GIBBON[1] and A.K. MERCER[2], [1]*Liverpool John Moores University, School of Education, Community and Social Science, Barkhill Road, Liverpool L17 6BD and* [2]*St Helens (NHS) Primary Care Trust Dental Health Promotion Unit, Latham Road Clinic, Latham Road, Huyotn, Merseyside L36 9XX*

Despite huge improvements in dental caries rates in children, this still remains a major drain on NHS resources despite being largely preventable. Dietary intake (frequent intake of sugars) is a major determinant of caries and dietary change is needed to bring about further improvements in children's dental health, as well as other aspects of their health. This project aimed to improve the eating habits of pre-school children through a short interactive programme of intervention for children and their carers.

A random sample of nursery schools and schools with reception classes was selected (twenty-five of the seventy-one schools in the district offered the programme) stratified according to average prevalence of caries (measured by dmft – decayed, missing, filled teeth) and proportion of children receiving free school meals. Knowledge and attitudes of carers who volunteered, and dietary intake of the children, were assessed before, immediately after and 2 months after the intervention, which was a short interactive session based on food tasting. Only paired data were analysed, using McNemar's and Wilcoxon's tests. About 1200 individuals were invited to take part, 650 completed the baseline, 247 participated on the day and 219 completed the 2 month follow-up assessment. Knowledge and attitudes were assessed by questionnaires based closely on the content of the intervention. Dietary intake was assessed by food intake questionnaire (FIQ) previously reported but slightly modified (Johnson *et al.* 1999) which gives the proportion (%) who claimed to have eaten each food on the previous day.

Baseline knowledge scores varied from 10 to 100%. On average, knowledge scores were 11% higher at follow-up (change from 79.7 to 88.2%, $P<0.05$), but some huge improvements occurred. Attitudes were also more positive towards diet and caries, with more acceptance that the 'first teeth' are important ($P<0.05$). The FIQ asked whether fifty-three food items had been consumed on the previous day. For five items (see Table) a significant improvement occurred in the proportion who claimed that their child had eaten the food ($P<0.05$) although claimed consumption of another thirty-five foods moved in a positive direction.

Dietary intake at:	Base-line (%)	Follow-up (%)
Brown breads	38.0	54.1
Sugar on food	31.3	23.0
Baked potatoes	10.6	23.4
Fresh fruit	83.6	92.2
Sugary fizzy drinks	41.6	27.2

The feedback on the intervention was almost universally positive. Everyone thought the session was useful to them, 97% said they had learned something new, 19% reported that they had tried at least one food for the first time and 62% wanted to know more.

This study has shown that a modest programme of intervention can achieve useful changes in knowledge and attitudes. It also suggests that a positive change in dietary intake occurred. Although knowledge about healthy eating is often suggested to be adequate, this study shows that great improvements can be made.

We would like to thank the teachers, parents, children and dental health promotion officers for their help.

Johnson B, Hackett A, Bibby A & Cross J (1999) *Journal of Human Nutrition and Dietetics* **12**, 307–316.

Characteristics of women with high and low eating frequency: dietary analysis of the UK Women's Cohort Study. By V.J. BURLEY, D.C. GREENWOOD and J.E. CADE, *Nutrition Epidemiology Group, The Nuffield Institute for Health, University of Leeds, 71-75 Clarendon Road, Leeds LS2 PL*

Suggestions that eating frequency might have implications for health were originated by research conducted by Fabry & Tepperman in the 1970s. Since then a steady flow of research on obesity, diabetes, hypercholesterolaemia and, more recently, colon cancer has suggested that eating frequency may be implicated in these conditions. However, the relationship with obesity is by no means consistent. Both high (snacking) and low eating frequencies (gorging) have been implicated in the promotion of excess body weight. This study aimed to investigate whether the anthropometric characteristics of women with a high or low eating frequency differed significantly.

The women studied formed a subsample of the UK Women's Cohort Study, which is a national, 10 year investigation of diet and cancer in 35 000 women initially aged 35–69 years. The baseline data on the cohort were obtained with a 217-item FFQ, with additional questions on health and lifestyle. A second contact has now been undertaken (Phase 2), 2–5 years after baseline, with all 35 000 women being sent a 4 d food diary and a further health and lifestyle questionnaire. The data obtained from 1214 subjects at this second contact forms the basis for this cross-sectional study. Measures of eating frequency (EF) were obtained by calculating the mean number of energy-yielding eating or drinking episodes per day from the food diaries. Weight, height, and waist and hip circumference were all self-reported measures. The women were divided into tertiles of eating frequency, and analysis of variance was conducted on the relationship between eating frequency and the anthropometric measures and other continuous variables.

Mean (SD)	**Low eating frequency** (*n*=418)	**Medium eating frequency** (*n*=415)	**High eating frequency** (*n*=381)	***P***
Eating episodes per day	5.6 (0.9)	7.7 (0.49)	10.4 (1.6)	
BMI	24.5 (4.3)	23.9 (3.8)	23.9 (4.2)	0.04
Waist circumference (cm)	80 (11)	79 (10)	79 (12)	0.19
Hip circumference (cm)	101 (10)	100 (9)	99 (10)	0.01
Waist : Hip ratio	0.79 (0.07)	0.79 (0.09)	0.79 (0.06)	0.78
Weight change (kg) per year since age 20	0.24 (0.3)	0.22 (0.3)	0.21 (0.3)	0.07
Body mass (kg)	66 (12)	64 (11)	64 (11)	0.01
Age (years)	56.3 (9.25)	54.8 (8.6)	53.0 (7.9)	<0.001

Despite a doubling of eating episodes from the lowest to the highest tertile of eating frequency, BMI was not elevated in the highest EF group. This suggests that eating more frequently does not automatically exacerbate obesity. On the contrary, although the absolute differences between groups were small, BMI, weight and hip measurements were significantly greater in those eating least frequently. Analysis of covariance suggested that adjustment for differences in age between the EF tertiles did not markedly affect the relationship with BMI. Although not statistically significant, women in the lowest EF tertile had gained the most weight since age 20 and also had the highest yearly rate of weight gain. Partial analysis of the food diaries showed that both fruit and vegetable intakes increased progressively with increasing eating frequency ($P<0.05$). However, the number of servings of red meat consumed, although low overall, was significantly greater in the low eating frequency group ($P=0.01$). There were no differences between the eating frequency groups in the number of servings of fish and fish dishes, meals containing pulses or nuts or servings of white meat consumed.

Further exploration of the food diaries is required to rule out the possibility that the eating frequencies determined are an artefact of under-reporting in the low frequency group. Alternatively, a low eating frequency may be conducive to weight gain or may be a reflection of the drive to reduce or maintain current body weight.

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Fabry P, Tepperman J (1970) *American Journal of Clinical Nutrition* **123**, 1059–1068.

Tracking of waist circumference and body mass index in young British children. By K.V. JARRETT[1], H.D. McCARTHY[1], I. ROGERS[2], P.M. EMMETT[2] and the ALSPAC STUDY TEAM[2], [1]*School of Health and Sports Science, University of North London, Holloway Rd, London N7 8DB and* [2]*Institute of Child Health, University of Bristol, ALSPAC, 24 Tyndall Ave, Bristol BS8 1TQ*

Little research has focused upon the dynamics of upper body fatness in early childhood. Waist circumference (WC) is a marker of upper body fat accumulation and a high WC in childhood has been linked to altered blood lipids, fasting insulin levels and blood pressure (Cowin *et al*. 2000; Freedman *et al*. 1999). WC percentile charts have now been developed for use with British children (McCarthy *et al*. 2001). However the tracking of WC in early childhood is unclear. This study compared the tracking of WC, body mass index (BMI) and conicity index (CON, a new marker of upper body fatness = WC/(0.109×√wt/ht); Valdez *et al*. 1993) in a sample of young British children.

454 boys and 385 girls from the Children in Focus cohort – a subset of the Avon Longitudinal Study of Parents and Children – were studied (ALSPAC; Golding *et al*. 2001). Waist circumference was measured midway between the 10th rib and the iliac crest. Height and weight were measured using standard procedures, and BMI and CON were calculated. Data were obtained at ages 3.1 (baseline), 3.6, 4.1 and 5.1 years. Correlation coefficients were calculated between baseline values and equivalent values at later ages. Secondly, subjects were divided into quartiles of WC, BMI and CON at baseline and cross-tabulated with age to determine the proportion of the cohort who remained within the same quartile.

Tracking correlation coefficients are shown in the Table.

	WC			BMI			CON		
Age	3.6 years	4.1 years	5.1 years	3.6 years	4.1 years	5.1 years	3.6 years	4.1 years	5.1 years
Boys 3.1 years	0.74*	0.70*	0.67*	0.88*	0.85*	0.81*	0.47*	0.36*	0.38*
Girls 3.1 years	0.79*	0.76*	0.71*	0.90*	0.87*	0.81*	0.45*	0.46*	0.46*

*P<0.01 for all correlations.

BMI tracked most strongly, followed by WC, but tracking decreased with increasing time span between measurements. Cross-tabulations showed that between 3.1 and 5.1 years of age, 13.6% of boys and 13.5% of girls remained in the highest quartile of WC and 13.1% and 17.2%, respectively, remained in the lowest quartile ($P<0.0001$). This compared with 17.0% of boys and 18.1% of girls who remained in the highest quartile for BMI and 15.9% and 17.8%. respectively. remaining in the lowest quartile, ($P<0001$). Tracking of both WC and BMI was lower (6.4–9.0% for WC and 8.1–13% for BMI) for the middle two quartiles. For conicity, tracking rates were even lower (the highest being 11.0% for girls remaining in the highest quartile).

Despite BMI tracking most strongly in early childhood, this gives no indication whether those with a high BMI have excess fat accumulation on the upper body. The findings for WC indicate that upper body fatness does track in some children throughout early childhood, although there is also a considerable degree of centile crossing. These results are in agreement with others on slightly older children (Mueller *et al*. 2001). Particular attention should be paid to those children who remain in the upper centiles for WC as they grow, and those who move up the centiles with increasing age, as these are the children who are gaining upper body fat at the greatest rate.

Cowin I, Emmett P and the ALSPAC Study Team (2000) *International Journal of Obesity* **24**, 330–339.
Freedman DS, Serdula MK, Srinivasan SR & Berenson GS (1999) *American Journal of Clinical Nutrition* **69**, 308–317.
Golding J, Pembrey M, Jones R and the ALSPAC Study Team (2001) *Paediatric and Perinatal Epidemiology* **15**, 74–87.
McCarthy HD, Jarrett KV & Crawley HF (2001) *European Journal of Clinical Nutrition* **55**, 902–907.
Mueller WH, Dai S & Labarthe DR (2001) *International Journal of Obesity* **25**, 1850–1855.
Valdez R, Seidell JC, Ahn YI & Weiss KM (1993) *International Journal of Obesity* **17**, 77–82.

The relationship between the nutritional and psychological status of pregnant adolescents and non-adolescents in Brazil. By P.H.C. RONDÓ, M.R. SOUZA, F. NOGUEIRA and M. TAGA, *Nutrition Department, Public Health School, University of São Paulo, Avenida Dr. Arnaldo 715, CEP-01246-904 and Institute of Mathematics and Statistics, Cidade Universitária, São Paulo, Brazil*

Pregnancy in adolescence means an increase in nutritional requirements, for the growth of the fetus and for the mother herself. In developing countries, many children with mild to moderate malnutrition survive to reach adolescence, when the malnutrition tends to remain mild but chronic, being detectable only by anthropometric measurements. On the other hand, relatively well-nourished children may develop malnutrition in adolescence as a result of acquired dietary habits, influenced by the obsession with thinness. The psychological status of pregnant adolescents may have an impact on their nutritional status, although the nature of the relationship is not clear, including possibilities that it can be mediated through pre-pregnancy weight and prenatal weight gain. In addition, stress/distress may also affect the nutritional status of these women indirectly, when it leads to unhealthy habits such as cigarette smoking, drinking alcohol or excessive coffee consumption. The objective of this study was to examine the association between the nutritional and psychological status of pregnant adolescents aged 13–19 years and non-adolescents (20–42 years) through three interviews (gestational age (GA) ≤16 weeks, 20–26 weeks and 30–36 weeks), considering also their toxic intake (smoking, alcohol and coffee consumption).

The data for this cohort study were derived from a population of 855 pregnant women who attended antenatal care between September 1997 and August 2000 at seventeen health centres in Brazil. The nutritional status of each woman was assessed by anthropometric measurements (weight, height, body mass index (BMI) and mid-upper arm circumference (MUAC)). Weight gain was calculated by subtracting self-reported pre-pregnancy weight from weight measured at the time of the interview. Stress/distress were assessed by the State Trait Anxiety Inventories (STAI; Spielberger *et al*. 1970), the General Health Questionnaire (GHQ; Goldberg, 1972) and the Perceived Stress Scale (PSS; Cohen *et al*. 1983).

Adolescents had lower pre-pregnancy weight ($P<0.001$), and lower mean BMI and MUAC (ANCOVA; $P<0.001$) at the time of the three interviews than non-adolescents, but higher mean scores on the Trait Anxiety Inventory (TAI) and PSS (ANOVA; $P\leq0.02$). Multiple linear regression analysis determined associations between weight gain and GHQ scores for both groups of women ($P\leq0.03$), and associations between BMI and GHQ scores ($P=0.003$) for non-adolescents, while controlling for cigarette smoking, alcohol and coffee consumption, GA, pre-pregnancy BMI, parity, education and per capita income.

The individual and interacting influences of nutritional and psychological factors on prenatal weight gain should be considered in future studies, especially in studies involving thin adolescents with high scores for stress/distress.

Cohen S, Kamarck T & Mermelstein R (1983) *Journal of Health and Social Behaviour* **24**, 385–396.
Goldberg DP (1972) *The Detection of Psychiatric Illness by Questionnaire*. London: Oxford University Press.
Speilberger CD, Gorsuch RL & Lushene RE (1970) *STAI Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologist Press.

Development of a dietary assessment tool for children (the PEARS project), for the Department of Health. By J.E. CADE[1], L. FREAR[1], K. LAWSON[1], D. GREENWOOD[1], J.D. THOMAS[1], P. SAHOTA[2] and M. RUDOLF[3], [1]*Nutrition Epidemiology Group, Nuffield Institute for Health, University of Leeds, 71–75 Clarendon Road, Leeds LS2 9PL,* [2]*Department of Nutrition and Dietetics, Leeds Metropolitan University, Calverley Street, Leeds LS1 3HE and* [3]*Academic Unit of Paediatrics and Child Health, University of Leeds, Belmont House, 3–5 Belmont Grove, Leeds LS2 9DE*

Promotion of fruit intake at school may be one important way to encourage healthy eating. The National School Fruit Scheme (NSFS) will provide fruit to young children aged 4–6 years. Currently there is no suitable tool to evaluate the effectiveness of this dietary intervention. The main aim of this work is to develop a concise, simple tool for use by non-specialists to assess diet in primary school children.

Six primary schools in Leeds, currently not part of the NSFS pilot work, with a wide range of socio-economic and ethnic backgrounds, have been selected for the project, with two classes from each school involved in testing the 24-h checklist questionnaire developed for the project. A total of 234 children out of a possible 379 (62%) consented to take part in the study. The study was carried out over the course of one day, the checklist for foods eaten at home was completed by the parent, the children completed forms for foods eaten at break-time and where possible a dinner helper completed the checklist for foods eaten at lunch. The comparison method was a 24-h semi-weighed food diary which was completed by the study team for foods eaten at school and by the parent for foods eaten at home. Each child also had their height and weight measured at school using a standardized technique. Growth data was converted into SD scores using the UK 1990 growth reference data, and international cut-off points were used to ascertain the number of overweight and obese children in the study (Cole *et al*. 2000).

	n	Mean age (years)	Fruit at school (%)	Fruit at home (%)	Body size (%)		
					Normal	Overweight	Obese
Girls	129	5.5	61	50	86	13	1
Boys	105	5.2	50	45	68	25	7

The checklist indicated that rather more girls than boys reported eating fruit at school or at home on the day of recording. The mean number of items of fruit eaten over the day for both girls and boys was 1.5. We found that 26% of boys and 29% of girls only had fruit at school but not at home; 20% of boys and 17% of girls had fruit at home but not at school; and 29% of boys and 22% of girls did not have fruit at all. More boys than girls were overweight or obese in the sample. Of the overweight or obese girls, 93% reported eating some fruit compared with 78% of the normal-weight girls; however, of the overweight or obese boys, only 67% reported eating some fruit compared with 76% of normal-weight boys. These results will be compared to the semi-weighed food diaries to explore the validity of the checklist technique.

Overall, a low fruit intake was reported, although most children reported eating some fruit. Women tend to eat more fruit than men do and it is interesting that this gender difference is appearing at such a young age. Children's fruit intake is related in complex ways to a number of psychosocial and environmental factors (Gibson *et al*. 1998). It may be important to explore different approaches for boys and girls in the promotion of fruit.

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Cole TJ, Bellizzi MB, Flegal KM & Dietz WH (2000) *British Medical Journal* **320**, 1240–1243.
Gibson EL, Wardle J & Watts CJ (1998) *Appetite* **31**, 205–228.

The impact of social class on a healthy diet: analysis from the UK Women's Cohort Study. By Y. BRAVO VERGEL, D.C. GREENWOOD and J.E. CADE, *Nutrition Epidemiology Group, Nuffield Institute for Health, University of Leeds, 71–75 Clarendon Road, Leeds LS2 9PL*

The UK Women's Cohort Study (UKWCS), a large national cohort investigating food consumption patterns and their relationship with the occurrence of cancers. The aim of this paper is to estimate the impact of social class (using the National Statistics Socio-Economic Classification, NS-SEC, based on the Goldthorpe Class Schema) on having a healthy diet.

The NS-SEC is better theoretically founded than the previous two socio-economic classifications used in Great Britain, given that it follows a well-defined sociological argument that employment relations and work conditions are central to delineating the structure of socio-economic positions in modern societies (Goldthorpe, 1997; Erikson & Goldthorpe, 1992). The level of education and training is also part of the definition of each position.

The Healthy Diet Indicator (HDI) was calculated using the dietary guidelines for the prevention of chronic diseases, defined by the WHO Study Group (1990). A multiple regression model with multi-category dependent variables was designed for the analysis of the HDI (multinomial logit model). To validate the results, we estimated a second multiple regression model using as the dependent variable an index of diversity of fruit and vegetable intakes. The linear regression analysis was carried out using the statistical package SPSS v.10; the logit estimates were computed using Stata v.6. The number of observations is 5691 for married women and 1804 for non married women.

The estimated odds of having an *average* healthy diet score rather than below average for single women belonging to the managerial and professional class is 1.56 times greater than those for the rest (intermediate and manual classes), after controlling for other variables included in our model (number of children, being vegetarian or vegan, taking dietary supplements, BMI, age and alcohol intake). The odds of having an *above* average healthy diet score rather than below average for single women belonging to the managerial class was similar at 1.85 times those for non-managerial women. Results for married women were not substantially different from those of the single women.

	Non Married Women		Married Women	
Social class + control variables	Average/ Below Average	Above average/ Below average	Average/ Below Average	Above average/ Below average
Non Married-Managerial class	1.33 (.18)	1.45 (.17)*		
Both partners managerial class			1.56 (.17)*	1.85 (.16)*
Wife managerial, husband lower class			1.41 (.22)	1.18 (.21)
Wife intermediate, husband managerial			1.47 (.16)*	1.60 (.15)*
Both partners intermediate			1.57 (.33)	1.05 (.32)
Wife intermediate, husband manual			1.43 (.19)	1.19 (.18)
Wife manual, husband higher class			1.12 (.21)	1.21 (.19)
Age	1.01 (.00)	1.01 (.01)	1.0 (.01)	1.0 (.005)
Taking dietary supplements	1.05 (.17)	1.48 (.16)	1.11 (.10)	1.53 (.09)*
Self-defined as vegetarian or vegan	1.23 (.26)	3.94 (.24)*	1.69 (.22)*	6.29 (.20)*
Consumption of alcohol	1.19 (.12)	1.37 (.11)*	1.31 (.07)*	1.33 (.07)*
Smoking	0.84 (.17)	0.78 (.16)	0.97 (.10)	0.94 (.09)
Overweight	0.73 (.20)	0.80 (.18)	1.09 (.11)	0.91 (.11)
Obesity	0.63 (.23)	0.50 (.22)*	1.15 (.16)	0.84 (.28)

*Statistically significant at the 0.05 level

From the results we can infer that women's social class has a strong effect on having a healthy diet. After being vegetarian or vegan, the strongest predictor was belonging to the managerial and professional class rather than being a manual worker. Social class is a robust indicator, significant and relevant independently of the marital status of women. It will be important to assess the impact of social class as related to diet on future health outcomes.

Erikson R & Goldthorpe JH (1992) *The Constant Flux*. Oxford: Clarendon Press.
Goldthorpe JH (1997) In *Constructing Classes: Towards a New Social Classification for the UK* [D Rose and K O'Reilly, editors]. Swindon: ESRC/ONS.
WHO Study Group (1990) Diet, Nutrition and the Prevention of Chronic Diseases. Geneva: WHO.

Underlying structure to dietary information from food frequency questionnaires: example from the UK Women's Cohort Study. By D.C. GREENWOOD[1,2], V.J. BURLEY[2] and J.E. CADE[2], [1]*Biostatistics Unit and* [2]*Nutrition Epidemiology Group, Nuffield Institute for Health, University of Leeds, 71–75 Clarendon Road, Leeds LS2 9PL*

Nutritional research often focuses on nutrient intake, making comparison with some recommended level for optimal nutrition or requirement to avoid malnourishment. From a public health perspective, individuals view what they eat as a collection of food products, not nutrients. By identifying patterns in food consumption, these two perspectives need not be contradictory, and results from epidemiological studies can be more easily applied (Hu *et al*. 2000; Osler *et al.* 2001; Schulze *et al*. 2001).

We take as our example the UK Women's Cohort Study. A cohort of 35 000 women have recorded dietary information using a 217-item food frequency questionnaire. The sampling methods used ensured a diverse range of intakes by including a high proportion of vegetarians and fish-eaters. These include many diets of particular interest to research into prevention of cancer and heart disease. To identify underlying patterns we used factor analysis with varimax rotation. Eight separate factors were identified on which an individual's diet could be scored. The underlying factors were:

Factor 1. A diversity of healthy foods, vegetables, nuts and pulses.
Factor 2. Meats.
Factor 3. Fish and seafood.
Factor 4. Foods rich in fat and sugar.
Factor 5. Diet, low calorie and low fat products.
Factor 6. Fruit.
Factor 7. Beans and vegetables.
Factor 8. Natural, pure and wholemeal foods, including nuts and grains.

Women with low intakes of foods contributing to each factor and therefore scoring in the lowest quartile (L) for each factor were compared to those in the highest quartile (H). Due to the large number of observations, upper and lower quartiles were significantly different from each other ($P<0.001$) for most factors and subject characteristics.

	Factor 1		Factor 2		Factor 3		Factor 4		Factor 5		Factor 6		Factor 7		Factor 8	
	Healthy		Meat		Fish		Rich		Diet		Fruit		Vegetables		Natural	
	L	H	L	H	L	H	L	H	L	**H**	L	H	L	H	L	H
Mean age	57	49	49	53	56	49	53	52	55	50	51	53	50	55	49	54
Mean BMI	25	24	23	25	24	25	24	24	24	25	25	24	24	25	25	24
% Vegetarian	17	39	90	0	22	36	26	32	29	25	28	28	25	32	33	33
% Supplement	54	61	64	51	61	54	60	55	54	61	56	59	57	60	51	68
% Smoking	10	12	9	13	7	16	13	9	13	9	16	8	12	10	14	8
% Energy fat	31	34	32	35	32	34	30	35	35	30	33	31	33	32	33	32

In addition, vitamin C intake was higher for those scoring highly on factors 1, 5, 6, 7 and 8 ($P<0.001$). Likewise fruit and vegetable consumption was also higher for women scoring highly on these factors.

These factors represent a range of food products crucial to characterizing differences between alternative approaches to diet. Scoring individuals on each factor could help discriminate between, for example, vegetarians eating a healthy range of products, and more lax vegetarians eating an otherwise "normal" diet. Alternatively, fish-eaters whose diets are much like meat-eaters could be separated from those who are more like vegetarians.

Investigating relationships between factor scores and subsequent illness and mortality could provide useful public health information that allows for the complex combination of food products making up people's diet. Knock-on effects of recommending certain dietary regimes could also be examined more easily using this approach.

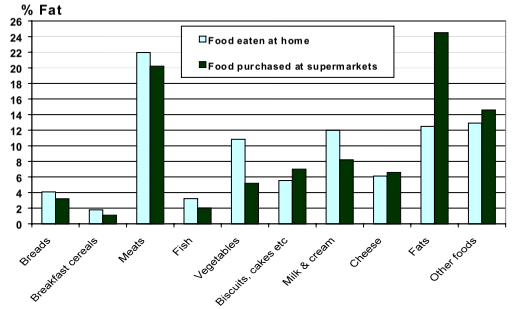
The UK Women's Cohort Study is funded by the World Cancer Research Fund.

Hu FB, Rimm EB, Stampfer MJ, Ascherio A, Spiegelman D & Willett WC (2000) *American Journal of Clinical Nutrition* **72**, 912–921.
Osler M, Heitmann BL, Gerdes LU, Jorgensen LM & Schroll M (2001) *British Journal of Nutrition* **85**, 219–225.
Schulze MB, Hoffmann K, Kroke A & Boeing H (2001) *British Journal of Nutrition* **85**, 363–373.

What are the main sources of fat in supermarket shopping? An analysis of the contribution food groups make to fat purchased at supermarkets and foods eaten at home. By J.K. RANSLEY[1], J.K. DONNELLY[1], H. BOTHAM[1], T.N. KHARA[1], H. ARNOT[1], J.E. CADE [2] and D.C. GREENWOOD[2], [1]*The Public Health Nutrition Unit, Trinity and All Saints, University of Leeds, Brownberrie Lane, Horsforth, Leeds LS18 5HD and* [2]*Nutrition Epidemiology Group, Nuffield Institute for Health, The University of Leeds, 71–75 Clarendon Road, Leeds LS2 9PL*

90% of the UK population purchase most of their domestic food from supermarkets (Caraher *et al.* 1998; Competition Commission, 2000). The UK diet continues to exceed recommended levels of fat (Ministry of Agriculture Fisheries and Food, 1999). To achieve a reduction in the fat content of the diet, knowledge of the amount of fat purchased from supermarkets and an analysis of the food groups from which it is derived is required..

For this study, 214 households were recruited from a random sample of Tesco Clubcard members in Leeds. Itemized receipts from all supermarket purchases were collected for a period of 28 d to estimate household food purchased from supermarkets and other retail outlets. Each resident of the participating households completed a 4 d weighed food record during this period.



Major sources of fat in food consumed at home compared with fat purchased at supermarkets

An average of 5.17 kg fat was purchased, per household, per month from supermarkets (mean household size was 2.4 people). Of this, 1.05 kg (20.2%) of fat was derived from meat and meat products and 1.27 kg (24.5%) was derived from fats and oils. A further 768 g (14.80%) of the fat was derived from milk, cream and cheese and 362 g (7.0%) from biscuits, cakes and puddings. 267 g (5.2%) was derived from the food group 'vegetables' which included potato products such as chips. 754 g (14.6%) of the fat in supermarket purchases was derived from 'other foods' including salad dressings and sauces. The sources of fat in food eaten at home follow a similar pattern to food purchased from supermarkets with the notable exception of 'fats and oils'. The contribution of fat to this category is recorded as 13% in foods eaten at home compared to 24.5% purchased from supermarkets, indicating possible under-reporting of the consumption of foods in this category.

This analysis has identified the key sources of fat in food purchased from supermarkets as meat and meat products, dairy products and fats and oils. This may help to provide a more specific focus for dietary interventions designed to reduce the fat content of the household diet.

The research was funded by the Department of Health and the MRC Nutrition Programme. Tesco Stores Ltd. have provided additional support. The views expressed are the authors' own.

Caraher M, Dixon P, Lang T & Carr-Hill R (1998) *Health Education Journal* **57**, 191–201.
Competition Commission (2000) *Supermarkets: A Report on the Supply of Groceries from Multiple Stores in the United Kingdom,* Vol 1. London: The Stationery Office.
Ministry of Agriculture Fisheries and Food (1999) *National Food Survey, 1998. Annual Report on Household Food Consumption and Expenditure*. London: The Stationery Office.

Differences in food intake of children within separate weight categories. By P. SAHOTA[1], J. CADE[2] and M.C.J. RUDOLF[3], [1]*Leeds Metropolitan University, Calverley Street, Leeds LS1 3HE,* [2]*Nuffield Institute for Health, Leeds University, 71–75 Clarendon Road, Leeds LS2 9PL and Leeds Community and Health Services Trust, 3–5 Belmont Grove, Leeds LS2 9NP*

The prevalence of childhood obesity is increasing (Rudolf *et al*. 2001) and has been designated a public health issue (WHO, 2000). The onset is often in childhood and, due to the possible tracking of obesity, including the tracking of associated risk factors from childhood to adulthood, preventative strategies need to be targeted at children. However, little information on effective obesity preventative strategies exists. The design of effective strategies requires knowledge of the specific eating habits of children, in particular whether any difference exists in the intake of children within separate weight categories.

Dietary data were collected by 24-h recall from 601 children (270 girls and 331 boys) aged 7–8 years in ten primary schools representative of the socio-demographic diversity of Leeds, northern England. The dietary data were categorized into five groups: foods high in fat, foods high in sugars, drinks high in sugars, fruit, and vegetables, and presented as mean number of portions consumed per day for each of the categories.

Height and weight were measured and BMI and SD scores calculated using the UK growth reference (Cole *et al*. 1995). International cut-off points as defined by Cole *et al*. (2000) were used to separate the children into normal, overweight and obese weight categories. The data were analysed using ANOVA to determine any relationship and are presented in the Table.

A comparison of children's intake of food from each of the food groups with their weight category. Data are presented as mean (95% CI) of number of portions eaten per day (*P< 0.05).

Food group		Normal		Overweight		Obese			
		Girl n=223	Boy n=283	Girl n=35	Boy n=36	Girl n=12	Boy n=12	Sig. (ANOVA) Girls	Boys
Foods high in fat	Mean	2.0	2.0	1.6	1.7	2.2	1.8	0.008*	0.06
	95% CI	(1.9, 2.1)	(1.9, 2.1)	(1.4, 1.8)	(1.5, 1.9)	(1.6, 2.9)	(1.4, 2.2)		
Foods high in sugar	Mean	4.6	5.5	3.3	3.6	4.2	3.6	0.02*	0.002*
	95% CI	(4.3, 5.0)	(5.1, 5.9)	(2.8, 3.8)	(2.8, 4.3)	(2.0, 6.4)	(1.5, 5.8)		
Drinks high in sugar	Mean	1.6	2.0	1.7	1.7	1.9	1.6	0.8	0.23
	95% CI	(1.5, 1.8)	(1.8, 2.1)	(1.3, 2.2)	(1.2, 2.1)	(1.1, 2.7)	(0.8, 2.3)		
Fruit intake	Mean	2.3	1.9	2.2	1.6	2.7	2.1	0.6	0.30
	95% CI	(2.1, 2.5)	(1.8, 2.1)	(1.8, 2.7)	(1.2, 2.0)	(1.8, 3.7)	(1.2, 3.1)		
Vegetable intake	Mean	0.60	0.6	0.50	0.4	0.7	0.1	0.3	0.47
	95% CI	(0.5, 0.7)	(0.6, 0.7)	(0.4, 0.6)	(0.4, 0.7)	(0.3, 1.1)	(0.1, 0.8)		

Both overweight girls and boys and obese boys consumed fewer foods high in fat compared with the normal-weight children; however, the difference was significant for girls only. Both overweight and obese boys and girls consumed significantly fewer foods high in sugar compared with normal-weight children. These differences may be attributed to under-reporting of these foods due to the awareness amongst children that these foods are linked to the development of obesity, although the differences could also be due to a genuine reduced consumption in an attempt to lose weight.

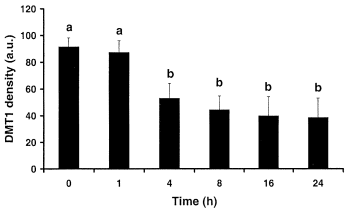
There were no differences in the consumption of drinks high in sugar. All children consumed fewer than the recommended five portions/d of fruit and vegetables. This is a concern for all children, as healthy eating habits are established during this period, but particularly for overweight and obese children who may be compensating their intake with other high energy density foods.

The results indicate that despite overweight and obese children reporting consuming fewer foods high in fat and sugar, interventions aimed at increasing fruit and vegetables are needed Due to the refractory nature of childhood obesity evidenced by the increasing prevalence of adult obesity, all children despite their present weight category should be targeted with healthy eating messages.

Cole TJ, Freeman JV & Preece MA (1995) *Archives of Disease in Childhood* **73**, 25–29.
Cole TJ, Bellizzi MB, Flegal KM & Dietz WH (2000) *BMJ* **320**, 1240–1243.
Rudolf MCJ, Sahota P, Barth JH & Walker J (2001) *BMJ* **322**, 1004–1005.
WHO (2000) *Obesity, Preventing and Managing the Global Epidemic.* Geneva: WHO.

Regulation of divalent metal transporter expression by non-haem iron in intestinal epithelial cells. By D.M. JOHNSON, K.J. BOXALL, J.P TENNANT and P.A. SHARP, *Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH*

Absorption of dietary non-haem iron by intestinal enterocytes is mediated by the divalent metal transporter (DMT1). Recent work from our laboratory has shown that the function and expression of DMT1 is decreased by increasing levels of iron (Sharp *et al*. 2002; Yamaji *et al*. 2002). The aim of the current work was to determine the time course and mechanism of these non-haem iron mediated events. Studies were carried out on Caco-2 cells 21 d post-seeding, at which time they were exposed to 100 μM $FeCl_3$ for up to 24 h. At the end of the experimental period, whole cell and plasma membrane protein was isolated and utilized for Western blotting and total RNA was extracted and subjected to RT-PCR for the two DMT1 splice variants (with and without a 3′ iron-responsive element (IRE) respectively). Band densities were semi-quantified using Scion Image software. Data are presented as mean ± SEM of 3–4 Western blotting experiments or six RT-PCR experiments and were analysed using either one-way ANOVA followed by Scheffé's *post hoc* test or Student's unpaired *t*-test where appropriate.



DMT1 protein is decreased by non-haem iron. Caco-2 cells were cultured for up to 24 h in the presence of 100 μM $FeCl_3$ and plasma membrane protein isolated and subjected to Western blotting. Band densities were analysed by Scion Image software and are presented as mean ± SEM. Different letters above the data bars denote a significant difference between these groups (P<0.05, one-way ANOVA and Scheffé's *post hoc* test).

Exposure of Caco-2 cells to elevated non-haem iron decreased DMT1 protein expression in the plasma membrane (see Figure). DMT1 was significantly reduced by 4 h (0 h, 91.4 (SE 6.7) a.u.; 4 h, 52.9 (SE 11.1) a.u.; P=0.025) and this decrease was maintained for the rest of the experimental period. Interestingly, whole cell levels of DMT1 were unaltered by iron exposure. Previous work demonstrated that iron-dependent regulation of DMT1 over 24 h occurred as a consequence of a decrease in the IRE-containing DMT1 splice variant (Yamaji *et al*. 2002). However, after 4 h exposure to iron, neither DMT1 isoform was regulated at the mRNA level. Taken together, these data suggest that the initial cellular response to elevated iron involves a decrease in apical membrane expression of DMT1, perhaps due to trafficking of the transporter away from the membrane. This possibility is currently under investigation in our laboratory.

Sharp PA, Tandy SR, Yamaji S, Tennant JP, Williams MR & Srai SKS (2002) *FEBS Letters* **510**, 71–76.
Yamaji S, Tennant J, Sharp P & Srai SKS (2002) *Journal of Physiology* **539**, 20.

Mercury in fish is an important source of environmental exposure in Brazil. By J.A. MORETON[1] and C.J. DORÉ[2], [1]*Nutrition Epidemiology Group, Nuffield Institute for Health, University of Leeds, 71–75 Clarendon Road, Leeds LS2 9PL and* [2]*Medical Research Council Clinical Trials Unit, 222 Euston Road, London NW1 2DA*

A series of studies have investigated environmental exposure to mercury in Brazil as a result of gold mining activities since the mid-1980s. This EU-sponsored study in four locations in the Tapajos valley in the Amazon Basin in 1990 (Cleary *et al.* 1994) provides the strongest evidence to date that organic mercury in fish poses more of a health problem than inorganic mercury or mercury vapour. Mercury accumulates in fish as organic mercury and is concentrated as it moves up the food chain, giving higher levels in larger fish and carnivorous fish. All but one of the 106 subjects in the study had mercury levels in either blood or urine that exceeded the upper 95% limits for non-exposed subjects (UK HSE, 1991), which are 3 μg l^{-1} Hg for blood and 2 nmol Hg/mmol creatinine (~4 μg l^{-1} Hg) for urine. The non-occupationally exposed subjects, living in Jacareacanga (JA), a fishing village on the river Tapajos 100 km from the nearest mining camp, had significantly higher mean blood Hg levels (P<0.004) than the occupants of two mining camps, Crepuri (CR) and Cuiú-Cuiú (CU), and a gold mining town, Itaituba (IT). This was assumed to be due to consumption of organic Hg in fish. There were marginally significant differences between the mean urine Hg levels for the four groups (P=0.04).

Location	Blood Hg (μg l^{-1})			Urine Hg (μg l^{-1})			Geometric mean Blood : Urine ratio
	Geometric mean	95% CI	n	Geometric mean	95% CI	n	
Jacareacanga	59	41–86	24	10	6–17	25	5.98
Crepuri	18	13–25	27	24	15–39	28	0.73
Cuiú-Cuiú	10	7–14	25	12	7–20	24	0.81
Itaituba	25	17–35	27	21	13–34	28	1.16

A multiple regression analysis was performed to compare the relationship between $\log_e$ blood Hg levels and $\log_e$ urine Hg levels in the four locations, since elimination of inorganic Hg occurs in faeces and urine, while organic Hg is mainly (90%) eliminated in faeces. The slopes of the four regression lines were not significantly different (P=0.16). A new model with parallel regression lines for all locations gave an estimated slope of 0.33 (SE=0.06, P<0.001) with highly significant differences between the intercepts for the four locations (P<0.001). The intercept for location JA was significantly higher (P<0.001) than that for CR but CU and IT were not significantly different from CR. For a given urine level, an individual in JA has a higher blood Hg level than an individual in CR. A further confirmation of the different relationship between blood and urine level at JA was found by comparing the geometric mean blood : urine ratios in the four locations, where JA was significantly higher (P<0.001) than the three other locations.

The geometric mean Hg concentration for the fish analysed in the EC study was 0.18 mg kg^{-1}, 95% confidence interval 0.12–0.26 mg kg^{-1}, n=52 (wet weight). Twenty-one (40%) of the fish samples exceeded 0.3 mg kg^{-1} Hg, the EC Environmental Quality Standard for "a basket of fish". Most of the fish analysed were relatively small. Larger fish would be likely to contain higher mercury levels.

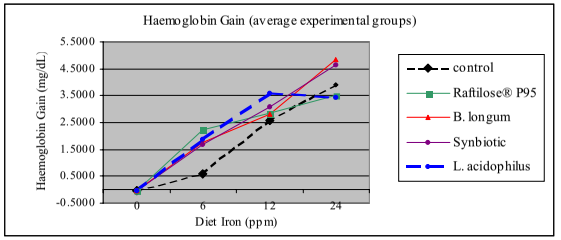
Previously the main concern has been the effects of occupational mercury exposure in gold miners, ancillary workers and traders, but this study demonstrates that gold mining affects the wider population both in mining areas and further afield.

Mercury analysis for the EU study was carried out by J.A. Moreton at the SAS Trace Element Unit, Level D, South Block, Southampton General Hospital, Southampton SO16 6YD, UK.

Cleary D, Thornton I, Brown N, Kazantzis G, Delves H & Worthington S (1994) *Nature* **369**, 613–614.
UK HSE (Health and Safety Executive) (1991) *Occupational Medicine and Hygiene Laboratory. Guidance on Laboratory Techniques in Occupational Medicine.* 5th ed. London: HMSO.

Effect of fructooligosaccharides, *Bifidobacterium longum* and *Lactobacillus acidophilus* on iron deficiency anaemia recovery in rats. By L.M. YBARRA[1], M.R. SILVA[1], G. DIAS[2], N.M.B. COSTA[2] and C.L.L.F. FERREIRA[1], [1]*Departamento de Tecnologia de Alimentos and* [2]*Departamento de Nutrição e Saúde, Universidade Federal de Viçosa, Viçosa (MG), CEP 36571-000, Brazil*

The increase of food fortification with minerals leads us to discuss alternative ways to increase their absorption without increasing ingestion. The use of prebiotic substances and/or probiotic microorganisms such as *Bifidobacterium longum* and *Lactobacillus acidophilus* could be an alternative choice, together with other benefits also conferred by probiotics. Increases in calcium and magnesium bioavailability have been reported when fructooligosaccharides (FOS) were used (Ohta *et al*. 1995; Morohashi *et al*. 1998). The objective of this study was to verify the effect of FOS (Raftilose® P95), *Bifidobacterium longum* ATCC 15707 and *Lactobacillus acidophilus* NCFM on iron absorption in anaemic rats, by determining the levels of haemoglobin and haematocrit (packed cell volume), and haemoglobin gain. A group of 128 weaned male Wistar rats were fed a depletion diet (iron-free) for 21 d (AOAC, 1984). The anaemic animals were divided into sixteen experimental groups (n=8), in a 5×3+1 factorial design. The five treatments included Control, Raftilose® P95, *B. longum, L. acidophilus* and Raftilose® P95 + *B. longum* (synbiotic), each one with three iron concentrations (6, 12 and 24 mg, as ferrous sulphate/kg diet), plus one group on an iron-free diet. Test diets were administrated for a repletion period of 14 d. Raftilose® P95 was incorporated into the diet (5%), while the probiotic microorganisms were given daily as a cellular concentrate (10^9 CFU of *B. longum*/ml and 10^{10} CFU of *L. acidophilus*/ml). Blood samples were collected by tail bleeding, at the end of both depletion and repletion periods. The increase in packed cell volume and haemoglobin levels, and also haemoglobin gain were dose-dependent. There was no statistical difference between treatments. Nevertheless, there was a positive trend observed with respect to the anaemia recovery in groups receiving pre-, pro- or symbiotics as compared to control groups. This trend towards an improved recovery from anaemia suggests a novel role of pre- and/or probiotics in mineral absorption. This indicates the necessity for further studies with these microorganisms and substances on mineral absorption for a larger period of intervention and in human subjects.



L. M. Ybarra is supported by a scholarship from Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior (CAPES)/ Brazilian Government.

AOAC (1984) In *Official Methods of Analysis of the Association of Official Analytical Chemists*, pp. 880–881. Washington, DC: AOAC.
Morohashi T, Sano T, Ohta A & Yamada S (1998) *Journal of Nutrition* **128**, 1815–1818.
Ohta A, Ohtuki M, Baba S, Adachi T, Sakata T & Sakaguchi E (1995) *Journal of Nutrition* **125**, 2417–2424.

Dietary phylloquinone (vitamin K_1) intake: comparison between adolescents living in Cambridge and a national British sample. By C.W. THANE, C.J. PRYNNE, F. GINTY, C. BOLTON-SMITH, S.J. STEAR, S.C. JONES and A. PRENTICE, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL*

A low dietary intake of phylloquinone (vitamin K_1) during adolescence may influence bone mineral accretion and attainment of peak bone mass, due to undercarboxylation of the vitamin K-dependent protein osteocalcin and impaired calcium binding (Shearer, 2000). In the present study, vitamin K_1 intake in adolescent boys and girls from Cambridge (Prynne *et al*. 2001) was compared with a nationally representative sample of adolescents, to further ongoing investigations into the relative importance of vitamin K_1 intake for bone health in this age group.

Dietary vitamin K_1 intake, and the relative contributions of different food groups, were compared between 16–18-year-old adolescents, assessed between 1995 and 1998 as part of the Cambridge Bone Studies (CABS), and adolescents aged 15–18 years who participated in the 1997 National Diet and Nutrition Survey (NDNS) of young people aged 4–18 years (Gregory *et al.* 2000). Prospective 7 d dietary records using standard household measures with food photographs were analysed for 212 participants in the former study, while 7 d weighed dietary records were analysed for 383 participants involved in the latter. Vitamin K_1 intake was estimated for each individual using content values for a comprehensive range of food and drinks (Bolton-Smith *et al*. 2000, plus unpublished data).

Vitamin K_1 intakes were positively skewed in both studies, with overall geometric means (95% CI) of 71 (67, 76) and 45 (43, 47) µg/d in the CABS and NDNS, respectively ($P<0.001$). It was found that 37% of CABS adolescents reported vitamin K_1 intakes below 1 µg/kg body weight/d (boys, 39%; girls, 36%) compared with 73% (boys, 74%; girls, 73%) in those from the NDNS ($P<0.001$). On the whole, CABS boys and girls had significantly higher vitamin K_1 intakes than their NDNS counterparts, with no significant variation in intake observed by region or season in the NDNS. Vitamin K_1 intake did not differ significantly by sex in either population, nor were the differences observed between the two studies influenced significantly by differences in likely under-reporting (energy intake:estimated BMR <1.2).

	Boys		Girls	
Vitamin K_1 intake and selected sources	CABS (*n* 111)	NDNS (*n* 178)	CABS (*n* 101)	NDNS (*n* 205)
Intake (µg/d)†	74*** (68, 81)	47 (44, 50)	68*** (62, 74)	43 (40, 46)
Intake (µg/MJ)†	6.5*** (6.1, 7.0)	5.0 (4.7, 5.3)	7.7** (7.0, 8.4)	6.5 (6.1, 6.9)
Intake (µg/kg body weight/d)†	1.11*** (1.02, 1.20)	0.70 (0.65, 0.76)	1.21*** (1.11, 1.32)	0.73 (0.67, 0.78)
Contribution from vegetables (%)‡¶	49 (46, 52)	54 (51, 56)	50*** (46, 53)	60 (57, 62)
of which: leafy green vegetables (%)‡¶	*24** (20, 27)*	*18 (15, 21)*	*27 (23, 31)*	*26 (23, 30)*
of which: potatoes and products (%)‡¶	*12*** (10, 14)*	*20 (18, 22)*	*9*** (7, 10)*	*16 (14, 18)*

†Geometric means (95% CI) obtained by back-transformation of $\log_e$-transformed data, ‡arithmetic means (95% CI). Significant differences between studies for boys and girls respectively: **$P<0.01$, ***$P<0.001$ (2-tailed *t*-test, ¶Mann-Whitney *U* test).

Vegetables contributed most to vitamin K_1 intake in both samples (54% overall). Compared with the NDNS adolescents, CABS boys derived a higher percentage of vitamin K_1 intake from leafy green vegetables (e.g. cabbage, broccoli, lettuce and spinach), whereas girls had similar values. In addition, CABS adolescents derived a lower percentage of vitamin K_1 intake from potatoes and potato products (mainly chips and crisps) and a higher percentage from milk and eggs, fruit and nuts, and miscellaneous foods (mainly sauces and soups).

Further investigations will determine the impact of vitamin K_1 intake and associated dietary patterns on bone mineral status and metabolism in the CABS boys and girls.

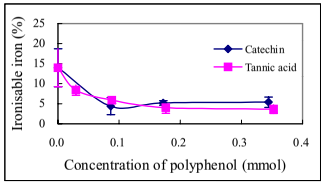
The Cambridge Bone Studies represent an addition to work supported by awards from the Department of Health/Medical Research Council Nutrition Research Initiative (boys study) and the Mead Johnson Research Fund (girls study). The views expressed in this publication are those of the authors and not necessarily those of the sponsors.

Bolton-Smith C, Price RJG, Fenton ST, Harrington DJ & Shearer MJ (2000) *British Journal of Nutrition* **83,** 389–399.
Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LV, Smithers G, Wenlock R & Farron M (2000) *National Diet and Nutrition Survey: Young People Aged 4 to 18 Years, Vol. 1: Report of the Diet and Nutrition Survey*. London: The Stationery Office.
Prynne CJ, Paul AA, Greenberg DC, Bolton-Smith C, Stear SJ, Jones SC & Prentice A (2001) *Proceedings of the Nutrition Society* **60,** 208A.
Shearer MJ (2000) *Current Opinion in Clinical Nutrition and Metabolic Care* **3,** 433–438.

The effect of polyphenols on iron availability: a comparison of *in vitro* methods. By Y. SHISHIKURA, P. KAJDA and S. KHOKHAR, *Procter Department of Food Science, University of Leeds, Leeds LS2 9JT*

Dietary iron availability depends on the chemical form and its interaction with the compositions of the diet. Rapid, relatively simple and inexpensive *in vitro* methods have been developed for the estimation of trace elements such as iron and calcium that is available for absorption. Three commonly used *in vitro* methods for estimating iron availability: the ionizable method, the soluble method and the diffusible method, were compared. Although all these *in vitro* methods involved a simulated digestion stage, the estimated iron availability varied depending on the method used.

The diffusible method showed similar estimated iron availability (5.05% (SD 3.55)) to *in vivo* whilist the iron availability measured by the ionizable method was slightly higher (11.72% (SD 6.03)), in the absence of polyphenols. Using the soluble method, the iron availability with or without adding polyphenols was considerably higher than that obtained by the other two methods. Earlier studies have indicated that diffusible method can be useful predictor of iron absorption (Hurrell *et al*. 1988). However, in the present study, the ionizable assay showed similar effect of polyphenols when compared with the effect in humans. This variation could be due to the fact that polyphenols bind iron in soluble complexes, not absorbable but readily diffusible through the dialysis membrane thus resulting in it being measured as dialyzable iron (Luten *et al*.1996).



Brune *et al.* (1989) reported that there was an effect of tannic acid but not of catechin in humans. However both tannic acid and catechin inhibited iron absorption strongly even at very low concentrations (0.1 mmol) [Figure1]. There was no difference in degree of inhibition between tannic acid and catechin at the concentration studied here. Khokhar and Apenten (2002) have suggested that the functional groups important for iron-binding are *ortho*-dihydroxyl groups or a large number of OH groups (e.g. tannic acid). This may support the present study.

Brune M, Rossander L & Hallberg L (1989) *European Journal of Clinical Nutrition* **43**, 547–557.
Khokhar S & Apeten RKO (2000) *Food Chemistry* (In Press).
Hurrell R, Lynch SR, Trinidad T P, Dassenko S A & Cook JD (1988) *American Journal of Clinical Nutrition* **42**, 102–107.
Luten J, Crews H, Flynn A, Deal PV, Kastenmayer P, Hurrell R, Deelstra H, Shen L,Fairweather-Tait S, Hickson K, Farre R, Schlemmer U & Frohlich W (1996) *Journal of Food Science and Agriculture* **72**, 415–424.

Ownership and use of domestic kitchen appliances related to cooking methods and macronutrient intake. By A. EFSTATHIOU, *School of Education, Community and Social Science, Liverpool John Moores University, IM Marsh Campus, Liverpool L17 6BD*

The ownership of domestic appliances for food preparation has increased in the last few years. This is thought to reflect an increased affluence and a desire to try new foods without using traditional cooking methods. The higher socio-economic groups are more likely to buy small kitchen appliances (Mintel International Group, 2000). Kitchen appliances, such as electric steamers, provide a means to alter traditional cooking methods and achieve a more nutritious diet; however, other appliances, such as deep-fat fryers, may have a detrimental effect on nutritional intake, providing a diet that contains a higher fat content.

The aim of the study was to assess the extent of ownership and use of kitchen appliances and relate this to cooking methods and dietary intake.

A questionnaire concerning ownership and use of small kitchen domestic appliances and cooking methods was distributed to 150 women from three socio-economic groups (thirty-eight professional, sixty-five skilled and forty-seven unskilled). Dietary intake (fourteen women) was determined using 3 d dietary records.

The majority (46%) spent 1–2 h/d cooking. The only difference between socio-economic groups and the frequency of the various cooking methods used was for deep-fat frying (P=0.002); 24% of the unskilled, 3.8% of skilled and 2.9% of professionals deep-fat fried every other day. Ownership of microwave ovens was 89%, deep-fat fryers 27%, food processors 49%, steamers 17% and bread-makers 11%. Ownership of deep-fat fryers (P=0.03) and steamers (P=0.015) was significantly higher in the unskilled group but that of food processors lower (P=0.047). Of the women surveyed, 62.5% used the microwave every day and 71% used their steamer 3–4 times a week, other appliances were used once a week or less; 60% of the unskilled group used the deep-fat fryer at least three times a week, whereas the majority of women in the other groups used this appliance less than once a week.

There were no significant differences between ownership of any domestic appliance and the percentage of energy intake from fats, carbohydrates or proteins.

Ownership of domestic appliances does not appear to reflect usage, but may reflect possible differences in cooking skills. However, the usage of domestic appliances may account for some of the differences in dietary intake between socio-economic groups, as observed in other studies.

Mintel International Group (2000) Consumer reports at http://consumer.mintel.com/ (accessed 17/08/2001).

Isoflavones affect antioxidant status *in vitro* but not *in vivo*. By R.M.B. MCLAUGHLIN[1], J.V. WOODSIDE[1], J. MCENENY[1], M.J. CAMPBELL[2], A.J.C. LEATHEM[2] and I.S. YOUNG[1], [1]*School of Clinical Medicine, Queen's University Belfast, Belfast BT12 6BJ and* [2]*Royal Free and University College London School of Medicine, London W1W 7EJ*

Isoflavones are plant compounds which have been proposed to play a role in cardiovascular disease and endocrine-responsive cancer. Isoflavones have a wide variety of physiological effects, but may be able to act as antioxidants, which could explain part of their ability to affect these diseases. We have used both *in vitro* and *in vivo* methods to assess the effect of isoflavones on lipid peroxidation and antioxidant status.

The antioxidant activity of the isoflavone genistein was assessed *in vitro* by examining the effects on copper-promoted low-density lipoprotein (LDL) oxidation (McDowell *et al*. 1995). Incubation of LDL with the isoflavone genistein caused a dose-dependent increase in time to half maximum ($t_{½}$), which is indicative of the susceptibility of LDL to oxidation. The mean $t_{½}$ (SD) (n 12 experiments) was as follows for concentrations of genistein (0 µmol/l 96.5 (4.1) min; 0.5 µmol/l 105.4 (4.6) min; 1 µmol/l 110.7 (5.5) min; 5 µmol/l 164.6 (12.2) min; 10 µmol/l 214.9 (16.2) min; P<0.001 for differences between groups, one-way ANOVA).

The *in vivo* study assessed the effect of 1 month isoflavone supplementation (86 mg/d) on antioxidant status in healthy pre- (n=16) and postmenopausal (n=7) women in a randomized, placebo-controlled crossover study with a 2-month washout period. The supplements used were purified isoflavone pills containing 43 mg total isoflavones per pill in the aglycone state (25 mg biochanin, 8 mg formononetin, 4 mg genistein, 5 mg daidzein; Promensil, Novogen, Australia). Fasting blood samples were collected at baseline and 28 d. Concentrations of α-tocopherol (Craft *et al.* 1992) and malondialdehyde (MDA, Young & Trimble, 1991) were measured by HPLC, while lipid hydroperoxides were measured by the spectrophotometric FOX assay (Woolf, 1994). Concentrations of the antioxidant enzyme glutathione peroxidase (GPX) were assessed by automated enzyme assay (McMaster *et al*. 1990). The change from baseline was compared between groups by the Wilcoxon signed rank test. There were no significant differences between the supplementation groups in any variable assessed.

		Postmenopausal (n 7)		Premenopausal (n 16)	
		Baseline	28 d	Baseline	28 d
α-tocopherol (µmol/mmol)	Placebo	35.3 (23.6)	34.9 (22.5)	39.6 (14.5)	34.0 (10.3)
	Supplement	33.2 (16.3)	32.1 (17.4)	33.9 (16.5)	34.6 (10.4)
MDA (µmol/mmol)	Placebo	2.41 (1.13)	1.97 (0.92)	4.36 (2.26)	3.87 (1.14)
	Supplement	2.68 (0.96)	2.15 (0.86)	3.69 (1.69)	4.18 (1.73)
FOX (µmol/mmol)	Placebo	3.41 (2.16)	3.18 (1.72)	1.05 (1.41)	1.02 (1.50)
	Supplement	3.04 (1.19)	2.58 (1.29)	0.80 (1.00)	1.00 (1.11)
GPX (µmol/l)	Placebo	330 (40)	322 (42)	300 (44)	285 (34)
	Supplement	318 (39)	318 (39)	284 (27)	285 (30)

Results reported per mmol triacylglycerol for α-tocopherol, MDA and FOX. Change from baseline was compared between intervention groups by Wilcoxon signed rank test.

This study shows that, despite *in vitro* antioxidant activity, isoflavones have little or no effect on lipid peroxidation products or antioxidant status *in vivo*. Subjects in this study had an initially good antioxidant status, and it may only be in those who are antioxidant-deplete that an antioxidant effect of isoflavones is observed.

Craft NE, Wise SA & Soares JH (1992) *Journal of Chromatography* **589**, 171–176.
McDowell IFW, McEneny J & Trimble ER (1995) *Annals of Clinical Biochemistry* **32**, 7–174.
McMaster D, Bell N, Anderson P & Love AGH (1990) *Clinical Chemistry* **36**, 211–216.
Wolff SP (1994) *Methods in Enzymology* **233**, 183–189.
Young IS & Trimble ER (1991) *Annals of Clinical Biochemistry* **28**, 504–508.

A comparison of the effect of soy isoflavonoids and fish oil on cell proliferation, apoptosis and expression of oestrogen receptor α and β in the mammary gland and colon of the rat. By F. KRAMER[1], E.K. LUND[1], V. BREINHOLT[1] and I.T. JOHNSON[2], [1]*Fødevaredirektorat, IFTB, Mørkhøj Bygade 19, 2860 Søborg, Denmark and* [2]*Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA*

Epidemiological studies indicate that both soy and fish oil protect against various cancers including breast and colorectal cancer. The active compounds in soy are suggested to be isoflavonoids; plant compounds with hormonal activity. Protection against breast cancer by soy may be due to its oestrogen-like activity. Expression of oestrogen receptor α and β has been found to be altered in breast and colorectal cancer as compared to healthy tissue. Oestrogen receptor β, to which isoflavonoids have a higher binding affinity than to oestrogen receptor α, is downregulated in breast and colorectal cancer (Campbell-Thompson *et al*. 2001). Additionally, isoflavonoids have been found to have a p53-dependent pro-apoptotic effect *in vitro* at concentrations above 25 μM/l (Leung & Wang, 2000) but their main activity is to block cancer cell lines in G2/M. The induction of apoptosis and reduction of mitosis are considered to be important in the cancer-protective activity of fish oil (Latham *et al*. 2001). The aim of the present study was to investigate the effect of soy isoflavonoids and fish oil on mitosis and apoptosis, and the expression of oestrogen receptor α and β in mammary gland and colon.

	Mitosis (cells/crypt)	Apoptosis (cells/crypt)
Control	9.1 (0.5)	0.7 (0.2)
Soy	6.0 (0.6)	0.7 (0.2)
Fish oil	5.8 (0.5)	1.18 (0.3)
Soy + fish oil	5.9 (0.5)	1.09 (0.2)

Mitosis and apoptosis in the female rat colon in response to dietary supplementation with fish oil or soy extract.

Female Sprague Dawley rats were exposed to a diet containing either 0.5% soy extract, Soylife *(genistin-2.5 mg/d, daidzin-5.2 mg/d, glycetin-4.6 mg/day of which 5-7% aglycone)*, or 8% fish oil (Menhaden Oil- enriched in eicosapentaenoic acid-Sigma UK), for a period of 2 weeks. Apoptosis and mitosis were assessed by morphological criteria in colonic crypt cells and this technique was then applied to terminal end buds, the most proliferative structures in the mammary gland. Expression of the oestrogen receptors was determined by RT-PCR, using Taqman. The present study revealed that soy isoflavonoids significantly increased mitosis in the mammary gland as compared to control animals, whereas mitosis was suppressed in colonic crypt cells. However, no effect of the soy extract on apoptosis could be detected. On the other hand, exposure to fish oil resulted in significantly decreased mitosis and increased apoptosis in both colon and mammary gland as compared to control animals. Associated with these changes it was observed that the soy extract caused an upregulation of α-receptor mRNA and a decreased expression of β-receptor mRNA in the mammary gland, whilst in the colon the reverse was seen, with a downregulation of α-receptor mRNA and an upregulation of β-receptor mRNA. Fish oil also caused a downregulation of the α-receptor and increase in β-receptor mRNA in the colon.

Changes in α-receptor mRNA paralleled changes in mitosis, suggesting a possible mechanism whereby both fish oil and soy extract modify mitosis. This hypothesis and the significance of these results with respect to cancer prevention require further investigation. Although soy had no effect on apoptosis in normal mammary tissue, genistein has been shown to cause cell cycle block and p53-dependent apoptosis in a rat mammary cancer model (Katdare *et al*. 1998).

Campbell-Thompson M, Lynch IJ & Bhardwaj B (2001) *Cancer Research* **61**, 632–640.
Katdare M, Osborne MP & Telang NT (1998) *Oncology Reports* **5**, 311–315.
Latham P, Lund EK, Brown JC & Johnson IT (2001) *Gut* **49**, 97–105.
Leung LK & Wang TT (2000) *Journal of Nutrition* **130**, 2922–2926.

Associations between dietary intake and bone mineral density in young British women. By D.J. PATTISON[1], J. CATTERICK[2], R.T. WEBB[1], J.A. BISHOP[2], A.D. WOOLF[3] and S.A. NEW[2], [1]*ARC Epidemiology Research Unit, University of Manchester, Manchester M13 9PT,* [2]*Centre for Nutrition and Food Safety, School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH and* [3]*Duke of Cornwall Rheumatology Unit, Royal Cornwall Hospital, Truro, Cornwall, TR1 3LJ*

Osteoporosis presents a major burden to society and health care services. A low peak bone mass may increase the risk of osteoporosis in women in later life, particularly in the postmenopausal stage. Most research to date has focused on dietary calcium, with only limited information available on the influence of other nutrients on bone health, particularly in young women. These data are preliminary results from the South-West cohort of the Study of Bone Health in Young Women in the UK. The aim of this investigation was to identify dietary factors associated with bone mineral density (BMD) in young women.

In this cross-sectional study, 175 women aged 20–29 years were recruited from general practices in Cornwall and 105 in Surrey using a two-stage random sampling process. Lifestyle, anthropometric and dietary information was collected via questionnaire, examination and estimated 7 d food diary (including MAFF Food Atlas standard portion size photographs) respectively. BMD was measured at the lumbar spine (BMD-LS) and femoral neck (BMD-FN) by dual energy x-ray absorptiometry (DXA) (QDR-4500, Hologic, MA). Food diaries were analysed using Diet 5, which is based on the McCance and Widdowson food composition tables.

These results are based on the analysis of the first forty food diaries from the South-West group only. Anthropometric, BMD and nutrient intake data are presented in the Table below.

	Median	IQR		Median	IQR	RNI
Age (years)	27.0	23.5, 29.0	Energy (MJ)	7.87	6.43, 9.23	8.10
Weight (kg)	63.1	59.6, 71.1	Protein (g)	59.4	52.5, 71.0	45
Height (m)	1.66	1.61, 1.71	NSP (g)	12.7	9.4, 15.3	18*
BMI (kg/m^2)	23.9	21.3, 26.5	Total carotene (μg)	1362	936, 1776	–
BMD-LS (g/cm^2)	1.038	0.947, 1.102	Potassium (mg)	2669	2208, 3176	3500
BMD-FN (g/cm^2)	0.846	0.790, 0.947	Calcium (mg)	657	550, 867	700
T-score-LS	–0.060	–0.910, 0.500	Vitamin D (μg)	1.6	0.98, 2.1	–
T-score-FN	–0.030	–0.530, 0.880	Phosphorus (mg)	1050	894, 1257	550
			Zinc (mg)	7.2	5.7, 8.2	7
			Magnesium (mg)	236	197, 285	270
			Iron (mg)	9.9	7.0, 11.1	14.8

IQR = Interquartile range; RNI = Reference nutrient intake; *Estimated average requirement

Mean daily intakes of energy, NSP, potassium, magnesium and iron were below the UK RNI values for women aged 19–50 years. Osteopaenia was found in 18% and 8% of women at LS and FN, respectively. BMD-LS was in the osteoporotic range (T-score <–2.5) in one subject. Linear regression was used to investigate associations between tertiles of nutrient intake (adjusted for height, weight and energy intake) and BMD at each site. The ratio of protein : potassium intake was negatively associated with BMD-LS and BMD-FN (test for linear trend; P=0.027 and P=0.005, respectively). Intake of total carotene was positively associated with BMD-LS (test for linear trend; P=0.009) only. NSP intake was positively associated with BMD-FN (test for linear trend P=0.012).

The dietary intake of several nutrients was found to be below the UK RNI for women of this age group. Despite a small sample size, these preliminary findings suggest that certain nutrients may be associated with bone health. These will be explored further in the complete data set.

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Dietary intake and sources of phylloquinone (vitamin K_1): regional differences in a national sample of British adults. By C.W. THANE and C. BOLTON-SMITH, *MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL*

Vitamin K, present in the diet mainly as phylloquinone (vitamin K_1), is essential for the functioning of a number of hepatic and extrahepatic proteins. Low vitamin K_1 intake may contribute to osteoporosis and increased risk of fracture through undercarboxylation of osteocalcin, whilst undercarboxylation of matrix Gla protein may be associated with vascular calcification and atherogenesis (Shearer, 1997). Reliable estimates of vitamin K_1 intake are required in order to translate the relationships between biochemical markers of vitamin K status and health outcomes into public health and dietary recommendations.

Dietary intake of vitamin K_1, and the relative contributions of different food groups, were estimated in a nationally representative sample of British adults aged 16–64 years who participated in the 1986–7 Dietary and Nutritional Survey of British Adults (Gregory *et al*. 1990). After excluding those who were unwell, with eating habits affected, 7 d weighed dietary records were analysed for 1936 participants (995 men, 941 women). Vitamin K_1 intake was estimated using vitamin K_1 content values for a comprehensive range of foods (Bolton-Smith *et al*. 2000, plus unpublished data) and examined according to sex, age group, season, occupational social class, smoking habit and region. Regional differences are reported here, with adjustment for these other factors.

Vitamin K_1 intakes were positively skewed, with an overall geometric mean (95% CI) of 72 (70, 74) µg/d (men, 79 (76, 82); women, 65 (62, 67) µg/d). In total, 47% of men and 48% of women had vitamin K_1 intakes below 1 µg/kg body weight per day. A significant North–South gradient existed in mainland Britain of increasing vitamin K_1 intake ($P<0.001$), with the lowest intakes found almost universally in Scotland. Regional differences in vitamin K_1 intake applied to both men and women, even after excluding likely under-reporters (energy intake : estimated BMR <1.2). Regional variation also existed in the dietary sources of vitamin K_1 as shown in the Table.

Vitamin K_1 intake and selected sources	Scotland (*n* 154–156)	North (*n* 493–496)	Central, SW & Wales (*n* 636–646)	London & SE (*n* 636–638)	*P**
Intake (µg/d)†	56^a (50, 62)	68^b (64, 71)	75^c (71, 78)	77^c (73, 81)	<0.001
Intake (µg/MJ)†	7.0^a (6.4, 7.7)	8.1^b (7.7, 8.5)	8.7^{bc} (8.4, 9.2)	9.2^c (8.8, 9.7)	<0.001
Intake (µg/kg body weight/d)†	0.83^a (0.75, 0.93)	0.97^{ab} (0.92, 1.02)	1.07^{bc} (1.02, 1.12)	1.12^c (1.07, 1.18)	<0.001
Contribution from vegetables (%)‡	52^a (49, 55)	63^b (61, 64)	65^b (63, 66)	65^b (64, 66)	<0.001
of which: cooked green vegetables (%)‡	*16^a (13, 20)*	*23^b (21, 25)*	*26^{bc} (24, 28)*	*28^c (26, 30)*	<0.001

†Geometric means (95% CI) obtained by back-transformation of $\log_e$-transformed data, ‡arithmetic means (95% CI). *Adjusted for season and other socio-demographic and lifestyle factors. Different superscripts indicate significant differences between regions ($P<0.05$, Scheffé test following ANOVA).

Vegetables contributed most to vitamin K_1 intake (63% overall), with 25% derived from cooked leafy green vegetables (cabbage >Brussels sprouts >broccoli >spinach) and 15% from lettuce and other raw salad vegetables. The main food groups of cereals, meat and fat spreads contributed 11%, 6% and 5%, respectively, to vitamin K_1 intake.

Vitamin K_1 intakes, sources and regional differences observed in this survey are comparable with those derived from further analysis of the more recent National Diet and Nutrition Survey (NDNS) of people aged 65 years and over (Thane *et al*. 2002). It will be pertinent to investigate whether vitamin K_1 intakes have changed between 1986–7 and 2000–1 when food consumption data become available from the NDNS of British Adults aged 19–64 years (currently being prepared by the Office for National Statistics). Regional differences in vitamin K_1 intake may be reflected in vitamin K status and influence the development of nutrition-related chronic diseases, such as osteoporosis and cardiovascular disease.

Bolton-Smith C, Price RJG, Fenton ST, Harrington DJ & Shearer MJ (2000) *British Journal of Nutrition* **83,** 389–399.
Gregory J, Foster K, Tyler H & Wiseman M (1990) *Dietary and Nutritional Survey of British Adults.* London: HMSO.
Shearer MJ (1997) *Proceedings of the Nutrition Society* **56,** 915–937.
Thane CW, Paul AA, Bates CJ, Bolton-Smith C, Prentice A & Shearer MJ (2002) *British Journal of Nutrition* **87,** 605–613.

A comparison of the estimated vitamin K_1 intake in older people from Cambridge, UK and Shenyang, PR China. By L. YAN[1], A. O'NEILL[1], B. ZHOU[2], D.C. GREENBERG, C.J. PRYNNE[1], C. BOLTON-SMITH[1] and A. PRENTICE[1], [1]*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL and* [2]*Shenyang Medical College, 146 Northern Huangher Street, Shenyang 110034, PR China*

Vitamin K is required for γ-carboxylation of the bone protein osteocalcin, which is important in bone mineralization. Low serum concentrations of vitamin K_1 (phylloquinone) have been linked to increased risk of hip fracture (Feskanich *et al*. 1999). The incidence of hip fracture in Shenyang, in north-eastern China is low compared with that in European countries (Yan *et al*. 1999). The aim of the present study was to investigate whether there is a difference in dietary vitamin K_1 intake between older people living in Cambridge and those living in Shenyang.

Dietary information from seventy-three British subjects (thirty-five men and thirty-eight women) was collected by a 7 d food diary and from 113 Chinese subjects (fifty-six men and fifty-seven women) by a 7 d food questionnaire, which has been validated against 5 d weighed intake. All subjects were from a large ongoing study of vitamin K and vitamin D status and bone health in older people aged 60–83 years conducted collaboratively by MRC Human Nutrition Research and Shenyang Medical College. Data used in this study were collected in the spring of 2000 and 2001. Vitamin K_1 intake was estimated using an in-house version of the vitamin K_1 composition of a comprehensive range of foods (Bolton-Smith *et al*. 2000; plus unpublished data) for both the British and Chinese subjects. Green leafy vegetables included spinach, rape leaves, broccoli, cabbage, Brussels sprouts, coriander, chives, Chinese leaves and lettuce. There was no gender difference in vitamin K_1 intake in either sample ($P>0.05$), so vitamin K_1 intakes and sources from men and women are presented together below.

	Chinese (*n* 113)			British (*n* 73)		
	Food wt. (g/d)	Vit K_1 (µg/d)	% of total	Food wt. (g/d)	Vit K_1 (µg/d)	% of total
Green leafy vegetables	167	285	82	45	59	50
Other vegetables	120	9	3	145	16	14
Potatoes	18	< 0.5	< 0.5	114	4	3
Fats and oils	30	37	11	17	2	2
Fruits	297	5	1	188	6	5
Cereal-based foods	295	3	1	273	11	10
Dairy foods and eggs	206	1	< 0.5	423	4	3
Meat and fish	100	2	< 0.5	163	6	5
Other foods	208	5	1	107	9	8
Vitamin K intake:						
Arithmetic mean (95% CI)		347 (313–380)			117† (103–131)	
Geometric mean (95% CI)‡		302 (272–334)			104† (93–116)	

† Significant difference between two populations, $P < 0.001$. ‡Antilog of $\log_e$-transformed data.

The average intake of vitamin K_1 by the Chinese was three times that of the British ($P<0.001$). The higher vitamin K_1 intake in Chinese subjects was due to the consumption of a large amount of green leafy vegetables and soybean oil, which are high in vitamin K_1. The vitamin K_1 intake of British subjects in the present study was higher than those reported in a Scottish population (arithmetic means: 69–76 µg/d) (Fenton *et al*. 1997) and in a national survey of British elderly people (geometric means: 50–73 µg/d) (Thane *et al*. 2001). These data indicate that a high vitamin K_1 intake can be achieved by a diet that is high in green leafy vegetables and vitamin K_1-rich oils. The influence of the higher vitamin K_1 intakes on bone mineral status and bone metabolism in the Chinese population is currently under investigation.

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Bolton-Smith C, Price RJG, Fenton ST, Harrington DJ & Shearer MJ (2000) *British Journal of Nutrition* **83**, 389–399.
Fenton ST, Price RJ, Bolton-Smith C, Harrington DJ & Shearer MJ (1997) *Proceedings of the Nutrition Society* **56**, 301A.
Feskanich D, Weber P, Willett WC, Rochett H, Booth SL & Colditz GA (1999) *American Journal of Clinical Nutrition* **69**, 74–79.
Thane CW, Paul AA, Prynne CJ & Bolton-Smith C (2001) *Proceedings of the Nutrition Society* **60**, 57A.
Yan L, Zhou B, Prentice A, Wang X & Golden MHN (1999) *Bone* **24**, 151–155.

An evaluation of a brief educational intervention to improve the management of obesity in primary care: the BiO Project. By H. MOORE[1], D. GREENWOOD[2], J. GRIFFITHS[3], M. HENDERSON[4], K. HESKETH[5], S. WOOLGAR[6] and A. ADAMSON[7], [1]*Centre for Research in Primary Care,* [2]*Biostatistics Unit, University of Leeds; Departments of Nutrition and Dietetics in* [3]*Leeds,* [4]*North Durham,* [5]*Newcastle,* [6]*Scarborough;* [7]*Human Nutrition Research Centre, University of Newcastle*

General practitioners and their teams have a key role in providing advice on weight management to individuals (National Audit Office, 2001). The National Audit Office (2001) stated that such activities needed to be undertaken more consistently and that there are opportunities for spreading good practice. A recent systematic review identified the need to investigate educational methods that encourage health care workers to improve delivery of weight management to their patients (Harvey *et al.* 1999).

This cluster randomized controlled trial assessed the effectiveness of a training programme (the intervention) promoting the evidence-based management of obesity, delivered by dietitians to general practice teams (unit of randomization). Forty-four practices in the North-East of England were recruited to the trial. Practice staff asked consecutive patients (BMI ≥30; aged 16–64 years) to participate, as they consulted over a 6-month recruitment phase and prior to randomization.

The training programme offered a model approach to obesity treatment, incorporating best evidence, and was judged brief enough to be implemented in primary care. The model advocated prescription of a moderate energy deficit diet and exercise. Practitioners were encouraged to see patients regularly (about every 2 weeks) until they had lost 10% of their original body weight, and then less regularly (about every 1–2 months) for weight maintenance.

The primary outcome measure was difference in weight between patients from intervention and control treatments at 1 year post-intervention. Secondary outcome measures included: difference in weight at 3 months, change in practitioner attitude towards and knowledge of obesity management, and an audit of obesity management activity extracted from patient notes.

The study was designed to have 80% power to detect a mean difference in weight between treatments of approximately 3–5 kg assuming 5% significance and an intra-practice correlation coefficient of 0.05.

Forty-four practices completed the trial and 840 subjects were randomized, with 564 patients (67%) providing follow-up at 1 year post-intervention. Despite the loss to follow-up, 80% power was maintained due to a negligible intra-practice correlation coefficient. Adequate balance of baseline characteristics was maintained for both patient (weight, BMI, age, sex) and practice (size of practice, presence of a dietetic service, socio-economic status of practice site) characteristics.

Preliminary results are shown in the Table.

	Intervention – control patients	95% confidence intervals	*P* value
Difference in weight at 3 months post-intervention	+0.66 kg	−1.99 to 3.30	0.63
Difference in weight at 12 months post-intervention	+1.04 kg	−1.87 to 3.96	0.48

General practices participating in this trial were likely to be a motivated subset of primary care and, similarly, patients opting to join the trial might be expected to be more interested in weight loss. Despite this, the intervention had no effect in terms of change in weight. This study calls into question the ability and motivation of primary care teams to deal with obesity within current resources for service delivery.

Harvey EL, Glenny AM, Kirk SFL & Summerbell CD (1999) *International Journal of Obesity* **23**, 1213–1222.
National Audit Office (2001) *Tackling Obesity in England. Report by the Comptroller and Auditor General.* London: The Stationery Office.

Dietary intake in post-myocardial infarction patients: self perception in relation to total fat intake. By W.S. LESLIE, D. CONNELLY, M.E.J. LEAN and C.R. HANKEY, *University of Glasgow Department of Human Nutrition, Queen Elizabeth Building, Glasgow Royal Infirmary, Glasgow G31 2ER*

Cardiac rehabilitation has a recognized role in post-myocardial infarction care and provides a natural opportunity for secondary prevention. There is now good evidence to support the value of specific dietary changes in reducing coronary risk (Committee on Medical Aspects of Food Policy, 1994). Dietary advice is routinely given as part of cardiac rehabilitation. Evidence exists, however, that nutritional knowledge does not automatically translate into practice (Lappalainen *et al.* 1997). Many people are unfamiliar with the composition of foods, have difficulty integrating advice within their own diet, and frequently overestimate the healthiness of their dietary habits (Margetts *et al*. 1997). The present study aimed to assess (1) knowledge of healthy eating, (2) perception of diet healthiness, and (3) compare perceptions with habitual dietary practices.

Seventy-six post-myocardial infarction patients attending cardiac rehabilitation and participating in a randomized controlled study to examine the effectiveness of intensive nutritional counselling were recruited. At baseline prior to dietary intervention all subjects completed a 7 d weighed intake food diary. A questionnaire designed to assess attitudes, beliefs and knowledge on health and diet (Lennernas *et al.* 1997) was also completed. Subjects were asked to describe the principles of healthy eating, and to rate the healthiness of their own diet. Using data from the 7 d weighed intake diaries, the study population was divided into two groups according to total fat consumption (>30% and <30% of total energy intake). Responses to the dietary questions were scored and compared with habitual dietary practices by group.

The majority of the study population were able to correctly cite the main dietary guidelines when describing a healthy diet. However, accurate knowledge of healthy eating guidelines did not appear to have influenced dietary practices, as more than half the study population (53%) reported a total fat consumption >30% of total energy. Comparison of responses to the dietary questions by high and low fat intake showed significant differences according to group. Subjects consuming >30% total fat (n=40) had a significantly lower score for the question on healthy eating (P=0.003), indicating a poorer knowledge of healthy eating. These subjects also tended to rate their diet as healthy and not requiring change in comparison with the <30% total fat group (P=0.0001).

Patients recovering from a myocardial infarction are clearly aware of the recommendations for healthy eating. However many overestimate the healthiness of their dietary habits, particularly those with a higher total fat consumption. Dietary counselling within cardiac rehabilitation programmes should not just provide information but should also enable patients to evaluate their own dietary habits, identify areas requiring change and assist patients with implementation.

Committee on Medical Aspects of Food Policy (1994) *Report of the Cardiovascular Review Group*. London: HMSO.
Lappalainen R, Saba A, Holm C, Mykkanen H, Gibney MJ & Moles A (1997) *European Journal of Clinical Nutrition* **51**, 36–40.
Lennernas M, Fjellstrom C, Becker W, Giachetti I, Schmitt A, Remaut de Winter A & Kearney M (1997) *European Journal of Clinical Nutrition* **51**, 8–15.
Margetts BM, McVey D, Oldfield K, Rogers L, Royle J, Speller V, Thompson R & Woodward N (1997) *Health Education Authority. Health and Lifestyle Survey 1993*. London: HEA.

The Mediterranean diet score: a useful means of evaluating and comparing differing dietary patterns? By C.R. HANKEY, V. DILIS, J.A. SCOTT, W.S. LESLIE and M.E.J. LEAN, *University of Glasgow Department of Human Nutrition, Queen Elizabeth Building, Glasgow Royal Infirmary, Glasgow G31 2ER*

The conventional approach to examining diet–disease relationships focuses on a single nutrient or a few nutrients or foods. Such an approach fails to recognize that people do not eat isolated nutrients but meals containing combinations of nutrients and non-nutrient components which may be either interactive or synergistic (Hu *et al*. 2000). The traditional Mediterranean dietary pattern, associated with longevity (Trichopoulou *et al*. 1995) and reduced cardiovascular complications after myocardial infarction (de Lorgeril *et al*. 1998), has been quantified as a simple-to-use diet score calculated from eight dietary components (Trichopoulou *et al*. 1995). A high score is defined as being ≥4.

The aim of the present study was to use the Mediterranean diet score (MDS) to evaluate the dietary patterns of a group of Scottish patients who had survived an acute myocardial infarction, were attending cardiac rehabilitation and participating in a randomized-controlled study to examine the effectiveness of intensive nutritional counselling. Subjects were randomized to receive either intensive dietary counselling or usual care. Dietary advice given to intervention subjects was of 4 h duration and aimed to encourage the consumption of a diet in accordance with the principles of the Mediterranean diet.

Seventy-five post-myocardial infarction patients, fifty-nine men and seventeen women with a mean age of 58 (range 40–75) years, were recruited. At baseline prior to dietary intervention and 12 weeks after the completion of cardiac rehabilitation all subjects completed a 7 d weighed intake food diary. Dietary intake data from the food diaries were used to calculate individual MDS (see Table) at the two time points. At baseline there was no difference between the groups either in the proportion of subjects achieving a high MDS (≥4) or in the mean MDS between the groups (see Table). However, at 12 weeks a greater number of subjects in the intervention group achieved a high score compared with those in the control group (P=0.032).

		Control group (n=38)			Intervention group (n=37)		
Food group	MDS score criteria (g per day)	Baseline %	12 weeks %	P^a	Baseline %	12 weeks %	P^a
Fruits	M >249, F >216	55	53	1.0	68	86	0.01
Vegetables	M >303, F >248	3	3	1.0	8	19	0.12
Legumes	M >60, F 49	40	29	0.42	30	32	1.00
Cereals	M >291, F >248	82	87	0.62	81	92	0.21
Meat & meat products	M <109, F <91	24	32	0.54	35	46	0.48
Milk & dairy products	M <201, F <194	13	13	1.00	19	30	0.28
Alcohol	M >10, F >0	24	18	0.77	32	30	1.00
MUFA/SFA ratio	>1.6	0	0	--	3	0	1.00
% achieving MDS score ≥ 4		13	16[b]	1.00	24	38[b]	0.22
Mean score (SD)		2.39 (0.97)	2.31 (1.16)	0.68[c]	2.60 (1.24)	3.24 (1.01)	0.003[c]

[a] McNemar test, [b]Mann-Whitney U test, [c] paired t-test.

This study was the first to examine the value of a diet score to evaluate changes in eating habits following a dietary intervention. The MDS was able to detect the improvements in the diet in a group of Scottish post-myocardial infarction patients receiving intensive dietary counselling. However, although this allowed the quantitative comparison of dietary habits with the traditional Mediterranean diet, it highlighted the low MDS for patients having received intensive dietary counselling.

de Lorgeril M, Salen P, Martin J, Monjaud I, Boucher P & Mamelle N (1998). *Archives of Internal Medicine* **158**, 1181–1187.
Hu F, Rimm E, Stampfer M, Ascherio A, Spiegelman D & Willett W (2000) *American Journal of Clinical Nutrition* **79**, 912–921.
Trichopoulou A, Kouris-Blazos A, Wahlqvist M, Gnardellis C, Lagiou P, Polychronopoulos E, Vassilakou T, Lipworth L & Trichopoulos D (1995) *British Medical Journal* **311**, 1457–1460.

Association of dietary variety with nutrient intake and body mass in women: results from the UK Women's Cohort Study. By J.E. CADE, V.J. BURLEY and D.C. GREENWOOD, *Nutrition Epidemiology Group, Nuffield Institute for Health, 71–75 Clarendon Road, Leeds LS2 9PL*

Dietary guidelines have long emphasized the importance of eating a variety of foods. This may improve eating patterns by providing access to the whole range of nutrients required for optimum health. On the other hand, results from animal studies and single-meal studies in humans show that access to a variety of foods is associated with increases in energy intake and weight gain (McCrory *et al*. 1999). This study aims to explore the impact of dietary variety on food intake and association with weight in free-living individuals. The UK Women's Cohort Study is a national cohort of 35 372 women, aged 35–69 years at baseline, who have all completed a 217-item food frequency questionnaire (FFQ). The FFQ recorded frequencies of eating foods in ten categories ranging from never to 6+ per day. Three diversity indices were created according to the number of individual foods on the FFQ which were recorded as being eaten every day (DI1), at least once a week (DI2) or ever eaten (DI3).

	Diversity index 1 (every day)		
	Low (n 12004)	Medium (n 10692)	High (n 12676)
Mean number of foods	5	8	14
Total calories (MJ)	8.2	9.6	11.6
% Energy from fat	33.1	32.5	31.7
Vitamin C (mg)	133	160	220
BMI (kg/m^2)	24.7	24.5	24.3

Subjects who consumed the highest number of the same foods every day ate more calories, more itamin C and had a greater variety of fruit and vegetables; they also had a lower percentage energy from fat and lower BMI than subjects who ate the lowest number of the same foods daily. Similar patterns were seen for increasing variety of foods eaten weekly or ever eaten for calorie intake, vitamin C and fruit and vegetable variety. However, percentage of energy from fat increased with increasing numbers of foods eaten weekly or ever. BMI increased with increasing numbers of foods eaten weekly and there was no difference in BMI with number of foods ever eaten. Differences were all highly statistically significant in a one-way analysis of variance. Adjustment for possible under-reporting did not materially affect the results. The most commonly consumed daily foods were milk (95%), tea (75%), coffee (54%), wholemeal bread (40%), apples (30%) and polyunsaturated margarine (26%). Important foods consumed weekly were again milk (94%), tomato (90%), carrot (86%), potato (83%), tea (83%) and apples (81%). Foods ever eaten by most people were carrots (99%), tomato (98%), lettuce (98%), cauliflower (97%), apples (97%), broccoli (97%) and boiled potato (96%).

Increasing the number of different food items consumed does appear to lead to a greater calorie intake; however, it also results in a greater variety of fruit and vegetables being consumed. The association with body size depends on whether more foods are eaten daily, weekly or ever. Measures of dietary variety may represent an additional factor to consider when studying healthy diets. The relationship of variety to health outcomes should be examined further, in particular in relation to obesity, heart disease and cancer.

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McCrory MA, Fuss PJ, McCallum JE, Yao M, Vinken AG, Hays NP & Roberts SB (1999) *American Journal of Clinical Nutrition* **69**, 440–447.

Interaction between changes in physical access and other factors influencing consumption of fruits and vegetables in a food desert. By D.L. WARM[1], B.M. MARGETTS[1] and N. WRIGLEY[2], [1]*Institute of Human Nutrition and* [2]*Department of Geography, University of Southampton, Southampton SO16 6YD*

Food deserts are geographical areas that have little or no retail provision of food. It has been hypothesized that the dietary patterns of individuals are compromised by living within a food desert (Department of Health, 1996). As part of an interdisciplinary cohort study investigating food shopping and consumption patterns within a known food desert located in a socio-economically deprived area of Leeds, the factors influencing fruit and vegetable consumption were assessed before and after the opening of a locally accessible superstore (Warm *et al*. 2001). The person in each household principally responsible for domestic arrangements completed a self-administered 7 d food diary and an interviewer-administered questionnaire. Following the opening of the superstore, data were collected from 615 (61%) respondents who had completed the initial questionnaire.

The interaction between changes in physical access to fruit and vegetables (as measured by use of the new superstore) and changes in other factors were examined within a developed framework in order to assess the effect on fruit and vegetable consumption. The other factors in the framework were changes in the: availability of fruits and vegetables; affordability of fruits and vegetables; attitude towards healthy eating; and other factors impinging on the buying and consuming of fruits and vegetables. Fruit and vegetable consumption has been calculated as a proxy measure for the intake of a healthy diet.

Change in factor in framework of fruit and vegetable consumption	Respondents switching to new superstore for fruit and vegetable shopping	
	Wave one (Mean±SD) (*n*)	Wave two (Mean±SD) (*n*)
Overall consumption	2.60±1.67 (218)	2.75±2.47 (218)
Increased availability	2.52±1.72 (73)	2.54±1.39 (73)
Increased affordability	2.33±1.45 (61)	2.62±3.64 (61)
Increased positive attitudes	2.91±1.74 (129)	2.87±2.74 (129)
Decrease in factors affecting	2.79±1.85 (91)	2.78±1.68 (91)
Decrease in factors limiting	2.99±1.98 (69)	3.11±3.55 (69)

Paired sample *t*-test: Statistical difference between consumption levels in wave one and wave two of those using/not using new superstore.

Overall, there was no significant change in consumption levels (2.88–2.92 portions/d; t –0.591, P 0.555, n 615). Of the respondents, 218 (35.4%) switched to using the new superstore for their fruit and vegetable shopping, and thus increased their physical access to fruit and vegetables. However, an increase in physical access to fruit and vegetables does not appear to have significantly affected fruit and vegetable consumption (2.60–2.75 portions/d; t 1.207, P 0.229, n 218). For those using the new superstore, a minority of people also experienced a positive change in any of the other factors in the framework, particularly with regard to the increased affordability of fruits and vegetables (n 61, 28%) or their availability (n 73, 33%). Furthermore, changes in other factors within the framework for those respondents using the new superstore does not appear to have affected fruit and vegetable consumption to a statistically significant level.

The way in which people buy and consume fruits and vegetables in a deprived area as in this study may be more complex than previously thought. It may be important to further examine the interaction between the different factors in the framework in order to understand the mechanisms by which fruit and vegetable consumption could be improved. Furthermore, other factors not in the framework may explain changes in fruit and vegetable consumption in a food desert.

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Department of Health (1996) *Low Income, Food, Nutrition and Health: Strategies for Improvement.* London: HMSO.
Warm DL, Margetts BM, Whelan AR & Wrigley N (2001) *Proceedings of the Nutrition Society* **60**, 177A.