

# Proceedings of the Nutrition Society

## Abstracts of Original Communications

*A Scientific Meeting was held at the University of Surrey, Guildford on 29 June–2 July 1998, when the following papers were presented.*

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

*The Editors of the Proceedings of the Nutrition Society accept no responsibility for the abstracts of papers read at the Society's meetings for original communications.*

**A comparison of the effect of two types of healthy eating dietary advice on perceived quality of life in Scottish men.** By SANDRA DRUMMOND<sup>1</sup>, TERRY KIRK<sup>2</sup>, JACQUELINE I. AARON<sup>2</sup> and DAVID J. MELA<sup>3</sup>. <sup>1</sup>Centre For Nutrition and Food Research, Queen Margaret College, Edinburgh, EH12 8TJ, <sup>2</sup>Consumer Sciences Department, Institute of Food Research, Reading RG6 2EF.

Long-term compliance with dietary advice may depend on the effect that trying to follow the advice has on quality of life. Often, "healthy eating" advice is perceived as being negative, involving the individual denying him/herself "bad" but enjoyable foods. If the dietary advice is positive, it may result in improved and long-term adherence to the diet. The present study aimed to compare the effects of two types of healthy eating advice on the subjects' responses to statements reflecting their quality of life. Eighty-nine non-dieting men (mean BMI 27.9 kg/m<sup>2</sup>), aged 40-53 years, were randomly allocated to one of three groups: CON, received no nutritional advice; group 1, received advice to reduce both dietary fat and non-milk extrinsic sugars (NMES); group 2, received advice to reduce dietary fat, allowing *ad libitum* NMES. Advice was given once, verbally, on a one-to-one basis, with additional dietary advice leaflets provided. It was emphasized that the advice was specific to each individual subject, to avoid discussion between groups. Compliance with the advice was measured by unweighed food diaries at 2, 4 and 6 weeks and 6 months. After 6 months, subjects were asked to complete a questionnaire aimed at assessing change in perception of quality of life over the preceding 6-month period. Subjects responded to a series of statements on a 7-point scale ranging from 1 = strongly disagree to 7 = strongly agree. Mean responses to each question, for each group are shown in the table.

	CON	Group 1	Group 2
1. Reduced risk of CHD	5.08a	6.32b	6.48b
2. Enjoy physical activity more	4.30a	5.45b	5.45b
3. Feel more energetic	4.33a	5.10b	5.33b
4. Feel more satisfied in general	4.67a	5.40b	5.50b
5. Feel more satisfied with appearance	3.90a	4.57b	5.03b
6. Feel more in control	4.10a	5.03b	4.97b
7. Improved quality of life	4.40a	5.12b	5.04b
8. Foods are easier to cook	4.04a	4.36ab	4.63b
9. Feel less worried about their health	4.70ab	4.47a	5.23b
10. Feel less worried about their weight	4.37a	4.33a	5.17b
11. Enjoy social situations	4.10a	4.50ab	4.73b
12. Feel happier in general	4.63a	5.00ab	5.30b

a,b Values within a row not sharing a common superscript were significantly different. (SPSS,  $P < 0.05$ , ANOVA, multiple range tests).

Both groups 1 and 2 similarly reduced percentage energy from fat ( $P < 0.005$ ) but group 1 could not maintain an initial reduction in percentage energy from NMES at 6 months despite advice to do so.

Both groups 1 and 2 agreed more strongly with statements 1-7 than CON (Table 1), which included the statement which indicated a perceived reduced risk of CHD. In addition, group 2 agreed more strongly than CON that the foods they were eating now were easier to cook. Group 2 were also less worried about their health than group 1 and less worried about their weight than CON or group 1. Group 2 enjoyed social situations more and felt happier in general than CON whereas the responses of group 1 did not differ significantly from those of the CON group. Advice given to group 2 allowing unrestricted NMES may have resulted in a more palatable low-fat diet, and produced a more positive attitude to quality-of-life statements than the advice given to group 1. Advice prioritizing a reduction in dietary fat, allowing *ad libitum* NMES, may help to create a positive attitude towards "healthy eating" and a feeling of well being which may promote dietary change long term.

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**Micronutrient status and other risk factors for CHD in a working male population.** By JAYNE V. WOODSIDE<sup>1</sup>, IAN S. YOUNG<sup>1</sup>, JOHN W.G. YARNELL<sup>1</sup>, DOROTHY McMASTER<sup>1</sup>, K. FRED GEY<sup>2</sup> and ALUN EVANS<sup>3</sup>. <sup>1</sup>Institute of Clinical Science, The Queen's University of Belfast, Belfast BT12 6BJ and <sup>2</sup>Institute of Vitamin Research, University of Bern, Switzerland

Dietary factors are believed to be a major underlying determinant of CHD risk. We have assessed micronutrient status (Catignani *et al.* 1983; Reynolds & Brain 1992; Thurnham *et al.* 1988; Ubink *et al.* 1991) in 765 working men aged 30-49 years in Belfast. The distribution of classic risk factors was also assessed, relating occurrence of these risk factors to micronutrient status. The Table shows the variables measured. The lipid-soluble micronutrients  $\alpha$ -tocopherol,  $\beta$ -carotene and retinol are presented as a micronutrient:cholesterol ratio.

Variables	n	Mean	SD
Age (years)	765	39.2	6.0
BMI (kg/m <sup>2</sup> )	765	26.1	3.1
Systolic BP (mmHg)	765	128	15
Diastolic BP (mmHg)	764	80	10
Total cholesterol (mmol/l)	765	5.83	1.11
LDL-cholesterol (mmol/l)	762	3.90	0.97
HDL-cholesterol (mmol/l)	763	1.09	0.28
Total triacylglycerol (mmol/l)	765	1.59	1.78
Smoking (% smokers)	761	23.3	-
Dietary supplements (% yes)	765	16.7	-
Homocysteine ( $\mu$ mol/l)	626	7.19	1.42
Folate (nmol/l)	611	11.2	1.5
Cobalamin (pmol/l)	610	256.4	1.6
Pyridoxal-5-phosphate (nmol/l)	565	38.7	2.8
$\alpha$ -Tocopherol ( $\mu$ mol/mmol)	537	5.91	1.80
Retinol ( $\mu$ mol/mmol)	534	0.55	0.22
Lycopene ( $\mu$ mol/l)	534	0.165	4.891
$\alpha$ -Carotene ( $\mu$ mol/l)	535	0.032	2.970
$\beta$ -Carotene ( $\mu$ mol/mmol)	494	0.070	0.055

There were several strong correlations among the variables measured. Plasma homocysteine was inversely related with serum levels of folate ( $r = -0.446$ ,  $P < 0.001$ ) and cobalamin ( $r = -0.377$ ,  $P < 0.001$ ). Age, BMI and systolic and diastolic blood pressure (BP) values were all positively associated with both serum cholesterol and serum triacylglycerol concentrations. Serum HDL-cholesterol level was inversely associated with BMI and triacylglycerol concentration and lowered in smokers. Folate levels were lower in smokers than non-smokers. There was a strong link between pyridoxal-5-phosphate (PLP) and smoking status, with PLP being higher in smokers and ex-smokers than never-smokers. Levels of PLP were strongly correlated with the number of cigarettes smoked per day ( $r = 0.271$ ,  $P < 0.001$ ). The link between smoking and PLP is previously unreported.

The study shows that a substantial proportion of this population is at risk of CHD both in terms of classic risk factors (80% either smoke, have high blood pressure or raised serum cholesterol levels) and sub-optimal micronutrient status (almost 30% are at moderately increased risk of CHD due to vitamin E levels). Although many of the weak correlations found within the micronutrients are likely to be due to common food sources, this study also shows that micronutrient status can interact with other risk factors for CHD.

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**Nutritional status and functional ability of older refugees.** By SIMONE PIETERSE, MARY MANANDHAR and SURAIBA ISMAIL. *Public Health Nutrition Unit, London School of Hygiene and Tropical Medicine, 49/51 Bedford Square, London WC1B 3DP*

Older people are an increasing proportion of the population in developing countries, yet virtually no information exists on their nutritional and functional status. In unstable situations, older people can be a particularly vulnerable group as conditions in refugee camps are harsh and social networks may have broken down. A cross-sectional study was undertaken among 828 refugees (50+ years) from rural East Rwanda in a camp in North-West Tanzania. Nutritional status was assessed by anthropometry and functional ability by simple physical tests and a questionnaire on independence in activities of daily living. Kyphosis was noted in 5% of the subjects; for these height was estimated from arm span. Nearly 72% were active in gardening in the camp and 42% were still involved in heavy household tasks. Only 4.1% were living alone or without younger adults.

The following factors should be borne in mind when considering the results of the present study: (a) the refugees were a self-selected group in that those with poorest nutritional status may have been left behind or have died on the way to the camp or soon after arrival; (b) there is reason to assume that the refugees had a reasonable nutritional status before departure; (c) the study took place in the rehabilitation phase.

	Sex	All ages			< 60 years			60-69 years			≥ 70 years		
		Mean	SD		Mean	SD		Mean	SD		Mean	SD	
Weight (kg)	m	55.8	6.9		57.2	6.8		55.0*	6.8		54.1*	6.6	
	f	52.5	7.6		54.4	8.1		51.2*	6.6		49.9*	6.6	
BMI (kg/m <sup>2</sup> )	m	20.2	2.0		20.4	1.9		20.0	2.1		20.1	2.1	
	f	21.3	2.9		21.7	3.1		21.0	2.6		21.2	2.7	
MUAC (cm)	m	25.1	1.9		25.5	1.8		24.9*	1.9		24.6*	2.1	
	f	26.1	2.8		26.6	3.0		25.8*	2.6		25.3*	2.6	
Handgrip (kg)	m	30.3	6.7		32.9	6.6		29.0*	5.7		26.2**	6.0	
	f	22.2	5.1		23.9	5.0		21.6*	4.6		19.0**	4.3	

MUAC, mid-upper arm circumference

Mean values were significantly different from those for < 60 years, \*P<0.05  
Mean values were significantly different from those for 60-69 years, \*\*P<0.05

The Table shows higher mean values in the youngest age group for weight, MUAC and handgrip strength. Underweight, defined as BMI lower than 18.5 kg/m<sup>2</sup>, was prevalent in 19.5% of the men and 13.1% of the women. In men the percentage underweight was significantly higher in older age groups (P<0.05). Using multivariate techniques and controlling for a wide range of variables, handgrip was strongly related to nutritional status indicators such as BMI and MUAC. Interestingly, studies from developed countries indicate that handgrip strength and anthropometric values are much higher among older European adults, but independence levels are lower (SENECA Investigators, 1996).

Guidelines regarding BMI cut-off points for older populations do not yet exist and therefore BMI classifications for adults were used (World Health Organisation, 1995). This study suggests that undernutrition does occur and that poor nutritional status is related to impaired functional ability. These findings are important if we want to promote the valuable role that many older people in developing countries play within and also beyond their families.

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**Variability over 6 years in blood homocysteine concentration in an elderly population in Hertfordshire and relationship with heart disease.** By JOHN M. JACKSON<sup>1</sup>, CAROLINE H. FALL<sup>2</sup>, REBECCA REYNOLDS<sup>2</sup>, SARAH L. DUGGLEBY<sup>2</sup> and ALAN A. JACKSON<sup>1</sup>. <sup>1</sup>*Institute of Human Nutrition and* <sup>2</sup>*MRC Environmental Epidemiology Unit, University of Southampton, Southampton General Hospital, Southampton SO16 6YD*

Plasma total homocysteine (Hcy) concentration has been related to the risk of vascular disease and a number of factors have been related to Hcy status, such as age, sex, dietary folate, vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, and presence of the thermolabile variant of the enzyme 5-methylenetetrahydrofolate reductase. (EC 1.7.99.5) We have measured Hcy in an elderly population in relation to heart disease and assessed the stability of Hcy in individuals over a 6-year period.

A fasting blood sample was taken from 634 men and 389 women of about 65 years of age and after 6 years total serum Hcy was measured in stored samples by HPLC with fluorimetric detection. Six years later, repeat measurements of Hcy were made in samples collected in 132 male subjects. On the first occasion Hcy concentration was measured in serum obtained from blood separated from erythrocytes at room temperature with an estimated delay of less than 45 min. On the second occasion Hcy was measured in plasma obtained from whole blood placed immediately on ice and separated from erythrocytes within 15 min.

The positive skew of the Hcy concentration distribution in both male and female subject groups was normalised by logarithmic transformation of individual values for statistical analysis. In the samples collected 6 years earlier, total Hcy was significantly greater (P < 0.001) in males (Mean 12.9 μmol/L, SD range 9.9 - 16.7 μmol/L) compared with females (Mean 11.2 μmol/L, SD range 8.3 - 15.1 μmol/L) and was significantly correlated with age in both sexes (P < 0.002). In a logistic regression allowing for age and sex, Hcy was strongly related to ischaemic heart disease, defined as angina or major Q waves or bypass surgery, (P < 0.002). There was a correlation between Hcy measured on the first and second occasions (r 0.81, P < 0.001). Differences between the first and second measurements might be attributed to blood sampling procedures, age-related changes or differences in dietary intake. Different blood sampling procedures might have allowed *in vitro* generation of Hcy in the initial measurement leading to a higher value in the first measurement. Age-related changes would have been expected to lead to a higher value in the second measurement. Despite these possible sources of variability, the close correlation between the first and second measurements over an interval of 6 years, suggests that there was some tracking in the level of Hcy. Further, the findings imply that any changes in the dietary intake of folate, vitamin B<sub>6</sub> and vitamin B<sub>12</sub> have contributed little to the variability of Hcy over this period of time.

**Malnutrition and dietary inadequacy among rural elderly Malays: simple indices to identify individuals at high risk.** By SUZANA SHAHAR<sup>1</sup>, JANE EARLAND<sup>1</sup> and ROBERT A. DIXON<sup>2</sup>, *Centre For Human Nutrition, University of Sheffield, Herring Road, Sheffield S5 7AU; <sup>1</sup>School of Health and Related Research, University of Sheffield, Regent Street, Sheffield S1 4DA;*

A large proportion of rural elderly Malays have been identified as malnourished using anthropometric and dietary indices (Shahar *et al.* 1997, 1998). Early recognition of those at high risk would allow possible interventions to prevent subsequent symptoms of malnutrition and thus enhance the quality of life and reduce the health care costs. Therefore, the present study was designed to identify predictors of malnutrition and dietary inadequacy that can be used to construct a simple, cheap and valid index to identify rapidly the most vulnerable elderly people.

A cross-sectional study was conducted among 350 elderly Malays aged 60 years and over in rural areas of Mersing district, on the East Coast of Malaysia, as has been previously reported (Shahar *et al.* 1997, 1998). The subjects were interviewed by six trained interviewers to obtain information on social and health aspects using a pre-tested questionnaire. Malnutrition was assessed using BMI, plasma albumin and haemoglobin on 285 subjects. Dietary adequacy [a examined using a validated dietary history questionnaire with a food frequency checklist on 337 subjects. Those who had four or more of the eight nutrients investigated (protein, vitamin A, thiamin, riboflavin, niacin, vitamin C, Ca and Fe) below 2/3 of the Malaysian RDA were classified as consuming an inadequate diet. Multiple logistic regression analysis was performed to identify significant predictors of malnutrition and dietary inadequacy from social and health factors and to derive appropriate indices based on these predictions. Before analysis, thirty-eight social and health factors recognized as determinants of nutritional or related problems among elderly people (for example, by Horwath, 1989 and Kohrs *et al.* 1989) were examined using Pearson's chi-square test, with only those found to be associated at  $P \leq 0.20$  included in the appropriate regression analysis.

As shown in the Table, the final regression models resulted in seven significant predictors of malnutrition with a sensitivity of 56% and a specificity of 84%; and five of dietary inadequacy with a sensitivity of 77% and a specificity of 47%. The predictors are all easy to recognize and collect in a population, and thus appropriate to use as an index to identify high-risk individuals. The regression coefficients were simplified to develop a scoring system which could be administered in any social setting. Although the sensitivity for identifying malnourished subjects was low (56%), the sensitivity for identifying subjects consuming an inadequate diet was high (77%), though correctly identifying only 42% of those not consuming an inadequate diet (specificity). Dietary inadequacy is the first stage of nutritional deficiency, therefore, the high sensitivity would allow early referral to those who are consuming an inadequate diet, and allow the early prevention of subsequent symptoms of malnutrition as a result of prolonged poor dietary intake. The high specificity for identifying non-malnourished subjects (84%) is desirable as it implies a low probability of unnecessary referral for those who do not need it. Although the low specificity for identifying subjects consuming an adequate diet (47%) implies a high probability of unnecessary referral for those who do not need it, low cost interventions at a community level can be provided. In conclusion, the indices can be used by public health professionals and community leaders as a simple, rapid and valid instrument to screen high-risk individuals who would benefit from early referral and treatment. The indices now need to be validated in other populations with similar nutritional problems.

Table. Predictors of malnutrition and dietary inadequacy as a result of the logistic regression analysis

Predictors of Malnutrition (n 285)	Predictors of Dietary Inadequacy (n 337)
Chewing ability	Chewing ability
Self-reported diagnosed joint disease	Ability to take public transport
Self-reported diagnosed hypertension	Consumption of fruit
Self-reported diagnosed respiratory disease	Appetite
Smoking status	Number of meals taken regularly in a day
Economic dependency	
Self-perceived weight loss	

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**The relationship between nutritional status and functional ability among older people in rural Malawi.** By DOROTHY M. CHILIMA and SURAJYA J. ISMAIL, *Public Health Nutrition Unit, Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, 49/51 Bedford Square, London WC1B 3DP*

The linking of anthropometry to functional ability of older people has been suggested by many scientists (Durmin, 1989; Kelly & Kroemer, 1990; World Health Organization, 1995) although very few studies have examined the relationship particularly in developing countries. A cross-sectional study was undertaken among older people in rural Malawi to examine the relationship between nutritional status and functional ability. The subjects were recruited using a multi-stage cluster sampling technique and selected anthropometric measurements were taken. BMI was computed using weight and height for non-kyphotic respondents (James *et al.* 1988). For kyphotic respondents, height was estimated from arm span using regression equations derived from the non-kyphotics (Chilima & Ismail, in press). Functional ability was assessed by questionnaire (activities of daily living) (Katz *et al.* 1963) and functional tests which assess manual dexterity, handgrip strength, psychomotor function and cognitive function (Manandhar, 1995; Bassey, 1990). A semi-structured questionnaire was also administered to obtain social, economic and health information. Furthermore, a basic clinical examination was carried out by a nurse.

	Males (n 94)		Females (n 190)	
	Mean	SD	Mean	SD
Age (years)	68.9	8.1	63.3**	6.1
MUAC (cm)	25.0	2.4	0.45	25.9*
BMI (kg/m <sup>2</sup> )	19.8	2.5	0.40	20.3
Handgrip strength (kg)	28.0	5.9	21.7**	4.5

r, Correlation coefficient between nutrition indicator and handgrip strength (all were statistically significant  $P < 0.0001$ ). Mean values were significantly different from those for males: \*  $P < 0.05$ , \*\*  $P < 0.01$ .

The Table shows that the men studied were older and stronger than women and that handgrip strength was positively correlated to both BMI and mid-upper arm circumference (MUAC) in both sexes. Even after controlling for potential confounders, functional ability (as assessed by handgrip strength) emerged as the most important predictor of BMI explaining 15.8% and 11.6% of the variation in BMI among males and females respectively. The prevalence of undernutrition (defined as BMI < 18.5 kg/m<sup>2</sup>) was 33.7% (95% CI = 23.8% - 43.6%) among males and 27.7% (95% CI = 21.2% - 34.2%) among females. Over 90% of all respondents were independent in all activities of daily living and men were generally faster than women in performing the manual dexterity test.

The study supports the hypothesis that poor nutritional status is associated with poor functional ability in both sexes. A pilot intervention study is needed to determine whether by improving nutritional status it is also possible to improve functional ability. Our study found also that most of the sample of the elderly people remained active, contributing substantially to the household economy. The ability to remain functionally active is thus important to this section of the population. The study was supported by the Association of Commonwealth Universities and University of Malawi.

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**Age-related changes in various biochemical measurements of metabolites and antioxidant nutrients in female volunteers aged over 65 years.** By M.C. MURPHY<sup>1</sup>, S.A. NEW<sup>1</sup>, J.P. WILSON<sup>1</sup>, A. CHAMBERS<sup>1</sup>, H. DAVIES<sup>1</sup>, H. CROKER<sup>1</sup>, K. WALMSLEY<sup>1</sup>, J. BARNHAM<sup>1</sup>, M.W.J. OLDER<sup>2</sup> and M. LUMBERS<sup>3</sup>. <sup>1</sup>Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH, <sup>2</sup>Department of Orthopaedic Surgery, Royal Surrey County Hospital, Guildford GU2 5XX, <sup>3</sup>Centre for Food and Health Management, Department of Management Studies, University of Surrey, Guildford GU2 5XH

Although there is general agreement that old age is related to reduced plasma levels of certain nutrients only limited data are available for associated age-related changes. Poor antioxidant status is a common characteristic of elderly patients (Schmuck *et al.* 1996), but further data are required on antioxidant nutrient status of this population group (Department of Health, 1992). In the present study age-related changes are reported for a variety of biochemical variables. Female volunteers were recruited from three centres: (1) orthopaedic patients (*n* 19) (mean age 84 (range 71–94) years), (2) day centre visitors (*n* 11) (mean age 77 (range 65–89) years) and (3) patients in a convalescent hospital (*n* 8) (mean age 85 (range 79–89) years). A non-fasting blood sample was taken, plasma and haemolysate were separated. Plasma albumin, C-reactive protein (CRP) and cholesterol levels were assayed spectrophotometrically using kits for the Cobas Mira clinical analyser (Roche Diagnostics). Plasma Se analysis was carried out by atomic absorption spectroscopy and haemolysate glutathione peroxidase activity according to St Clair & Chow (1996). Plasma for vitamin C determination was treated with TCA (100mmol/l), frozen at -70° and analysed (Omeye *et al.* 1979). Total antioxidant status was measured as described by Miller *et al.* (1993). Pearson correlation coefficients between age and the biochemical measurements are shown:

	Analyte concentrations		Pearson correlation coefficient	
	Mean	SD	R <sup>2</sup>	P value
Albumin (g/l) and age	3.98	0.49	0.19	0.004
CRP (mg/l) and age	0.32	0.39		NS
Cholesterol (mmol/l) and age	5.74	1.74		NS
Se (µmol/l) and age	0.69	0.28	0.14	0.012
Glutathione peroxidase (mmol/min/mg Hb) and age	27.36	10.72	0.10	0.049
Vitamin C (mmol/l) and age	2.15	1.42		NS
Antioxidant status (TEAC†) and age	1.76	0.07		NS
Albumin (g/l) and CRP			0.30	0.0001
Vitamin C (mmol/l) and antioxidant status (TEAC)			0.14	0.04

†TEAC, units of antioxidant status expressed as trolox equivalent antioxidant capacity.

Significant negative correlations were found between age and plasma albumin, Se and haemolysate glutathione peroxidase levels, but no significant relationships were found between age and plasma CRP, cholesterol, total antioxidant status or vitamin C levels. The decrease in albumin concentrations may be explained by the increase in CRP levels as would be predicted from the literature (Gersovitz *et al.* 1980). Selenium values were low compared with published reference ranges (Verlinden *et al.* 1983) as were glutathione peroxidase levels compared with studies in younger adults. These data suggest that the status of certain antioxidant nutrients is reduced with age, after 65 years, and more research is required to ascertain if this is due to either poor intake, increased utilization or some other factors.

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**Relationship between smoking, antioxidant intake and status indices in older people.** By CATHERINE M. WALMSLEY, CHRIS J. BATES, ANN PRENTICE and TIM J. COLE, *MRC Dunn Nutrition Unit, Downhams Lane, Milton Road, Cambridge CB4 1XJ*

A number of studies have reported both lower intakes and blood concentrations of antioxidant nutrients in cigarette smokers (Margetts & Jackson, 1996; Marangon *et al.* 1998), but little of this work has been carried out in older people who may be vulnerable to micronutrient deficiencies, due to poor quality diets, high prevalence of disease and the physiological effects of ageing.

The aim of the present study was to examine relationships between smoking, antioxidant nutrient intake and status indices using data from the National Diet and Nutrition Survey of people aged 65 years or over in mainland Britain (Finch *et al.* 1998). Individuals (*n* 2060) living in private residences or institutions participated in the cross-sectional survey during 1994–5, having been selected using a stratified, random design. The study was restricted to 1191 subjects who completed a full 4 d diet diary, provided a blood sample, and gave information by questionnaire on their smoking habits.

Nutrient intake*	Never smokers (n 286-311)		Cigar/pipe smokers (n 35-36)		Cigarette smokers >20/d (n 98-111)		Test for trend:	P value
	Mean	SD	Mean	SD	Mean	SD		
Vitamin C (mg/d) <sup>†</sup>	49.0	49.2	50.2	40.1	36.7	0.0001		
Total carotenoids (µg/d) <sup>‡</sup>	1517	1449	1352	1079	1047	<0.0001		
Vitamin E (mg/d) <sup>‡</sup>	7.04	6.90	6.53	5.91	5.02	<0.0001		
Nutrient status indices in plasma <sup>§</sup>								
Vitamin C (µmol/l)	32.7	34.3	31.5	26.6	24.5	0.0002		
β-Carotene (µmol/l)	0.358	0.343	0.285	0.279	0.286	0.0006		
Lycopene (µmol/l) <sup>¶</sup>	0.195	0.191	0.230	0.135	0.149	0.0002		
α-Tocopherol:cholesterol (mmol/mol) <sup>‡</sup>	6.12	6.05	5.83	6.03	5.73	0.2		

Means weighted to correct for oversampling of certain age/sex groups in the main survey (Finch *et al.* 1998). Number of subjects given as a range due to variation in numbers between different indices.

\* Adjusted for age category, sex and domicile.

† Adjusted for age category, sex, domicile and nutrient intake.

‡ Variables were transformed where necessary, and means back-transformed: <sup>1</sup> log, <sup>‡</sup> square root.

The Table shows that after adjustment for age, sex and domicile (private residences or institutions) cigarette smoking was inversely correlated with intake of vitamin C, total carotenoids and vitamin E. After adjustment for age, sex, domicile and nutrient intake, cigarette smoking was inversely correlated with blood levels of vitamin C and the carotenoids, but not with vitamin E status. These associations, except that for vitamin C intake, remained significant (*P*<0.05) after further adjustment for total energy intake, alcohol intake, social class, region, receipt of benefits, depression score, self-reported health score and use of certain drugs. Mean antioxidant nutrient intake and status of never smokers did not differ significantly from those of previous smokers or cigar/pipe smokers (results not shown).

Older people who smoke cigarettes are at increased risk of suboptimal antioxidant nutrient intake and status. The lower intakes found in cigarette smokers only partly explain their reduced blood indices, which could be largely a consequence of the smoke-related oxidant load (Mezzetti *et al.* 1995). These conclusions are in line with those previously reported for younger adults.

CMW was supported by the Ministry of Agriculture, Fisheries and Food.

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**Food choice event frequencies predictive of individual fat intake: towards a self-assessment tool.**  
By DAVID A. BOOTH, KAREN R. ROWE and AISLING ARMSTRONG, *Nutritional Psychology Research Group, University of Birmingham, Edgbaston, Birmingham B15 2TT*

The aim of this project was to develop a tool for estimating the individual's usual current intake of fat, in a form that can be used for self-assessment. The tool would provide feedback so that the person could change the frequency of food choices in order to bring fat intake into line with nutritional recommendations. Food-frequency questionnaires, even when long, account for, at best, about 30% of the variance between individuals in fat intake estimated from weighed food records (Bingham *et al.* 1994; Paisley *et al.* 1996; Roe *et al.* 1996). Memory is contextually prompted and so recall of food intake is more likely to be accurate if the occasion is named as well as the food. Thus, we have begun to evaluate an adaptation of the food-frequency approach based on the recall of food choices at particular meal times or periods between meals.

Twenty sets of weighed food records collected in 4 periods of 4 different days of the week (Bingham *et al.* 1994) were selected from the control group of a community project, ten men and ten women with half of each sex having the group's highest fat intakes (mean 41.8, sd 4.0 % energy from fat) and half the lowest (mean 31.8, sd 4.5). Foods and drinks for each of six meal or between-meal periods of the day were collapsed into categories similar in fat and carbohydrate contents and in culinary concept. The number of times that an occasion-food category was recorded by an individual over the 16 d was then used as a variable in factor analysis within or across the periods of the day. This detected not only common food combinations at a meal but also associations between foods eaten on different occasions, reflecting some habit, preference or attitude prevalent in these people. The frequencies of these occasion-food items, combinations or associations were then entered as predictors in multiple regression across the twenty individuals *v.* their total fat intakes in g/d or percentage of energy.

These exploratory analyses of a few records provided only some occasion-food frequency question items to be validated in a larger sample - for example, "When did you last have a cooked breakfast including sausage or egg?" Nevertheless, their qualitative outcomes are of some interest.

The strongest high-fat predictors included, not only breakfast sausage and egg, a cooked meat meal at lunch, tea or coffee in the afternoon with whole milk and a fried supper or meat and rice, unsurprisingly, but also tea with semi-skimmed milk at breakfast and lunch and bread, salad, banana or coffee at lunch or during the afternoon, or a cooked meal for supper that included meat. Some items were predictive in the opposite direction in other periods of the day, further supporting the occasion-food approach.

In addition, at breakfast, white toast predicted high daily fat intake whereas wholemeal toast predicted low fat intake. This was presumably because the direction of choice between white and wholemeal is associated with attitudes to eating fatty foods. We have proposed that such food-specific attitudes be used to estimate under-reporting of energy intake. For similar reasons, bias in the estimates of fat intake by this tool might be reduced by asking questions about occasion-food associates and not solely about high-fat foods.

This research project is funded by the Ministry of Agriculture, Fisheries and Food, London.

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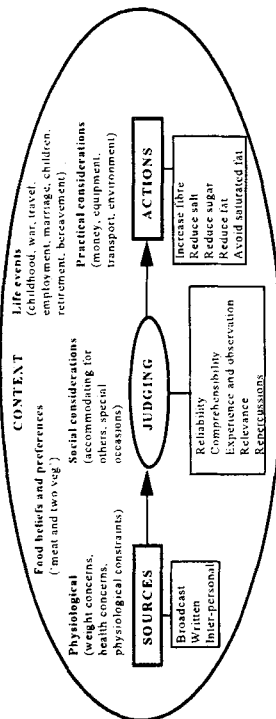
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**Food choice in older adults: the role of information.** By P. E. BELDERSON<sup>1</sup>, F. E. WOOD<sup>2</sup> and M. E. BARKER<sup>3</sup>, <sup>1</sup>Centre for Health Information Management Research, The University of Sheffield, Western Bank, Sheffield S10 2TN, <sup>2</sup>Department of Information Studies, The University of Sheffield, 211 Portobello Street, Sheffield S1 4DP, <sup>3</sup>Centre for Human Nutrition, The University of Sheffield, Northern General Hospital, Herries Road, Sheffield S5 7AU

The Nutrition of Elderly People report (Department of Health, 1992) has emphasized that older people should share in the knowledge about maintaining health through good nutrition. However, data are lacking on how best to achieve effective dietary change in this group. Therefore the present study aimed to explore the channels by which dietary information may be received by this population and to evaluate the role that these information sources played in food choice.

Participants in this study were forty-seven community dwelling older adults aged 65-74 years. The sample consisted of sixteen men and thirty-one women, the average age of whom was 70 years. Participants were all residents of the Manor Estate, a deprived area of Sheffield with a high rate of chronic disease. A qualitative approach was employed, involving in-depth, semi-structured interviews conducted at the respondents' own homes. Interview transcripts were analysed by means of an exploratory 'open coding' process (Glaser & Strauss, 1967), using the software package NUD\*IST (Qualitative Solutions & Research Ltd, La Trobe University, Australia).

The analysis resulted in the formation of a conceptual model displayed below:



Key components of the model are:

- (1) Actions, relating to actual dietary changes. Most participants had made changes to their diet (to a greater or lesser degree) for health reasons.
- (2) Context. Contextual factors mentioned relating to food choice encompassed physiological, personal, social and practical factors as well as life events. These factors impacted in various ways on information use and perceptions of information, often acting as either triggers or barriers to action.
- (3) Sources. The participants had gathered and utilized information from a variety of sources, including written, broadcast and interpersonal channels. However, although much nutrition information was received, only some provoked action.
- (4) Judging. The central strategy intervening between source reception and actions was described by participants as "using your judgement". This notion expresses the way in which participants assessed information in relation to their own lives. In doing so, they considered the following issues - reliability of information, comprehensibility of information, the extent to which the message corresponded with personal experience and observations; the perceived relevance to one's own situation, and potential repercussions of uptake.

Thus, although information was being received and utilized by this sample of older adults, the relationship between acquisition and action was by no means a straightforward one. A recognition of this complexity may help to contribute to effective nutrition education.

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**Measurements of energy density from lean and obese consumers' self-reported dietary intakes: variation and associations with other dietary components.** By DAVID N. COX, LYNN PERRY, PETER B. MOORE, and DAVID J. MELA. *Consumer Sciences Department, Institute of Food Research, Reading RG6 6BZ*

The energy density (ED, kJ/g) of dietary intake may be an important factor in the regulation of food and energy intake, and possibly play a role in body-weight status. The measurement of ED has rarely been reported and no consensus exists on how ED should be calculated from food records, and the implications of different methods have rarely been explored.

In a study, lean (LE; BMI 20-25 kg/m<sup>2</sup>) and obese (OB; BMI ≥30 kg/m<sup>2</sup>) healthy adult consumers, not on weight-loss diets nor actively losing weight, kept 4 d weighed dietary intake records. Results for three different methods of measuring ED are presented in the Table for subjects with (recorded energy intakes/estimated BMR) >1.06 (Goldberg *et al.* 1991). There were no significant differences in mean reported total food energy or macronutrient intakes between the two groups.

Method	Lean (n=41)		Obese (n=34)	
	Mean	SE	Mean	SE
(A) All foods and beverages (kJ/g)	3.2	0.08	3.5	0.1
(B) Protein, carbohydrate and fat (kJ/g)	21.0	0.4	20.8	0.6
(C) All food, including milk, excluding other non-alcoholic beverages (kJ/g)	5.9	0.1	6.5	0.2

As expected, ED varied considerably with method of calculation, and specifically with the exclusion of low-energy-dense non-alcoholic beverages and water. In addition, method of calculation influenced the absolute and relative differences in ED between the weight status groups. We also observed between-group differences in the CV for total food weight (OB, 30% v. LE, 18%) and, more importantly, water intake (OB, 34% v. LE 20%).

By any method ED and fat tended to be significantly correlated whereas for other macronutrients the correlations tended to be both method- and group-dependent. Significant correlations between ED and percentage food energy from fat were found with method B (LE  $r$  0.481,  $P$  < 0.01 and OB  $r$  0.713,  $P$  < 0.01) and method C (LE  $r$  0.543,  $P$  < 0.01 but not OB  $r$  0.323, NS). There were significant and strong inverse correlations between ED and carbohydrate (both as mean percentage energy and g weight) for method A. However, by all methods, mean fat intake (g) correlated significantly with ED for LE but not OB.

Measures of ED need to be carefully defined and handling of non-energy- and low energy-dense-items carefully considered for their impact on ED values and group comparisons.

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**Developing a national point-of-purchase guide to healthier food choices.** By MONIQUE M. RAAFS, JULIE ROYCE and LYNN STOCKLEY. *Health Education Authority, Trevelyan House, 30 Great Peter Street, London SW1P 2HW*

Partnership for health gain with the food industry has spawned a number of national point-of-purchase healthy eating guides across the world. These include the National Heart Foundation's 'Pick the Tick Scheme' (Australia), the National Food Administration's 'Green Keyhole Scheme' (Sweden), the American Heart Association's 'Food Certification Program' (USA), and the Heart and Stroke Foundation of Canada's 'Health Check Food Information Program' (Canada). Learning from these, the Health Education Authority (HEA) has been developing a national point-of-purchase guide to healthier food choices for the UK. A two-phase detailed feasibility study is being carried out; it includes consumer research; consultations with health professionals, food retailers, food manufacturers and other key stakeholders.

A recent review of effectiveness of health promotion interventions to promote healthy eating (Roe *et al.* 1997) concluded that interventions instigated at point of purchase should be supported with more detailed printed guides and promotion within the outlet. Such programmes should use simple shelf signs to identify healthier food choices, rather than providing numerical information for all food choices. The signs should be in place long term and be regularly updated; co-ordination with food outlets is required to ensure a consistent message and address both commercial and health considerations.

A qualitative study, involving over 200 consumers (120 semi-structured one-to-one interviews and 10 focus groups) and, was conducted as part of the HEA's feasibility study. Consumers were receptive to the concept of product endorsement. It was felt that it would be beneficial in helping them to save time and in making healthier choices within specified product categories. It was also found that current UK supermarket schemes are selectively recognized and used on a product-specific basis, highlighting a "healthier choice" alternative. Schemes with a clear image, backed by a verbal claim were viewed most positively. The study concluded that an endorsement symbol must: state its backing by a credible and authoritative source whilst remaining independent of the government and, importantly, the manufacturer; be clearly recognizable, bold, eye-catching, not too detailed and complicated; and contextually appropriate, in keeping with, rather than contradicting, current schemes. The HEA was perceived to be in a unique position between the government, the manufacturer and the consumer. A product carrying the HEA logo was widely expected to have been independently tested by HEA representatives, not the manufacturer. Most assumed the testing would occur on a random basis at least once annually. Many felt that providing the manufacturer with guidelines for testing would not be sufficient to avoid corruption; this independence needs to be maintained by a clear statement of testing guidelines and procedures.

Existing of national point-of-purchase healthy eating guides are said to have an impact on making healthier products available through reformulation and product development. Evidence from Australia suggests that the 'Pick the Tick Scheme' is viewed as a positive marketing tool for industry. Based on the findings of the feasibility study the HEA is continuing to invest in developing the project.

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**The impact of a nutrition education intervention to increase fruit and vegetable consumption in an area of urban deprivation.** By ANNIE SANDERSON, ALI BELL and RITA CALLANDER, *Centre for Applied Nutrition Research, Matthew Building, University of Dundee, Perth Road, Dundee DD1 4HT*

Diet and disease patterns vary by socio-economic circumstances and area of residence. One reason for these findings may relate to the access and availability of a range of good quality foods (particularly fruits and vegetables (FAV)) at a reasonable cost (Department of Health, 1996). Many community based food initiatives to assist low-income consumers to change dietary patterns have developed over the last decade, enabling some of the perceived barriers to healthy eating to be addressed at a local level. A recent audit of Community Food Initiatives in Scotland (Anderson *et al.* 1996), highlighted a wide range of food-related activities which include educational interventions, but it is recognized that these have rarely been evaluated in a systematic manner (Nelson, 1997).

In order to evaluate an educational intervention within a community food initiative (a food co-operative) aimed at increasing FAV consumption in an area of urban deprivation in Dundee, a mail-based programme (with additional opportunities for food-based interactive learning sessions) was developed, and assessed 5 months after implementation. Adults (n=200; randomly allocated into control and intervention groups) who lived in households with children, resident in Whitfield, Dundee were studied. Subjects participated in pre- and post-intervention validated portion sheet measurements (Cox *et al.* 1997) and questionnaire studies, and provided details on perceived confidence about ability to prepare FAV (nine items), knowledge about recommended portions for consumption (one item) and FAV purchase (forty-one items).

Repeat questionnaires were returned and completed on both occasions by 78% of the sample and validated portion measure sheets by 23%. The majority (79%) of the intervention group reported receiving the mailed packages and the most widely remembered component of the packages (total of thirteen items) was a locally produced cookery booklet which 68% claimed to have read. Self-reported portion measures showed that the proportions of subjects achieving "5-a-day" increased from 27% to 40% in the intervention sample compared with a rise of 34% to 42% in the control sample (NS). Usage of the FAV co-op increased in the intervention group from 6% to 21% pre-post intervention compared with 2% to 11% in the control group (NS). Changes in items relating to cooking confidence were significantly better in the intervention group compared with control groups but there were few other significant differences between the groups for changes in household purchases and no difference in knowledge of recommended portions during the intervention period.

Variable	Intervention		Control		Significance of differences compared: P=
	Pre-Post difference	Mean	Pre-Post difference	Mean	
Confidence in preparation of root vegetables	+0.45	1.03	+0.08	1.20	0.005
Confidence in preparation of vegetable soup	+0.51	1.53	-0.22	1.61	0.04
Household Purchase of FAV (kg/week)					
Lettuce	-0.16	0.27	-0.07	0.29	0.045
Mandarin	+0.21	0.37	+0.11	0.26	0.055

Scale -3 to +3  
These results suggest some impact of the intervention on process aspects of increasing FAV but limited effect on outcome in terms of consumption.

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**Within-subject reproducibility of dietary restraint questionnaires.** By D.A. HUGHES, C. REID and R.J. STUBBS, *Rowett Research Institute, Greenburn Road, Aberdeen AB21 9SB*

It is important to characterize the degree of restraint in subjects participating in feeding studies because restrained eaters show differences in their feeding patterns relative to unrestrained eaters. Restraint is subjectively determined using the Dutch eating behaviour questionnaire (DEBQ; van Strien *et al.* 1986) and the three factor eating questionnaire (TFEQ; Stunkard & Messick 1985). These questionnaires are primarily used to predict restrained eating behaviour in short-term studies. However, in order to use restraint in longer-term studies the temporal stability of the restraint construct should be demonstrated. If not, its utility in longer-term studies would be compromised. We hypothesized that both questionnaires would demonstrate within-subject and between-questionnaire reproducibility in both the short and medium-long term. The present study determined the short (day to day, Expt. 1) and medium-long term (week to week, Expt. 2) within-subject reproducibility of the restraint factor of the DEBQ and TFEQ in a UK population.

In Expt. 1, eight women, (weight 61.92 (SD 10.88) kg; height 1.67 (SD 0.08) m; age 30.63 (SD 9.42) years) and eight men, (weight 75.88 (SD 10.67) kg; height 1.78 (SD 0.07) m; age 25.13 (SD 6.10) years) were studied over a 1-week period. Twice during this 1-week period subjects completed both the DEBQ and TFEQ, at the same time. In Expt. 2, eight lean women, (weight 61.28 (SD 5.91) kg; height 1.65 (SD 0.06) m; age 37.44 (SD 11.62) years) and sixteen overweight women, (weight 72.62 (SD 6.76) kg; height 1.62 (SD 0.06) m; age 38.37 (SD 6.81) years) were studied over a 4-week period. Subjects completed both the DEBQ and TFEQ in a single session which was repeated 4 weeks later. All questionnaires were completed under laboratory conditions.

Bland and Altman analysis revealed that there were no significant changes in restraint response between days (Expt. 1) and between weeks (Expt. 2) for either questionnaire. There was no tendency for bias in subjects who had high or low restraint responses. Regression analysis (see Table) suggested that both the DEBQ and TFEQ had higher within-subject reproducibility between days than over 4 weeks. Overweight women also produced more consistent restraint scores between weeks than lean women. Regression analysis of the pooled data from both experiments revealed a strong positive correlation ( $r^2$  80.5 %,  $p < 0.001$ ) between the DEBQ and TFEQ when completed in the same session, although the tabulated data revealed greater within-subject reproducibility in the DEBQ. The lower correlation coefficients in lean women (see Table) can be explained by high variation in one subject.

Expt.	Group	Within-subject reproducibility between repeated questionnaires		TFEQ score	
		adjusted $r^2$ (%)	SE †	adjusted $r^2$ (%)	SE †
1	Men (lean) <sup>c</sup>	88.2 ***	2.46	80.7 **	1.61
	Women (lean) <sup>c</sup>	96.3 ***	1.73	88.7 ***	1.81
2	Women (lean) <sup>c</sup>	74.2 **	3.64	59.7 *	2.12
	Women (overweight) <sup>d</sup>	85.7 ***	2.89	74.8 ***	2.31

† SE of the observations \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

These data suggest that both the TFEQ and the DEBQ show good within-subject reproducibility both across time (up to 4 weeks) and between questionnaires in this study population. This suggests that in nutritional intake studies (without extensive weight loss) restraint is a useful and stable construct.

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**Fruit, vegetable and fat consumption of people who believe their diet is healthy.** By J.M. LAWRENCE and B.M. MARGETTS, *Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX*

A recent study (Kearney *et al.* 1997) of the perceived need to alter eating habits amongst adults in the European Union found that 25% of UK subjects agreed with the statement, "I do not need to make changes to the food I eat, as it is already healthy enough." Healthy eating campaigns may be unsuccessful if they are not felt to be relevant to people who believe that their diet is already healthy.

We compared the dietary and demographic characteristics of a nationally representative sample of 5553 (70% response rate) English adults (Margetts *et al.* 1997), 24% of whom believed that they did not need to change their diet as it was already healthy enough. The total fat and fruit and vegetable content of the diet was assessed using the DINE (Roe *et al.* 1994) questionnaire. The fruit and vegetable content of the respondent's diet was also the basis for three groups: low intake (fruit or vegetables 4 d/week), moderate intake (fruit or vegetables each day), high intake (fruit and vegetables each day). Cluster analysis was used to summarize the demographic characteristics of the respondents. The greatest differences between the groups were achieved using four clusters.

Fruit and vegetable content of diet Dietary characteristics	Average fat intake (g/d)			High
	Low	Medium	High	
Total sample (n 5553)	110*	102 <sup>b</sup>	94 <sup>c</sup>	94 <sup>c</sup>
Made "healthy" changes* (n 2765)	101*	95 <sup>b</sup>	90 <sup>c</sup>	90 <sup>c</sup>
Believed diet healthy (n 1324)	99	99	94	94
Cluster 1, young, educated	97	98	96	96
Cluster 2, younger, worse off	97	101	115	115
Cluster 3, educated, better off	93	100	89	89
Cluster 4, older, less educated	107	97	97	97

\* Mean values in each row with unlike superscript letters were significantly different, P<0.0001.

<sup>a</sup> Changed diet in past three years to make it healthier.

The table presents the comparison of mean fat intakes by fruit and vegetable content. Respondents believed that their diet was already healthy (24%) or changed their diet to make it healthier (52%). In the total sample average fat intake fell as fruit and vegetable consumption rose; this was also true for adults who made healthy changes. Fat consumption by people who believed that their diet was healthy was less in the group who ate the most fruit and vegetables. When the group who believed that their diet was healthy was broken into demographic clusters, younger worse-off respondents (cluster 2) showed a fat consumption that increased with consumption of fruits and vegetables. These respondents may be confused about healthy eating messages or they may be making pragmatic decisions about feeding a young family on a low budget. These results suggest that high fruit and vegetable intake is associated with the belief that the diet is healthy. People may find it difficult to include an assessment of both fruit and vegetable intake and fat intake when thinking of a healthy diet. Respondents who believed that their diet was healthy were less likely to include low fat (14%) in their description of a healthy diet than respondents who changed their diet to make it healthier (20%). Dowler *et al.* (1995) looked at lone parents demographically similar to our cluster 4 group. Having a parent who looked for "freshness" when choosing food was associated with higher fruit and vegetable variety in children's diets but there was a need to "fill people up", "children demand to be fed". The high-fat, high-fruit-and-vegetable diet consumed by these cluster 4 respondents may meet an immediate health need for energy.

In promoting the concept of a healthy diet are we in danger of assuming that those who do not conform are not healthy?

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**An evaluation of the Wessex Healthy Schools Award: healthy eating.** By R.L. THOMPSON<sup>1</sup>, A. MOON<sup>1</sup>, M. MULLEE<sup>2</sup>, V. SPELLER<sup>1</sup> and P. RODERICK<sup>1</sup>, <sup>1</sup>Wessex Institute for Health Research & Development, and <sup>2</sup>Medical Statistics and Computing, University of Southampton, SO16 6YD

Healthy Schools Awards aim to promote the development of a health-promoting school. The Wessex Healthy Schools Award (WHSA) was inaugurated in 1992 as an alliance between Health and Local Authorities in Wessex (Rogers *et al.* 1993). Healthy eating is one of the nine key areas, and the award aims to increase the focus of healthy eating in the curriculum and increase the availability of healthy food within the school. To date, the effectiveness of Healthy Schools Award Schemes in promoting positive health-related change in school management, policy and process and in pupil knowledge, attitudes and behaviour has not been assessed.

An evaluation of WHSA was carried out using a non-randomized controlled trial. Schools were recruited from those joining the WHSA in the Autumn of 1995. We aimed to recruit twelve intervention and twelve control schools matched according to the percentage of pupils entitled to free school meals, and social status. Two samples of pupils were studied: a cohort of Year 7 pupils (aged 11-12 years) at baseline who were surveyed again as Year 8 (12-13 years) at follow-up, and a cross-sectional survey of Year 11 pupils (15-16 years) carried out in 1995 and again 15 months later on a separate cohort of pupils. The data presented here are taken from a self-administered questionnaire which was completed by pupils in the classroom.

Eleven intervention and five control schools were recruited (one intervention school subsequently dropped out). The Tables below show results at baseline and the difference at follow-up (follow-up - baseline) for questions relating to fruit and vegetables.

	Males (Year 8 - Year 7)				Females (Year 8 - Year 7)			
	Intervention (n 10)		Control (n 5)		Intervention (n 10)		Control (n 5)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Chose fruit and vegetables as healthy* (%)	58.2	11.9	59.9	6.8	79.8	9.8	77.4	13.6
Change	0.5	17.8	1.4	8.6	-2.7	15.6	3.1	13.3
Reported eating fruit 6-7 d/week (%)	43.5	11.0	41.6	5.4	51.5	20.8	44.7	13.5
Change	0.4	12.3	-0.7	9.1	-2.6	11.7	-2.7	13.4
	Males (Year 11)				Females (Year 11)			
	Intervention (n 10)		Control (n 5)		Intervention (n 10)		Control (n 5)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Chose fruit and vegetables as healthy (%)	59.5	13.5	58.1	12.5	72.5	16.9	81.0	2.4
Change	0.5	15.7	4.8	15.4	11.2	17.9	2.8	5.5
Reported eating fruit 6-7 d/week (%)	31.0	14.1	33.8	3.6	42.2	7.7	46.8	14.3
Change	-2.2	16.1	-4.4	10.1	14.6	12.6	-5.8	12.3

\*Question: Write down five foods you think would be healthy for you.

The Tables show that the WHSA had little impact on knowledge about fruit and vegetables or on reported fruit consumption. Females in Year 11, however, showed a more favourable result. In comparison with the control schools the intervention schools showed a 20% increase in the percentage of Year 11 females reporting that they ate fruit 6-7 d/week. This evaluation overall did not show change in knowledge about or behaviour towards fruit and vegetables in schools achieving the WHSA.

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**Costs of an (un)healthy diet: analysis from the UK Women's Cohort Study.** By HENDRIKE UPMEIER\*, JANET E. CADE and CLAIRE CALVERT, *Nuffield Institute for Health, University of Leeds, 71-75 Clarendon Road, Leeds LS2 9PL*

There is a belief within the population that eating healthy food is expensive (Health Education Authority, 1995). However, very little research has explored the truth of this belief (Cade & Booth, 1990). In the present study we investigated the marginal cost differences between eating a healthy and an unhealthy diet. The data for this analysis came from baseline data of the UK Women's Cohort Study. This cohort is designed to study women aged 35-69 years with a wide range of food habits (vegetarian, meat and fish eating). Subjects in this analysis were from the first 15 191 women who responded to a detailed lifestyle and food-frequency questionnaire (217 food items) (Calvert et al, 1997). A healthy diet indicator (HDI), with values from 0 (lowest) to 8 (highest), was developed based on the WHO dietary recommendations (WHO Study Group, 1990) for fats, carbohydrate, sugars, fibre, fruit and vegetables, pulses, nuts and seeds. Direct monetary cost of the diet was calculated using prices from the National Food Survey 1995 and Tesco home shopping catalogue. Indirect costs, such as location of shops, frequency of shopping etc., were assessed by telephone interviews focused on fifty-two subjects with HDI score 0 and fifty-two with score 8.

HDI	Vegetarian		High education level (%)	Shop only 1 or 2x/week* (%)	More expensive to eat healthy? * (% yes)	Cost of food/d (£)	
	n	(%)				Mean	SD
0 (lowest)	122	20	24	60	40	2.33	0.72
8 (highest)	75	78	50	35	29	3.81	0.96
Overall	15 185	42	28	-	-	3.43	1.30

\*Based on 104 telephone interviews.

Women in the healthy diet group were four times as likely to be vegetarian and to have a higher educational level. For direct costs, the difference between the most extreme HDI groups was £1.48/d (equivalent to £540/year), with fruit and vegetable expenditure being the main items making a healthy diet more expensive. Of the food budget 49% was spent on fruit and vegetables in HDI group 8 compared to 29% in HDI group 0. Interestingly, 51% of those questioned in both extreme HDI groups did not think that it was difficult to eat healthily.

To achieve a particularly healthy diet the women spent more money and more time on shopping for food, than women with a less healthy diet.

This work was supported by the World Cancer Research Fund and the UK Women's Cohort Study Steering Group.

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**Nutritional knowledge and attitudes to healthy eating amongst Surrey schoolchildren: preliminary data.** By SUSAN A. NEW, ANNA L. MATTHEWS, JANET CATTERICK, SARA THORNTON, AND JACKI A. BISHOP, *Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH*

The dietary habits of schoolchildren, particularly those in secondary school education, have generated a considerable amount of concern. Evidence in the literature points to a number of key behavioural patterns including frequent meal skipping, regular snacking on high-sugar, high-fat products (including confectionery and soft drinks), an intense popularity of fast and take-away foods and a high incidence of dieting among adolescent girls (Adamson et al. 1996). The consequences of these habits are likely to affect not only short-term health but may also hinder health in the longer term.

As part of a large investigation into dietary intake and lifestyle patterns among Surrey schoolgirls (Catterick et al. 1998), a second study is currently underway to establish levels of nutritional knowledge, the relationship between knowledge and dietary habits and the factors affecting food choice among adolescent girls and boys aged 11-16 years. A total of 500 subjects from a variety of schools in the Surrey area (separate from those in our main study) are being targeted. Randomly selected subjects were asked to complete, on separate occasions, two self-administered questionnaires on nutritional knowledge and healthy eating together with background information on dietary habits and socio-economic status. From the data collected, a nutritional knowledge score (NKS) and healthy eating behaviour score (HEBS) were calculated. Appropriate non-parametric statistics were used to analyse the data. Although the HEBS cannot be assumed to be an accurate indicator of healthy eating practices, it does have value as a means of ranking groups to enable comparisons to be made (Watt & Sheiham, 1996). Preliminary data are presented on 103 subjects (fifty-three males, fifty females) from the five year groups in one of the schools targeted: Year 7 (11-12 years), eleven male, seven female; Year 8 (12-13 years), eleven male, seven female; Year 9 (13-14 years), eleven male, eleven female; Year 10 (14-15 years), eleven male, thirteen female; Year 11 (15-16 years), nine male, twelve female.

Nutritional knowledge was found to vary considerably, even within the same age and sex groups. At the whole-group level, a significant positive correlation was demonstrated between knowledge and healthy eating behaviour ( $P < 0.01$ ). Taste was reported to be the prime influence on food choice (32%), followed by 'good for you' (23%), cost (19%), 'easy to eat' (14%) and availability (12%). Those subjects who indicated 'good for you' had a significantly higher HEBS ( $P < 0.001$ ). Females had higher HEBS than males ( $P < 0.01$ ) and an average of 39% of females reported wanting to weigh less. School lessons and the family were identified as the main sources of information regarding healthy eating, with the school canteen being selected by only 10% of subjects.

Further research in the study population is required. However, the finding that nutritional knowledge does influence dietary behaviour and the apparent consideration given to 'healthiness' in food choice may have important implications for the design of future dietary intervention studies.

We are indebted to the staff at the Bishop Reindorp Secondary School, Guildford, Surrey for their help with subject recruitment, and in particular, Mr J.C. Maton, Headteacher, Miss C. Foley, Mrs T. Green, Miss S. Hitchcock, Mr A. Mamen and Miss H. Tank.

JC holds a Daphne Jackson Fellowship, funded by SmithKline Beecham Pharmaceuticals, Research and Development.

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**Adverse reactions to foods in asthmatic young children.** By M.Y. NOORAINI, S.M. HAMPTON and JANE MORGAN, School of Biological Sciences, University of Surrey, Guildford GU2 5XH

There has been a steady increase, over the past 10-15 years, in public awareness and interest in the importance of diet and health associated with the rising incidence of "food allergy" (Lessoof, 1994). Unfortunately, the term "allergy" is often associated with the public with any type of adverse reaction be it immunologically based or not. The aim of the present study was to determine the extent of adverse reactions to foods that induced asthmatic episodes in young children as perceived by parents, and food avoidance practices as treatment for asthma.

Seventy-one volunteers aged between 2 and 15 years, clinically diagnosed with mild to moderate asthma, participated in this study. Parents completed a structured questionnaire which included adverse reactions of subjects to foods and drinks, avoidance of any particular foods or drinks in the treatment of their asthma, and parents' perceived views of food avoidance. The results are shown below:

**Table 1.** Adverse reactions to different types of foods in asthmatic children as reported by parents

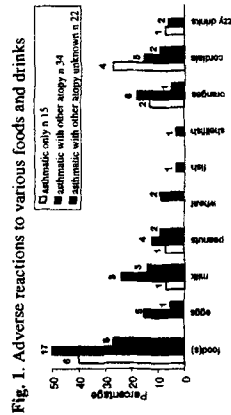
Study groups:	n	No. of different foods			
		0	1	2	3
1	15	9	3	3	-
2	34	17	8	4	2
3	22	16	3	1	1
1+2+3	71	42	14	8	3

n number of children in study groups.

Study group 1 = asthmatic alone.

Study group 2 = asthmatic with other atopy.

Study group 3 = asthmatic with other atopy unknown.



**Fig. 1.** Adverse reactions to various foods and drinks

**Table 2.** Current and past avoidance of foods by asthmatic children who reported having food allergy

Study groups:	n	n <sub>1</sub> (%)	Avoided foods for asthma	
			current	past
1	15	6 (40)	1 (17%)	0
2	34	17 (50)	8 (47%)	5 (29%)
3	22	6 (27)	1 (17%)	0
1+2+3	71	29 (41)	10 (35%)	5 (17%)

n number of children in study groups.

n<sub>1</sub> number of children with adverse reactions to foods.

Study groups 1, 2 & 3 - refer to study groups in Table 1

Results show that 41% (n 29 of 71) of parents observed that their children had adverse reactions to foods in relation to their asthmatic condition. Fig. 1 gives details of the number and percentage of children reported having adverse reactions to various foods and drinks. Table 1 shows that fourteen (48%) children were affected by one type of food. Fewer children reported having adverse reactions to more than two types of food.

Of those considered to have adverse reactions to foods, 35% (n 10 of 29) avoided the food(s) at the time of recruitment and 17% (n 5 of 29) had avoided the foods in the past for their asthma as reported by questionnaire (see Table 2).

The results indicate that self-diagnosed adverse reactions to foods and self-imposed allergy diets are relatively common in asthmatic children. The use of an elimination diet without professional advice from dietitians should be a cause for great concern as it will affect the quantity and quality of food intake particularly in asthmatic children who are in danger of an inadequate diet (Nooraini *et al.* 1998).

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

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**Characterizing dietary supplement use in women.** By SARA F.L. KIRK<sup>1</sup>, JANET E. CADE<sup>1</sup>, MARK CONNER<sup>2</sup> and JENNIFER BARRETT<sup>3</sup>, <sup>1</sup>Division of Public Health, Nuffield Institute for Health, Leeds LS2 9PL, <sup>2</sup>Department of Psychology, University of Leeds, Leeds LS2 9JT and <sup>3</sup>ARC Epidemiology Research Unit, Storrford Building, University of Manchester, M13 9PT

Dietary supplements are increasingly being used, despite the lack of evidence to suggest that they are generally needed to meet deficiency of nutrients. According to Mintel (1997), the main consumers in the vitamins and supplements market tend to be women in their forties, fifties and sixties, with higher than average disposable incomes. *The Dietary and Nutritional Survey of British Adults* (Gregory *et al.* 1990) reported that those who took dietary supplements paradoxically had higher recorded nutrient intakes from food sources alone than those who did not take supplements. Given that supplement users are more likely to consume sufficient nutrients from dietary sources, an understanding of the characteristics of dietary supplement users could be useful in discouraging unnecessary use of dietary supplements. The UK Women's Cohort Study is a national study being conducted on 33 000 women (Woodhouse *et al.* 1997). So far, data on supplement use, lifestyle factors and nutrient intakes are available on 13 822 women, 61% of whom have reported taking dietary supplements. This high level of usage provides a unique opportunity to study dietary supplement use in relation to other health behaviours in a large group of women. Thus the aim of the present study was to describe the characteristics of supplement users within this cohort.

Analysis was carried out using a logistic regression model. Lifestyle factors for inclusion in the model were selected on the basis of the hypothesis to be tested that supplement users would have a healthier lifestyle profile than non-users, using the variables age, BMI, smoking status, dietary habits, alcohol intake, intake of fruit and vegetables and activity (see Table). These variables had been found through univariate analysis to be statistically significantly different between users and non-users. Education status was not included as it was not significantly different between the two groups, a finding which may be explained by the nature of the recruitment of the sample, as subjects were not randomly selected and generally comprised highly educated subjects.

	Mean age (years)	Mean BMI (kg/m <sup>2</sup> )	Never vegetarian/vegan (%)	Never smoked (%)	Eating >4 helpings fruit and/or vegetables/d (%)	High levels of physical activity (%)	Drinking <1 unit alcohol/week (%)
Users (n 8409)	51.4	23.7	18	35	12	23	22
Non-users (n 5413)	50.1	24.2	11	23	6	13	14

Logistic regression modelling found that, after controlling for the other independent variables, all the variables included had a significant effect on whether subjects took dietary supplements. Subjects with a BMI over 25 were less likely to use dietary supplements, while those with a BMI below 20 were more likely to use supplements, compared with those in the normal range. Supplement use was also associated with being vegetarian, vegan or fish-eating as opposed to eating meat, consuming more fruit and vegetables, being more physically active and being less likely to drink or smoke. This fits in with our hypothesis, that supplement users are more likely already to have a healthier lifestyle than non-users and that supplement use could be unnecessary for the majority of supplement users.

This research was supported by the Ministry of Agriculture, Fisheries and Food and by the World Cancer Research Fund. We acknowledge the contribution of the UKWCS Steering Group to the cohort study.

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**Impact of diet intervention on the growth of asthmatic children.** By M.Y. NOORAINI, S.M. HAMPTON and JANE MORGAN, *School of Biological Sciences, Guildford GU2 5XH*

Currently, little is known about the impact of asthma on diet, however, food-related complaints have important nutritional implications. The present study examined the impact of diet intervention (devoid of eggs and milk for 8 weeks attempt to reduce the occurrence of severity of asthmatic symptoms) on the growth status of the asthmatic children.

Twenty-two children aged between 3 and 14 years (5.9 (SEM 0.6) years), clinically diagnosed with mild to moderate asthma participated in this diet-intervention prospective study. Thirteen children were allocated to the diet group (DG) who excluded eggs and milk from the diet for 8 weeks, and nine children were allocated to the non-diet group (NDG) who continued with their customary food intake. Weight and height of all children were measured during the pre-diet and post-diet assessment. All children in the DG were requested to consume a daily helping of Marmite (4 g) for riboflavin and a CaCO<sub>3</sub> supplement (Calcichew, 500 mg tablet) on alternate days. The children were provided with hypoallergenic milk supplement (Pepti-Junior) whenever requested. Parents were given a booklet that lists various commercial food items that are egg and milk free.

The results show that there was no significant weight gain or loss in either the DG or the NDG after 8 weeks without eggs and milk (Table 1). In addition, both groups appeared to have significantly gained in height (Table 2).

**Table 1:** Pre-study and post-study intervention weight (kg) in the diet group and the non-diet group

Study groups:	n	Pre-study weight (kg)			Post-study weight (kg)		
		Mean	SD	Range	Mean	SD	Range
Diet group	13	23.8	8.4	14.4-46.0	23.6	8.6	14.1-47.1
Non-diet group	9	21.8	3.8	15.7-27.8	22.2	3.7	16.9-27.8

**Table 2:** Pre-study and post-study intervention height (m) in the diet group and the non-diet group

Study groups:	n	Pre-study height (m)			Post-study height (m)		
		Mean	SD	Range	Mean	SD	Range
Diet group	13	1.190	0.172	0.958-1.619	1.197**	0.170	0.975-1.620
Non-diet group	9	1.121	0.079	1.005-1.238	1.126**	0.082	1.007-1.247

Mean value was significantly different from that for the corresponding pre-study value, \*\*P<0.01.

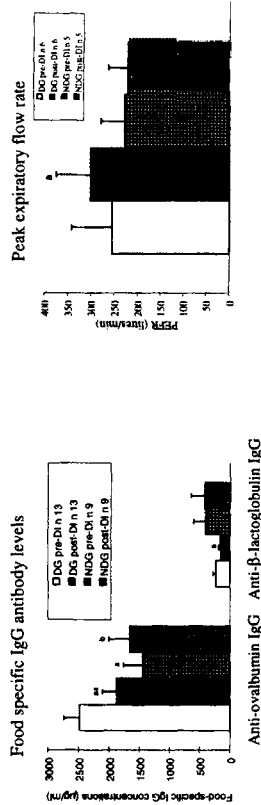
In conclusion, this study indicated that proper advice and close supervision from the dietitians in any diet elimination study was important to maintain and achieve good nutritional status of the individuals. However, for parents to place their children on an elimination diet without the professional guidance of dietitians may produce negative effects on the growth status of the individual, especially children. Therefore, to maintain and/or achieve good nutritional status, advice is needed from dietitians to provide important practical information on: (1) inexpensive and alternative foods that are available in supermarkets or shops; (2) foods that may hide the food allergen under known and unknown names; (3) ensuring diet adequacy to prevent any weight loss and nutrient deficiency; (4) how to achieve patient compliance.

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**Diet intervention (DI) in the treatment of asthma in young children.** By M.Y. NOORAINI<sup>1</sup>, A.S. MALLIK<sup>2</sup>, P. NAIR<sup>2</sup>, S.M. HAMPTON<sup>1</sup> and JANE MORGAN<sup>1</sup>, *School of Biological Sciences, University of Surrey, Guildford GU2 5XH and <sup>2</sup>Frimley Park Hospital, Frimley GU16 5UJ*

Current understanding of the role of excluding foods in the treatment of asthma in young children is still limited and controversial. The present study examined the benefits of an exclusion diet to reduce the occurrence of severity of asthmatic symptoms.

Twenty-two children aged between 3 and 14 years (5.9 (SEM 0.6) years), clinically diagnosed with mild to moderate asthma participated in this single blind, prospective study. Parents were given the option to volunteer their children either to the diet group (DG), who excluded eggs and milk from the diet for 8 weeks, or to the non-diet group (NDG), who continued with their customary food intake. Thirteen children were allocated to the DG and nine children to the NDG. All children were assessed by trained paediatricians and a blood sample was collected at the beginning and at the end of the study period. The serum was analysed by indirect ELISA for the determination of the anti-ovalbumin immunoglobulin G (IgG) and anti-β-lactoglobulin IgG (Hampton *et al.* 1990). Of the twenty-two children, six in the DG and five in the NDG were able to perform the peak expiratory flow rate (PEFR) correctly. The results are illustrated below. As the data were not normally distributed despite being logarithmically transformed, the statistical analyses were performed using non-parametric, Wilcoxon Signed Rank Sum tests.



Mean values were significantly different from DG pre-intervention: \*P<0.05, \*\*P<0.01. Mean values were significantly different from NDG pre-intervention: \*P<0.05.

The mean pre-DI anti-ovalbumin IgG level in the DG was significantly higher than that for the ovalbumin IgG level was significantly higher than the mean post-DI value, 2483 (SE 259) µg/ml v. 1876 (SE 223) µg/ml respectively (P<0.001). However, the mean pre-DI anti-ovalbumin IgG level in the NDG was significantly lower than the mean post-DI value, 1447 (SE 318) µg/ml v. 1659 (SE 344) µg/ml respectively (P<0.05). In the DG, the mean pre-DI anti-β-lactoglobulin IgG level was significantly higher than the mean post-DI value, 223 (SE 51) µg/ml v. 149 (SE 30) µg/ml respectively (P<0.05). However, the mean pre-DI anti-β-lactoglobulin level in the NDG was not significantly different from the mean post-DI value, 399 (SE 200) µg/ml v. 415 (SE 228) µg/ml respectively. In the DG, the mean pre-peak expiratory flow rate (PEFR) was significantly lower than the mean post-value, 235 (SE 35) litres/min v. 301 (SE 30) litres/min respectively (P<0.05). However, the mean pre-PEFR in the NDG was not significantly different from the mean post-value, 228 (SE 23) litres/min v. 222 (SE 19) litres/min respectively.

Our observations suggest that the well-being of asthmatic children may be improved with dietary restriction of potent allergens such as eggs and milk.

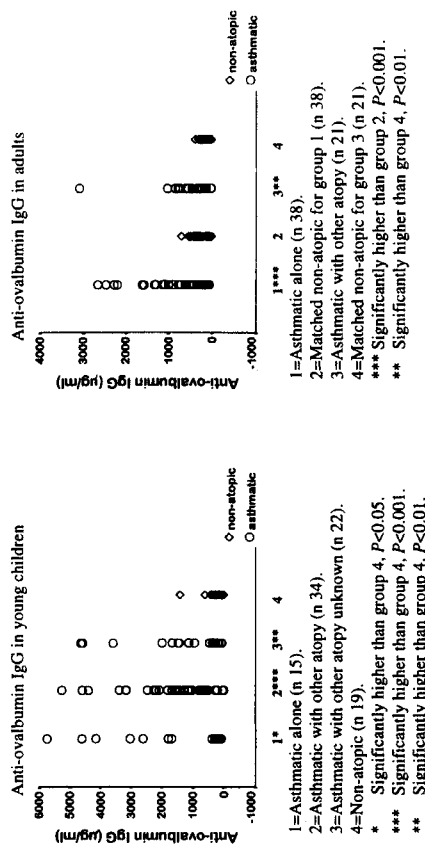
This research was funded by The Malaysian Government. We acknowledge the assistance of Frimley Children's Centre, Frimley Park Hospital and The Royal Surrey County Hospital.

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**Specific food immunoglobulin G (IgG) antibody levels to ovalbumin in asthmatic and non-atopic young children and adults.** By M.Y. NOORAINI, S.M. HAMPTON and JANE MORGAN, *School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

Adverse reactions to foods (by immune response) are more common in the child than in the adult populations (Lesof, 1994). This may be partly because of the large intake of food proteins, and the increased permeability of the less mature gut in children than in adults. However, our understanding of the role of foods in asthmatic individuals is limited. The purpose of the present study was to investigate specific food antibody levels to ovalbumin in asthmatic young children and adults and compare them to those in non-atopic young children and adults.

Recruited into the study were seventy-one children, aged 2-15 years, and fifty-nine adults clinically diagnosed as asthmatic; nineteen non-atopic children; and forty-three non-atopic adults matched for sex and age (sixteen non-atopic adults were matched twice against the two groups of asthmatic adults). A casual blood sample was collected and serum analysed by indirect ELISA for the determination of the anti-ovalbumin IgG (Hampton *et al.* 1990). The results are shown below. As the data were not normally distributed despite being logarithmically transformed, the statistical analyses were performed using non-parametric, Mann-Whitney U tests.



The results show that there were significantly higher levels of the anti-ovalbumin IgG in asthmatic children and adults compared with non-atopic controls. Similar observations were reported in a study on asthmatic children compared with age- and sex- matched healthy children (Nooraini *et al.* 1998).

The findings reported here show that in this study of asthmatic children and adults, there were differences in the food antibody IgG levels to ovalbumin, which suggest that asthmatic children may be more likely to have adverse reactions to foods than asthmatic adults.

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**Longitudinal observations of weight change in inoperable lung cancer.** By JOCELYN A. KRAMER and MARINOS ELJIA, *Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH*

Nutritional support is often advocated in patients with cancer because weight loss is associated with deterioration in body function and well being. Surprisingly, longitudinal patterns of weight change from diagnosis to death are poorly documented, even in patients with common malignancies such as lung cancer (Costa *et al.* 1980; Lindsey & Piper, 1985; Sarna *et al.* 1994; Palomares *et al.* 1996), where the presence of oedema has been ignored.

The aims of the present study were to document the extent and pattern of weight change from diagnosis to death in patients with inoperable lung cancer, and to examine two hypotheses: (1) weight loss rarely exceeds 10% of the pre-illness weight and BMI rarely falls below 20 kg/m<sup>2</sup>; and (2) oedema is frequently present and confounds interpretation of the weight change.

Sixty patients with histologically proven inoperable lung cancer (forty-five male, fifteen female; median age 72, range 45-86 years) were studied every 8 weeks from diagnosis. Measurements included weight, height, and grade of oedema (absent, mild, moderate, severe).

Data from forty-six (77%) patients who had died were available for analysis. Median time from diagnosis to first measurement was 41 (range 15-97) d, and from last measurement to death was 29 (range 3-106) d. Median duration of the observation period was 136 (range 26-587) d. Forty-four patients (96%) lost weight from pre-illness; mean change from self-reported pre-illness weight to first measurement was -5.7 (SD 6.0) kg and from first to final measurement was -4.7 (SD 7.4) kg. These values represent mean losses of 7.4% and 6.5% respectively. Fourteen patients (30%) lost > 10% of their pre-illness weight by the first measurement; eighteen (39%) lost >10% of their first measured weight while under observation. The proportion of patients with BMI < 20 kg/m<sup>2</sup> rose from 6% at the first observation to 28% at the last measurement before death.

Oedema was observed in twelve patients (26%) at the first assessment and in twenty-three (50%) at the last. Severity of oedema increased with disease progression, from four patients (9%) having moderate/severe grades initially, to fourteen (30%) moderate/severe at the last measurement. Despite this, a general pattern of progressive weight loss was seen throughout the illness (Table).

	Mean	SD
Pre-illness	26.4	4.9
First measurement	24.4 ***	4.9
Mean during illness	23.9 ***	4.7
Final measurement: all patients	22.7 ***	4.5
: those without oedema (n 23)	21.6 ***	4.3

Significantly different from pre-illness value, \*\*\*P<0.0001 (repeated measures ANOVA).

Mean BMI values in the Table fail to show that individual patterns of weight change varied widely. Twenty-five (54%) patients gained at least 0.5 kg from their first measured weight, usually after a course of anti-cancer treatment. This was generally followed by a decline, even in the presence of oedema, although severe oedema sometimes caused a terminal increase in weight. Unrelenting weight loss occurred in only nine (20%) patients. Of thirty-two patients with four or more observations, twenty-two (69%) had a quadratic pattern of weight change during the measurement period.

The findings of this study did not support the hypothesis that severe weight loss is rare in patients with inoperable lung cancer. Frequency and severity of oedema increase with disease progression, affecting 50% of the final weight measurements and resulting in the extent of undernutrition being underestimated.

We wish to acknowledge the contribution of Dr Susan Ibb.

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**Luminal short chain fatty acid concentration and apoptosis in colonic crypts from rats exposed to dimethyl hydrazine(DMH).** By E.K. LUND, R.L. BOTHAM, D. BECKLEY, J.M. GEE and I.T. JOHNSON, *Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA*

Diets containing high levels of non-digestible complex polysaccharides are associated with a reduced risk of colon cancer. It has been proposed that this is due to the induction of apoptosis in colonic crypts by short-chain fatty acids (SCFA) produced as a result of bacterial fermentation (Hague *et al.* 1993). This hypothesis is based on the observation that SCFA induce apoptosis in cultured colorectal adenocarcinoma cells. However fermentable fibre is also known to increase crypt-cell proliferation, which might theoretically increase the risk of colon cancer (Johnson, 1995). Butyrate, often regarded as the most biologically active SCFA produced in the colon, has been shown to promote proliferation, induce differentiation or increase apoptosis in different *in vitro* models (Heerd *et al.* 1994; Sing *et al.* 1997). To compare these effects in normal and damaged cells *in vivo* we used the DMH treated rat model to look at cellular responses to SCFA exposure.

Rats were fed on diets containing cellulose (C), maize starch (MS) or an amylase-resistant starch (RS) for 5 d. At 36 h before death, half the animals in each group (*n* 8) were injected with DMH (30 mg/kg body weight) whilst controls (*n* 8) received an equal volume of saline. Weight gain and food intake were recorded and the gut contents collected and frozen before analysis of SCFA content by GC. Tissue samples from the proximal and distal colon were fixed in ethanol-acetic acid (75:25) and stained with Feulgen's reagent. Crypts were microdissected and the numbers of mitotic and apoptotic cells per crypt recorded. Total SCFA concentrations were highest in the rats fed with RS, intermediate in those fed MS and lowest in those fed C.

Food intake and weight gain were not significantly different between groups. High total SCFA concentrations in rats fed on RS and MS were not associated with any increase in apoptosis or mitosis in the proximal colon, but there was a small increase in apoptosis in the distal colon (C, 0.19 (SE 0.08); RS, 0.30 (SE 0.06) apoptotic cells/crypt) in DMH-treated rats. In DMH-treated MS-fed rats there was elevated apoptosis in both distal tissue (MS, 0.45 (SE 0.02) and proximal tissue (C, 0.29 (SE 0.01) MS 0.43 (SE 0.02) apoptotic cells/crypt). This was matched by raised mitotic rates. Crypt cell proliferation rates were not significantly affected by feeding rats on the RS or by any dietary manipulation in the absence of DMH.

The data do not support the hypothesis that SCFA derived from polysaccharides increase cell proliferation in the colon, but suggest that they may increase apoptosis. This increase in apoptosis may explain the reduction in tumour yield seen previously on feeding high-fibre diets to rats exposed to DMH (Heitman *et al.* 1992). However the highest level of apoptosis was associated with a higher valerate (C5) concentration, rather than with the highest butyrate level. The effects of valerate on apoptosis require further investigation.

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**Nutritional status at diagnosis in a national cohort of children with acute lymphoblastic leukaemia.** By JOHN J. REILLY<sup>1</sup>, JENNIFER WEIR<sup>1</sup>, JOHN H. MCCOLL<sup>2</sup> and BRENDA E.S. GIBSON<sup>3</sup>, *University of Glasgow Departments of <sup>1</sup>Human Nutrition, <sup>2</sup>Statistics, and <sup>3</sup>Department of Haematology, Yorkhill Hospitals, Glasgow G3 8SJ*

The prevalence of undernutrition at diagnosis in childhood acute lymphoblastic leukaemia (ALL) is an important clinical issue which is unresolved at present. Some previous studies have concluded that undernutrition is common, others that it is rare, but these have generally been based on small, selected samples. The primary aim of the present study was to estimate the prevalence of undernutrition at diagnosis in a large, national cohort of patients (*n* 1019) treated on the same protocol between 1986 and 1991 in the UK, obviating concerns over sample size and patient selection. Since the reference data for nutritional assessment recommended for use in the UK have not been widely used and are essentially untested, a secondary aim was to statistically test some of their underlying assumptions.

BMI, expressed as a standard deviation (SD) score relative to revised UK reference data (Cole *et al.* 1995), was used as the index of nutritional status. Undernutrition was defined as a BMI SD score < 2.0 (Cole *et al.* 1995; Reilly *et al.* 1997). The cohort consisted of standard risk patients. Reference data were tested by investigating whether or not the variance of BMI SD scores was significantly different from 1.0 (Chi-squared test) and whether the BMI SD scores were distributed normally (Anderson-Darling test).

Prevalence of BMI SD scores below the cut-off value in both boys (7.6%) and girls (6.7%) exceeded expected frequencies. These differences were statistically significant (Chi squared goodness of fit test,  $P < 0.001$ ), 95% CI 5.8-9.0%. However, variance of the SD scores exceeded 1.0 and this difference was statistically significant ( $P < 0.01$ ). In addition, the distribution of BMI SD scores differed significantly from normality ( $P < 0.05$ ).

We conclude that undernutrition at diagnosis was probably common in this cohort, since the prevalence of children with BMI below the cut-off value was approximately three times higher than expected. However, there was evidence that variances for the UK reference data from BMI are too low and this may have artificially increased the estimated prevalence of undernutrition. While the major long-term nutritional problem for these patients is overnutrition (Odame *et al.* 1994), screening for undernutrition at diagnosis is indicated and could be achieved using measurements which are made routinely at diagnosis.

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**Effect of meal size and carbohydrate content on gastric emptying, energy intake and satiety in man.** By S. LONG<sup>1</sup>, B. AMAEE<sup>1</sup>, A. KESIDIS<sup>1</sup>, A. SUTTON<sup>2</sup>, N. SPYROU<sup>1</sup>, P. ROGERS<sup>3</sup>, and L. MORGAN<sup>1</sup>, <sup>1</sup>University of Surrey, Guildford, GU2 5XH, <sup>2</sup>Royal Surrey County Hospital, Guildford, GU2 5XX, <sup>3</sup>Institute of Food Research, Reading, RG6 6BZ

Although individuals may report greater feelings of fullness following preloads of increasing energy content, this is not often accompanied by a concurrent reduction in energy intake unless measurements are made soon after the preload. The present study investigated the relationship between a meal's energy and carbohydrate (CHO) content, gastric emptying, and satiety. As glucagon-like peptide-1 (GLP-1) has been shown to delay gastric emptying (Weitgren *et al.* 1997), and has recently been implicated as a satiety factor in man (Gutzwiller *et al.* 1997), its role in postprandial satiety responses was also investigated.

Nine male subjects (age 23-28 years, BMI 20-25 kg/m<sup>2</sup>) participated on three occasions. Following a standardized meal consumed before 22.30 hours they fasted overnight before the study. At 09.30 hours subjects were given one of three 450 ml milkshakes (premeal) containing 1004 kJ/10 g CHO, 1674 kJ/50 g CHO or 2510 kJ/100 g CHO. Gastric emptying of the premeals was measured using an electrical impedance epigastrogaph. Subjects were asked to complete self-rating behaviour scales to assess hunger and satiety immediately before the meal and then at 30 min intervals post-prandially for 2.5 h. Venous blood was sampled throughout. At 2.5 h after the preload subjects were offered an *ad libitum* buffet meal from which food intake was recorded.

	1004 kJ (A)		1674 kJ (B)		2510 kJ (C)	
	Mean	SD	Mean	SD	Mean	SD
Hunger 30 min after premeal (cm)	6.1	2.8	5.6	2.5	4.7*	2.3
Buffet intake (kJ)	8005	2995	7380	2374	7532	2136
Gastric half-emptying (min)	20.1	5.9	28.9*	5.0	35.3**	8.8
AUC (0-2.5 h) GLP-1 (pmol/l.h)	55.0	26.8	52.1	27.6	87.0**	36.1

AUC, area under curve  
\* mean values were significantly different from A # mean values were significantly different from B

Repeated measures ANOVA showed that premeal did not have a significant effect on overall hunger and satiety ratings, or buffet meal intake (*n* 9). However hunger at 30 min was significantly greater after premeal A than premeal C (*P* = 0.02). A further nine subjects underwent the preload/buffet intake protocol only, and combined energy intake data (kJ) premeal A 7322 (SD 3124), premeal B 6816 (SD 2680), premeal C 6544 (SD 2716), (*n* 18) and brought the difference between buffet intakes into significance (*P* = 0.049). Gastric half-emptying time (T50) of the premeal increased significantly with increasing energy content and notably premeal A had a T50 less than 30 min, while that of premeal C was greater than 30 min. GLP-1 secretion increased with increasing premeal energy content. Total GLP-1 secretion and half-emptying times were correlated (*r* 0.42, *P* < 0.05), as was energy entering the duodenum and circulating GLP-1 at that time point (*r* 0.73, *P* < 0.001). There was a negative association between circulating GLP-1 and self-rated hunger 30 min following the premeal (*r* -0.41, *P* < 0.05). These findings suggest that feelings of satiety following a preload are influenced by rate of gastric emptying, and are consistent with a role for GLP-1 as a physiological mediator of gastric emptying and satiety in man.

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**Effect of glucagon-like peptide-1 infusion on gastric emptying, energy intake and satiety in man.** By S. LONG<sup>1</sup>, A. SUTTON<sup>2</sup>, A. KESIDIS<sup>1</sup>, B. AMAEE<sup>1</sup>, N. SPYROU<sup>1</sup>, P. ROGERS<sup>3</sup>, and L. MORGAN<sup>1</sup>, <sup>1</sup>University of Surrey, Guildford, GU2 5XH, <sup>2</sup>Royal Surrey County Hospital, Guildford, GU2 5XX, <sup>3</sup>Institute of Food Research, Reading, RG6 6BZ

Centrally administered glucagon-like peptide-1 (GLP-1) has been shown to inhibit feeding in fasted rats (Turton *et al.* 1995), although this has recently been questioned as the peptide has also been shown to produce a conditioned taste aversion in these animals (Thiele *et al.* 1997). As the role of GLP-1 in human appetite remains largely unknown the present study investigated the effect of peripheral GLP-1 infusion on gastric emptying, satiety and food intake in man.

Ten male subjects (age 22-29 years, BMI 20-27 kg/m<sup>2</sup>) were infused in a randomized single-blind within-subject cross-over design, using saline infusion as a control. After consumption of a standardized meal, followed by a 6 h fast, subjects received either a GLP-1 infusion (1.2 pmol/kg per h) or a saline infusion for 1 h at 18.00 hours. At 20 min after starting the infusion the gastric emptying of a 400 ml water load was measured using an electrical impedance epigastrogaph. Venous blood was collected for GLP-1, insulin and glucose measurement, and subjects were asked to complete behavioural self-rating scales to assess hunger and satiety at 20 minute intervals during the infusion period. At 40 min after the start of the infusion subjects were given an *ad libitum* buffet meal and their food intake recorded.

	Saline infusion		GLP-1 infusion	
	mean	SD	mean	SD
GLP-1 concentration (pmol/l)	11.1	5.7	116.0*	33.2
Glucose concentration at 40 min (mmol/l)	4.9	0.4	4.1*	0.4
Gastric half-emptying (min)	7.0	1.6	11.8*	3.5
Buffet meal intake (kJ)	5894	1137	5475.8	1705

\* Mean values were significantly different from those for saline; \**P* < 0.05

GLP-1 infusion raised circulating GLP-1 to approximately twice post-prandial levels, whilst GLP-1 during the saline infusion remained at basal levels. A transient rise in insulin at the start of the GLP-1 infusion was noted although this was not significant, and glucose levels dropped below basal during the GLP-1 infusion from 4.9 (SD 0.1) to 4.1 (SD 0.1) mmol/l (*P* < 0.01) by 40 minutes. GLP-1 infusion also significantly delayed gastric emptying of the water load (*P* < 0.01). Self assessment of hunger and satiety was unaffected by the infusion before the buffet meal, although subjects tended to be less hungry after the buffet meal following the GLP-1 infusion (hunger rating 2.6 (SD 0.7) for saline v 1.4 (SD 0.2) for GLP-1, *P* < 0.09). Mean buffet energy intake was lower following GLP-1 infusion but differences failed to achieve significance (*P* = 0.27).

In conclusion GLP-1 infusion was shown to delay gastric emptying, but had a minimal effect on food intake and satiety. Effects on hunger following the buffet could be due to GLP-1 induced delay of gastric emptying. It is possible that raised circulating plasma glucose, as would normally occur post-prandially, might be necessary in order for GLP-1 to increase satiety.

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**Effect of habitual dietary protein intake on appetite and satiety.** By S. LONG, A. JEFFCOAT and D.J. MILLWARD *Centre for Nutrition and Food Safety, University of Surrey, Guildford, GU2 5XH*

It is generally recognized that protein is the most satiating macronutrient. Part of the reason for this may reflect both the limited capacity for protein storage, and the potential direct influence of amino acids within an amino static mechanism of appetite regulation (Millward, 1995). Given that rates of amino acid oxidation and the consequent amplitude of the diurnal cycle of postabsorptive losses and postprandial gains vary with the habitual protein intake, it might be predicted that the satiating effect of protein will vary according to habitual protein intakes reflecting both a greater capacity for postprandial deposition and an increased capacity for postprandial amino acid oxidation.

We have tested this hypothesis with measurements of hunger and satiety in response to three successive high-protein meals in 1d in subjects with either relatively high or low protein intakes and in subjects after a switch from a high to a low intake aimed at depleting body protein stores. Fourteen healthy non-obese subjects (seven male, seven female) were recruited, and habitual protein intake was assessed from food intake diaries and 24 hr urinary N excretion rates. On this basis subjects were divided into two mixed sex groups of low protein (LP) and high protein (HP) intakes. Satiety was measured on five occasions, initially in the two groups (LPa, and HPa), after a dietary manipulation of 13 d aimed at increasing the difference in protein intakes between groups, reducing intake of the LP group from 1.0 to 0.75 g/kg per d with dietary advice (LPb) and increasing intake of the HP group from 1.38 to 1.96 g/kg with high protein food supplements (tuna, HPb). The HP group was then switched to a low-protein diet (0.75 g/kg per d) for 2 d to deplete tissue protein stores (HPc). Subjective ratings of hunger and satiety were used to track responses during the satiety test day involving the feeding of a high-protein breakfast (08.00 hours), lunch (13.30 hours), and evening meal (17.45 hours), each meal providing 2.4 MJ containing (% energy) 35% protein 33% fat and 32% carbohydrate. Subjective ratings of hunger, satiety and other indices, obtained on visual analogue scales, were completed before and after each meal and hourly throughout the day.

On all occasions eating was shown to result in large differences in hunger and satiety measurements, with similar patterns of response in hunger, desire to eat, prospective consumption, urge to eat and thoughts of food, and with a reciprocal response for satiety. Of these indices, differences in satiety were most marked and these are discussed here. Comparisons between groups were made using analysis of covariance, with time as a repeated measures factor and group as the independent variable. Mean values for the satiety response for LPa were higher than for HPa as predicted (5.6 (SD 2.6) v 4.6 (SD 2.8) but  $P > 0.05$ ). There was however a significant difference in the satiety response between LPb and HPb ( $P = 0.025$ , 6.0 (SD 2.8) v 4.6 (SD 2.7)), while LPb v HPc approached significance ( $p = 0.076$ , mean (SD) 6.0 (2.8) v 4.6 (2.9)). There was no difference in satiety after breakfast, lunch or supper, tested for both LP and HP groups together or separately.

These results strongly support an important influence of prior protein intake on appetite control as would be expected from an aminostatic mechanism acting within a protein-stat mechanism of regulation of the lean body mass.

Millward DJ (1995) *Nutrition Research Reviews* 8, 93-120.

**The effect of phenylalanine supplementation on food intake and self-rated appetite scores.** By W.L. HALL<sup>1</sup>, P. J. ROGERS<sup>2</sup>, D.J. MILLWARD<sup>1</sup>, and L.M. MORGAN<sup>1</sup>, *Centre for Nutrition and Food Safety, University of Surrey, Guildford GU2 5XH and <sup>2</sup>Institute of Food Research, Reading RG6 2EF*

Protein appears to be the most satiating macronutrient, suppressing subsequent food intake to a greater extent than fat or carbohydrate (Barkeling *et al.* 1990), amino acids have also been implicated in the suppression of food intake via neurotransmitter-mediated mechanisms (Hrboticky *et al.* 1985). Studies that show a suppression of food intake by aspartame suggest that ingestion of phenylalanine may affect satiety (Rogers & Blundell, 1994). The aim of the present study was to investigate the effect of encapsulated phenylalanine on subsequent food intake and self-rated scores for hunger, desire to eat and fullness.

Nine healthy volunteers (five female and four male; 23-31 years; BMI < 25 kg/m<sup>2</sup>) were given 0 g, 400 mg, 2 g or 5 g phenylalanine in random order on four separate occasions at 18.00 hours. The amino acid was given as ten capsules, each capsule made up to 5 g with cornflour. Subjects had previously consumed their normal breakfast and nothing else apart from a standard commercial pasta ready-meal between 12.00 and 13.00 hours. Capsules were consumed with 300 ml cold and 200 ml warm water. Satiety and mood ratings were assessed with 100 mm visual analogue scales, before giving the capsules and at intervals over the following 60 min. At 19.00 hours the subjects were separated from each other and given *ad libitum* access to a range of previously weighed palatable cold foods, and encouraged to eat until they were comfortably full. Subjects were allowed to take home what they did not eat to prevent over-eating. Energy intake, food intake in terms of weight, macronutrient intake, and satiety measurements were analysed by repeated measures ANOVA using the Statistica computer package.

Phenylalanine dose	Energy intake (kJ) per kg body weight		Food intake (g) per kg body weight		Protein intake per kg body weight (g)		Fat intake per kg body weight (g)		Carbohydrate intake per kg body weight (g)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	76.57	6.61	9.00	0.72	0.60	0.05	0.77	0.08	2.20	0.17
400 mg	76.15	7.11	8.80	0.74	0.56	0.05	0.77	0.09	2.18	0.18
2 g	72.38	4.44	8.30	0.63	0.54	0.05	0.73	0.04	2.08	0.15
5 g	66.53	4.90	7.60*	0.41	0.48***	0.04	0.66	0.05	1.91	0.15

\*Significantly different from control dose, \* $p < 0.05$ , \*\* $p < 0.005$ .

Food intake was suppressed in a dose-related manner as indicated by lower mean values with increasing phenylalanine dose, a reduction which was significant for food intake weight after 5 g encapsulated phenylalanine. Mean values for protein, fat and carbohydrate were all lower with increasing phenylalanine dose and this was highly significant for protein after 5 g phenylalanine. There was no main effect of dose on measures of hunger, desire to eat or fullness.

These results suggest that oral phenylalanine can suppress food intake at large doses, and suggest that it has a particularly significant effect on protein intake. The fact that food intake was more significantly affected in terms of weight in grams compared with energy suggests that it may be the satiating effect of the bulk of food in the stomach and duodenum that is potentiated by the prior ingestion of phenylalanine supplements, possibly through cholecystokinin-mediated mechanisms. Further work will attempt to elucidate the physiological mechanisms behind this satiety effect.

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Hrboticky N, Leiter LA, Anderson GH (1985) *Nutrition Research* 5, 595-607.

Rogers PJ and Blundell JE (1994) *Physiology and Behavior* 56, 247-250.



**The effects of high-fat and low-fat diets on mood, energy expenditure, neurohumoral function and blood lipids.** By REBECCA MOORE<sup>1</sup>, ANITA S. WELLS<sup>1</sup>, IAN A. MACDONALD<sup>2</sup>, CARRIE RUXTON<sup>3</sup> and NICHOLAS W. READ<sup>1</sup>. <sup>1</sup>Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Sheffield, S5 7AU; <sup>2</sup>University of Nottingham NG7 2UH, Queens Medical Centre, Nottingham; UK. <sup>3</sup>The Sugar Bureau, Dolphin Square, London, SW1V 3PW.

Fat and carbohydrate (CHO) have differing effects on alertness, mood (Wells *et al.* 1995) and energy levels (Keith *et al.* 1991), with fat-rich meals causing a greater decline in alertness and performance than CHO-rich meals while increasing feelings of friendliness. Recent dietary intervention has shown that a reduction in dietary fat from 41 to 25% energy may also affect mood (Wells *et al.* 1998). Food consumption has also been shown to increase sympathetic nervous system activation (Welle 1995) with sympathetic activity being higher after glucose than after isoenergetic fat-based drinks (Fagius & Berne, 1994).

The present study aimed to investigate the effects of CHO and fat on energy expenditure (EE), mood and activation of the autonomic nervous system in healthy free-living adults. Nine males (mean BMI 23.5 kg/m<sup>2</sup>, mean weight 76.3 kg) and nine females (mean BMI 24.4 kg/m<sup>2</sup>, mean weight 66 kg) consumed two isoenergetic diets (HF: 55% energy from fat, 30% energy from CHO, or LF: 25% energy from fat, 60% energy from CHO) for a period of 2-weeks, each separated by a 2-week washout. Daily energy requirements were assessed and the diets matched these to ensure energy balance. During the intervention periods, subjects wore a heart rate monitor (Polar Advantage NV, Kepele, Finland) and hip actometer (Caltrac caloric counter, Birmingham, UK) to measure EE. At the end of each week subjects completed a bipolar profile of mood states. Weight, percentage body fat by impedance (Bodystat, Isle of Man, UK) and BMI, were recorded at the beginning and end of each 2-week intervention. Fasting blood samples were also collected to assess blood lipids for dietary compliance, leptin and thyroid hormone concentrations. Fasting urine samples were assessed for catecholamine concentrations and excretion rates to provide a measure of sympathetic nervous system activity.

There was no difference in mood ratings or EE between the two diets (mean kJ/h HF 525.2 (SE 7.2), LF 501.0 (SE 8.7) (HR monitors); HF 489.4 (SE 4.4), LF 484.5 (SE 5.1) (actometer)). There was no change in mean weight or percentage body fat during the intervention indicating no changes in activity. Leptin, triiodothyronine and thyroxine concentrations remained the same on both diets but thyroid stimulating hormone concentrations (mIU/l) fell following the HF intervention (mean changes over diet periods; HF -0.28 (SE 0.1), LF 0.00 (SE 0.11) F 7.27, df 1,15, P<0.02). Mean triacylglycerol (TAG) concentration (mmol/l) increased after the LF intervention (HF 0.04 (0.38), LF 0.28 (0.11) F 5.21, df 1,16, P<0.05) while mean cholesterol (mmol/l), HDL (mmol/l) and LDL concentrations (mmol/l) decreased following a low fat diet (HF 0.10 (SE 0.12) LF -0.48 (SE 0.07) F 16.50, df 1,16, P<0.001); HF 0.04 (SE 0.38), LF -0.37 (SE 0.36) F 39.34, df 1,16, P<0.0001); and HF 0.09 (SE 0.04), LF -0.22 (SE 0.03) F 13.44, df 1,16, P=0.002 respectively). Mean fasting urinary adrenaline concentrations (nmol/l) and adrenaline excretion rates (nmol/h) showed no changes following the LF intervention while mean noradrenaline concentrations and excretion rates (nmol/h) fell after the HF diet (HF -3.82 (SE 5.79), LF 1.46 (SE 4.08); and HF -0.44 (SE 1.42), LF -0.22 (SE 1.11) respectively).

This study did not reveal any medium-term effect of diet on mood, EE or sympathetic activity but indicates that the high-fat diet caused a decrease in thyroid stimulation, and TAG levels.

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**Physiological and psychological effects of fat and carbohydrate: influence of the rate of delivery.** By ANITA S. WELLS<sup>1</sup>, N. W. READ<sup>1</sup> and I. A. MACDONALD<sup>2</sup>. <sup>1</sup>Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Herring Road, Sheffield S5 7AU and <sup>2</sup>Department of Physiology and Pharmacology, Medical School, Queen's Medical School, Nottingham NG7 2UH

Meals and gastric infusions rich in carbohydrate (CHO) have been reported to induce greater increases in heart rate (HR) (Hesseltine *et al.* 1990) and resting energy expenditure (EE) (Schwartz *et al.* 1985) and have differing effects on mood and sleepiness (Wells *et al.* 1998) than meals and gastric infusions rich in fat. The present investigation examined the possibility that these differences may be related to the rate at which these nutrients enter the duodenum.

Eight healthy male subjects were each tested on 2 d, with each test day separated by at least 2 d. On arrival at the Centre for Human Nutrition, at 08.00 hours, an enteral feeding tube was inserted via the nose into the duodenum. Starting at 10.15 hours, subjects received an intraduodenal infusion lasting 3.75 h. Subjects were blinded to the nature of the infusion. Sucrose solution (100% energy CHO) was infused on one day and lipid emulsion (100% energy fat, 20% Intralipid, Kabi Pharmacia Ltd, Milton Keynes, Bucks) was infused on the other day. The infusions were given in a randomized order, at a rate of 502 kJ/h (120 kcal/h). Measures of HR, EE (using the ventilated hood system), mood and sleepiness (Wells *et al.* 1998) were collected before the infusions and then every 0.5 h during the infusion.

	F value, ANCOVA repeated measures						
	Infusion df (1,17)	Time df (6,42)	Infusion x time df (6,42)	Mean post-infusion change		Lipid SE	
				Mean	SE		
EE (kJ/kg per 24 h)	1.19	4.99 †	1.29	7.27	0.79	6.50	1.41
RQ	97.36 †	<1	1.73	0.10*	0.01	-0.01	0.01
HR (beats/min)	12.26 †	5.06	1.09	3.44*	1.26	8.70	1.24
Sleepiness	<1	4.14 †	<1	-0.79	0.54	-0.25	0.56
Autentive-dreany	<1	7.19 †	<1	-10.55	5.44	-2.57	5.29
Energetic arousal	2.84	4.85	<1	0.11	1.47	-0.52	1.68
Tension	<1	<1	<1	-0.21	1.45	-0.36	1.06
Hedonic tone	1.01	1.25	<1	0.29	1.41	-0.37	1.26
Hungry-satiated	<1	<1	1.23	1.34	3.87	0.23	3.33

\* significantly different from lipid (P<0.05), † F value significant (P<0.01), ‡ F value significant (P<0.001).

The Table shows that when sucrose and lipid are infused into the duodenum at the same rate, they have similar effects on EE, mood and sleepiness. Lipid induced a significantly greater increase in HR than sucrose. These results suggest that the rate at which nutrients leave the stomach influences postprandial HR, EE, mood and sleepiness.

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**Effect of graded levels of exercise on body weight, appetite and food intake in normal-weight women consuming their normal diet in their natural environment.** By M. FLEMING<sup>1</sup>, A. SEPP<sup>1</sup>, A. M. JOHNSTONE<sup>1</sup>, D. A. HUGHES<sup>1</sup>, C. A. REID<sup>1</sup>, N. KING<sup>2</sup>, J. BLUNDELL<sup>2</sup> and R. J. STUBBS<sup>1</sup>, <sup>1</sup>Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen and <sup>2</sup>Biopsychology Group, Department of Psychology, Leeds University, Leeds LS2 9JT.

Government recommendations suggest that the public should increase physical activity (PA) levels. A recent study in men suggested that changes in energy expenditure (EE) through PA are an effective strategy to alter medium-term energy balance provided subjects are able to attain high levels of EE, while living in their natural environment and consuming their normal diets. Women may be different. The present study was therefore conducted to assess the effect of graded increases in EE through PA on appetite, energy intake (EI), approximate estimates of EE and body weight in women living in their normal environment. Six lean young women (mean age 23.0 (SD 0.6) years; weight 58.4 (SD 3.3) kg; height 1.65 (SD 0.02) m; BMI 21.4 (SD 1.0) kg/m<sup>2</sup>), were each studied three times during a 9 d protocol, corresponding to no-exercise (control) (NEX; 0 MJ/d), medium exercise level (MEX; 2x40 min sessions; about 1.3 MJ/d) and high exercise level (HEX; 3x40 min sessions; about 2.6 MJ/d). On days 1-2 subjects were given a medium fat (MF) maintenance diet, (1.5 x resting metabolic rate). On days 3-9, they self-recorded dietary intake using a food diary and PETRA weighing system which has recently been validated in this laboratory (Johnstone *et al.* 1998). Each subject completed a pre- and post-treatment sub-maximal fitness test on a bicycle ergometer to (i) monitor fitness levels, and (ii) calibrate heart rate (HR) using the POLAR HR monitor against O<sub>2</sub> consumption, measured using the VMAX metabolic cart (Sensor Medics, USA). Subjects attended the Human Nutrition Unit to exercise daily to a required HR. EE was assessed using the modified FLEX method (Ceesay, 1989). Body weight was recorded each morning before eating and after voiding. Subjects could entirely determine their own meal time, frequency and composition. They completed hourly hunger ratings during waking hours to record subjective sensations of hunger and appetite. ANOVA was conducted on EI, EE, body weight and hunger, using treatment and day as factors and subject and run as blocking factors. Mean daily EI, EE and total weight change are given in the Table.

	NEX	MEX	HEX	F	SED
Energy Intake (MJ)	8.87	9.23	9.96	F (2,10) 4.80	0.035
Energy expenditure (MJ)	9.20	10.95	12.09	F (2,10) 5.67	0.023
Body weight change (kg)	-0.36	-0.47	-0.77	F (2,10) 1.65	NS
					0.23

Markedly increasing EE through exercise produced significant compensation and subjects consumed 1.58, 1.64 and 1.78 X BMR on the NEX, MEX and HEX treatments respectively. There was no treatment effect on hunger appetite or body weight, but they lost weight on each treatment. This was significant for the MEX and HEX treatments. These data suggest that while the women showed a tendency to compensate, this amounted to only about 33% of the increased EE through PA, precipitating mild weight loss. This suggests that changes in EE through PA are an effective strategy to control energy balance. Comparison with an identical protocol in men suggests that men and women may behave differently in their feeding responses to altered EE through exercise.

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**Effects of different sugars on mood and cognitive performance.** By P. J. ROGERS, P. POLET, A. AHERNE, E. A. ATKINSON, N. J. RICHARDSON and J. E. L. DAY, *Consumer Sciences Department, Institute of Food Research, Reading RG6 6BZ*

Popular beliefs and scientific theories predict a variety of different and partly contradictory effects of sugar on behaviour, including alerting and calming effects, relief from depression, and either improved or slowed mental performance (Rogers, 1998). In the first of the two studies reported here, free-living male and female participants aged between 18 and 60 years consumed a novel, fruit-flavoured drink once daily for 3 weeks. The 250 ml drinks contained 31.75, 19.50, 9.50 or 0 g sucrose, were made equally sweet by the addition of increasing amounts of aspartame and acesulfame-K, and were given double-blind according to a between-subjects design (n 22 per group). Participants were instructed not to eat or drink for at least 1 h before and 1 h after consuming the test drink. Analysis of responses on an eighteen-item mood questionnaire (Rogers *et al.* 1995) showed only small and inconsistent effects of sucrose content on mood. For example, although there was a greater increase in 'liveliness' 15 and 60 min after consuming 31.75 g compared with 0 g sucrose (P<0.05, least significant difference test (LSD)), ratings of liveliness were highest of all 60 min after 9.5 g sucrose. The second study was carried out in a controlled laboratory setting according to a between-subjects design. It compared the effects of water with the effects of drinks sweetened with 50 g sucrose, 50 g glucose, 50 g fructose, or 25 g glucose + 25 g fructose, or intense sweeteners (all drinks 250 ml). The participants, thirty-four males and forty-two females aged between 18 and 32 years, received their drink for four consecutive days starting at 16.00 hours each day. For 2 h before and 2.25 h after 16.00 hours they were not permitted to consume any other food or drink. Measures of mood and mental and psychomotor performance (simple reaction time (SRT), rapid visual information processing and tapping tasks; Green & Rogers, 1995) revealed no significant effects of the sugars, except for fructose. The Table shows that SRT was significantly faster and ratings of 'calmness' significantly lower 2 h after 50 g fructose compared with water.

	Sucrose		Fructose		Glucose+		Aspartame+		Water	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
SRT (ms)	386.6	22.4	370.16	14.6	342.94*	8.3	381.3	13.7	377.1	17.0
Calmness†	4.58	0.21	4.45	0.22	4.02	0.21	4.64	0.21	4.70	0.22

Mean values were significantly different from water control, \*P<0.05 (post hoc LSD comparisons).  
 † Rated on a seven-point scale, means adjusted for pre-drink (baseline) ratings.

Taken together, the results of these two large studies suggest that the mood and cognitive performance effects of sucrose and glucose (and aspartame and acesulfame-K) are generally weak. This conclusion is supported by the results of the second study which confirmed the sensitivity of the methods used by detecting significant effects of fructose. The relative improvement in SRT performance after fructose may be explained by the mild adverse reactions (related to fructose malabsorption, slower gastric emptying, etc.) that are known to occur following consumption of large pure fructose loads (Riby *et al.* 1993). That is, the slight arousing/alerting effects accompanying these symptoms (fructose also made subjects feel less 'calm') could be expected to benefit performance on this easy, but boring and fatiguing task. Consistent with this, another recent study found that 60 g fructose, but not glucose, significantly reduced sleepiness in sleep-deprived individuals (Lennernas *et al.* 1997).

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**The effect of chewing nutritive and non-nutritive material (modified sham feeding model) on subsequent appetite sensations and food intake.** By R.J. STRATTON, A. NUGENT, C. RUTGERS and M. ELIA, *MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH*

The modified sham feeding model in human subjects, in which food is tasted, chewed and subsequently spat out, has been reported to elicit a cephalic phase response, which includes a reduction in rated hunger (Le Blanc & Soucy, 1996). Furthermore, patients in whom oral food intake is contraindicated because of disease, may still feel hungry and retain the desire to eat despite receiving their estimated nutritional requirements in full by enteral or parenteral feeding (Stratton *et al.* 1998). To relieve these appetite sensations some patients chew and spit food and a smaller proportion chew non-nutritive material (e.g. gum). The aim of the present study was to test the hypothesis that chewing nutritive material affects appetite sensations and subsequent energy intake to a greater extent than chewing non-nutritive material. The study involved twelve healthy, weight-stable men (age 40 (SD 13) years, BMI 22.7 (SD 2.61) kg/m<sup>2</sup>). After an overnight fast, on three mornings (each separated by 1 week), subjects consumed one of the following test meals over 10 min, in a random order: sandwiches (150 g, 1.5 MJ, 40% energy from fat, 45% carbohydrate, 15% protein) which were chewed and swallowed; the same type of sandwiches which were chewed and spat out (A); tasteless latex dummy which was chewed (B). Appetite was assessed using 100 mm visual analogue scales (e.g. 'not at all hungry' (score 0) to 'as hungry as I have ever felt' (score 100)) before the test meal, every 2.5 min during the test meal, and thereafter, every 5 min for 0.5 h and then at 15 min intervals up to 85 min. The test period was immediately followed by an *ad libitum* lunch (each food item 550 kJ/100 g, 30% energy from fat, 47% carbohydrate, 13% protein) from which subsequent energy intake was calculated. Pleasantness and satisfaction ratings were obtained for the test meal and for the subsequent lunch. Repeated measures ANOVA with contrasts and Student's paired *t* test were used to analyse normally distributed data, and Friedman's *k*-related samples and Wilcoxon signed ranks test were used for non-parametric data. Appetite sensations before starting the modified sham feeding models were not significantly different (e.g. median desire to eat: A 53, B 60). During the modified sham feeding test meal period, chewing the nutritive material was associated with a significantly greater increase in fullness than the non-nutritive material (dummy) (mean difference +9,  $P < 0.003$ ), which persisted until the end of the test period (mean difference +7,  $P < 0.005$ , see Table). Both models of modified sham feeding had similar effects on hunger, both during the meal and until the end of the test period. The chewing of the nutritive material, which was rated as significantly more pleasant and satisfying than the dummy ( $P < 0.01$ ), was also accompanied by a greater rise in desire to eat (mean difference +7,  $P < 0.002$ ) (see Table). By comparison, ingestion of the test meal produced much larger changes in hunger (-10), desire to eat (-10) and fullness (+16) ( $P < 0.006$ ).

	Model A, nutritive		Model B, non nutritive	
	Mean	SD	Mean	SD
Subsequent lunch energy intake (kJ)	6953	482	7164	472
Change in hunger score (0 to 85 minutes)	7	21	3	15
Change in desire to eat (0 to 85 minutes)	7	17	0*	15
Change in fullness (0 to 85 minutes)	9	20	2*	18

\* Values significantly different from model A,  $P < 0.005$  (paired *t* test)

Subsequent energy intakes at lunch were no different after either the chewing of non nutritive or nutritive material, and 10% and 13% greater respectively than following meal ingestion ( $P < 0.04$ ). No order effect was evident for energy intakes at lunch and pleasantness and satisfaction ratings did not differ significantly on the three occasions. This study in healthy subjects suggests that chewing nutritive material (modified sham feeding model A), has different short-term responses to the chewing of non-nutritive material (model B), producing greater increments in sensations of fullness and desire to eat without significantly affecting hunger sensations or subsequent energy intake at lunch.

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**Investigation of salivary hormone secretion following food intake in man.** By B.A. MESSENGER, M.N. CLIFFORD and L.M. MORGAN, *Centre for Nutrition and Food Safety, University of Surrey, Guildford GU2 5XH*

Immunoreactive peptides of gut origin have been previously reported in the salivary glands (Tseng *et al.* 1993) their function is not known, but recent feeding experiments suggest that they influence postprandial carbohydrate and lipid metabolism. Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) both act to potentiate insulin secretion. A study was therefore conducted to measure the concentrations of these hormones in blood and saliva following a swallowed meal and sham feeding in human subjects.

The study was a random within-subject crossover design with twelve subjects. The differences in hormonal response following either chewing and swallowing a mixed meal or chewing and expectorating ('sham feeding') were compared in saliva and plasma. GIP, GLP-1 and insulin were measured in both blood and saliva samples taken at identical time points before and for 90 min after the meal.

Fasting salivary insulin levels were found to be 28 (SE = 6) pmol/l compared with plasma fasting levels of 40 (SE = 25) pmol/l. At 60 min after eating and swallowing, insulin in saliva had risen to 96 (SE = 18) pmol/l and to 270 (SE = 66) pmol/l in plasma. The saliva insulin concentrations rose more slowly than those in plasma. Following 'sham feeding', insulin did not change in either plasma or saliva. GLP-1 was not detected in saliva. Plasma GLP-1 rose after feeding; no response was seen after 'sham feeding'.

GIP was found in saliva, with basal levels of 183 (SE = 23) pmol/l. At 15 min after the swallowed meal this level had dropped to 98 (SE = 17) pmol/l ( $P < 0.02$ ) and to 63 (SE = 18) pmol/l after the 'sham fed' meal ( $P < 0.01$ ). At 90 min after the swallowed meal salivary GIP levels were 111 (SE = 17) pmol/l compared with 112 (SE = 25) pmol/l after the 'sham fed' meal. This compares with plasma GIP fasting levels of 20 (SE = 7) pmol/l and peak postprandial levels of 117 (SE = 15) pmol/l. Plasma GIP levels were unchanged following the 'sham fed' meal.

From this study it was concluded that the hormones GIP and insulin but not GLP-1 were present in saliva. The lower levels and the slower response of insulin in saliva compared with plasma could suggest that salivary insulin may be an ultrafiltrate of blood. In contrast the equivalent comparison for GIP suggests that GIP is secreted from salivary glands.

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**The effect of marine fish oil supplementation on insulin sensitivity in the dog.** By A.J. IRVINE<sup>1</sup>, R. BUTTERWICK<sup>2</sup>, T. WATSON<sup>2</sup>, D.J. MILLWARD<sup>1</sup> and L.M. MORGAN<sup>1</sup>, <sup>1</sup>Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH and <sup>2</sup>Waltham Centre for Pet Nutrition, Freaby Lane, Waltham-on-the-Wolds, Melton Mowbray, Leicestershire LE14 4RT

Obesity is increasingly common in dogs, with observed incidence between 24 and 44% in the UK. As with human subjects, obesity in dogs is associated with fasting hyperinsulinaemia, impaired glucose tolerance and an increased insulin response to a glucose load, which worsens with the degree of obesity and age. Obesity confers an increased risk of cardiovascular disease and maturity onset diabetes mellitus. Recent human studies have shown that dietary supplementation with *n*-3 polyunsaturated fatty acids (PUFA) can improve insulin sensitivity and reduce the risk of cardiovascular disease. The present study was carried out to establish a suitable method for assessing insulin sensitivity in dogs and to investigate the effect of dietary *n*-3 PUFA on insulin sensitivity in these animals.

Insulin sensitivity was assessed using an oral glucose tolerance test (OGTT, 4 g glucose/kg body weight), an intravenous glucose tolerance test (IVGTT, 500 mg glucose/kg body weight) and an insulin tolerance test (ITT, 0.1 U insulin/kg body weight). Five dogs were tested in random order, at least 1 week apart, following an overnight fast. The OGTT was found to be unsuitable due to delayed gastric emptying, which caused mean plasma glucose levels to be elevated 3 h following the glucose load (basal glucose 4.08 (SD 0.17) mmol/l, after 3 h 5.47 (SD 0.72) mmol/l,  $P=0.053$ ). The ITT was quick and easy to perform, but there was the possible risk of the dogs becoming hypoglycaemic. The IVGTT was similarly easy to perform, without risks of hypoglycaemia. Glucose disappearance rates (K) for the ITT were 7.41 (SD 0.76) %/min (CV 10.3%) and for the IVGTT 4.89 (SD 1.2) %/min (CV 24.4%). Repeatability studies (one animal, four repeated tests) showed intraindividual CV of 12.8% and 24.5% respectively. The IVGTT was used for further studies because of the potential to apply kinetic modelling to glucose and insulin data, and because of the risk of hypoglycaemia with the ITT.

To determine the effect of *n*-3 PUFA on insulin sensitivity six Labrador dogs, aged 6 (SD 2) years were placed on a standard weight-maintenance diet (Canned Diet; kJ metabolic energy/d = 460 x body weight (kg)<sup>0.75</sup>) enriched with marine fish oil (MFO) containing 0.08g eicosapentaenoic acid and 0.06g docosahexaenoic acid/100 g for 7 months. These animals were tested with an IVGTT following an overnight fast with six matched dogs that had been on the same standard diet without the MFO supplementation. One control dog failed to complete the IVGTT. Comparison of the K values between the MFO diet (mean 2.60 %/min, SD 1.24) and control diet (mean 2.19 %/min, SD 0.47) showed no significant difference ( $P=0.744$ ). Similarly, no significant differences ( $P=0.482$ ) were found when comparing the integrated area under the curve (IAUC) for the glucose response. A trend ( $P=0.053$ ) towards higher plasma insulin on the MFO-supplemented diet was found when comparing the IAUC for the insulin response. However using minimal model analysis, no significant differences were found in insulin sensitivity (Si) and glucose utilisation (Sg) between diets.

A diet enriched with MFO at this level therefore had little effect on insulin sensitivity in dogs. The lack of effect could be because the animals used were non-obese, healthy and regularly exercised and had a high basal insulin sensitivity before dietary intervention.

**Influence of a reduced saturated fat and isoennergic increase in carbohydrate intake on the insulin sensitivity of glucose disposal in middle-aged men.** By K.A. SLEVIN<sup>1</sup>, A.C. FEREDAY<sup>1</sup>, E. AH-SING<sup>1</sup>, J.WRIGHT<sup>1</sup>, A. IRVINE<sup>1</sup>, L.M. MORGAN<sup>1</sup>, C.M. WILLIAMS<sup>2</sup> and D.J. MILLWARD<sup>1</sup>, <sup>1</sup>Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH, <sup>2</sup>Hugh Sinclair Unit of Human Nutrition, Department of Food Science and Technology, University of Reading, Reading RG6 6AF

Epidemiological studies have linked high saturated fat (SFA) intakes with insulin resistance and an increased risk of cardiovascular disease. While there is general agreement that SFA intakes in the UK population should be reduced, there is debate about how to do this: i.e. replacement of SFA with carbohydrates or unsaturated fat. We have conducted a study in which the intake of SFA has been reduced and replaced isoennergically with carbohydrate in order to investigate the effects on the insulin resistance of macronutrient disposal. We report here preliminary results of the influence of this dietary change on the insulin resistance of glucose disposal. The effects on lipid responses and postprandial lipaemia are reported in an accompanying abstract (Fereday *et al.* 1998).

The experimental design was a single intervention with initial habitual diet as control. Non-smoking and non-exercising middle-aged men were recruited locally and screened with blood tests, anthropometry and a diet history. Twelve were selected on the basis of fasting insulin levels and a SFA intake typical of the current UK diet (16% of dietary energy). Initial fat intakes were measured with a 7 d food diary and baseline measurements of insulin resistance made. Intakes of SFA were then reduced to 8 % energy by means of individual dietary advice on replacing SFA isoennergically by carbohydrate for a 10-week period. The intervention utilized food products available in supermarkets and so reflected changes achievable by the general public. Compliance was assessed by 7 d food diaries after 2 and 8 weeks of the intervention and by personal interview. Measurements of erythrocyte phospholipid profiles showed significant decreases in total SFA and monounsaturated fatty acids at 4 and 10 weeks. Insulin sensitivity was measured by the short intravenous insulin tolerance test following i.v. injection of 0.1 U insulin/kg after a standardized meal consumed at 20.00 hours the previous day followed by an overnight fast. Blood sampling at 1 min intervals, as forearm venous blood "arterialized" by hand warming, was terminated at 15 min with a glucose drink.

The rate constant for glucose disappearance, calculated from the ln glucose-time plot between 4 and 15 min varied widely in the first test from -4.9 to -1.8 %/min, mean -3.2 (SD 0.83) %/min. There was a variable response to the intervention according to initial insulin resistance. The seven most insulin-resistant subjects all increased insulin sensitivity and glucose disposal rates from -2.7 (SD 0.50) to -4.1 (SD 1.02) %/min, ( $P=0.005$ ,  $n=7$ ), whilst the least insulin-resistant subjects showed no significant overall response: i.e. rates of -4.1 (SD 0.56) %/min control and -3.3 (SD 0.55) %/min intervention, ( $P>0.05$ ,  $n=5$ ). Our reproducibility studies for this assay indicate that the responses are real and not a reflection of assay variation and regression to the mean.

These results demonstrate that in markedly insulin-resistant middle-aged men, a reduction in saturated fat intake and replacement with carbohydrate improves insulin sensitivity whilst having no effect on more insulin-sensitive subjects.

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**The effect of the daily ingestion of inulin on fasting and postprandial lipid metabolism in middle-aged men and women.** By K.G. JACKSON, G.R.J. TAYLOR, A.M. CLOHESSY, J.E. LUFF, C.S. THOMAS and C.M. WILLIAMS. *Hugh Sinclair Unit of Human Nutrition, Department of Food Science and Technology, University of Reading, Reading RG6 6AP*

A number of animal studies have demonstrated marked reductions in fasting and postprandial triacylglycerol (TAG) and cholesterol (TC) levels when diets containing significant amounts of fructo-oligosaccharides (FOS, e.g. inulin) are fed (Roberfroid, 1993). This is associated with suppression of the *de novo* synthesis of TAG-rich lipoproteins, inhibition of hepatic fatty acid synthase (EC 2.3.1.85) and down-regulation of the gene expression of this lipogenic enzyme (Delzenne, 1998). This TAG-lowering property of FOS is important in view of TAG being identified as a risk factor for CHD (Zilversmit, 1995). However, very little is known about the effects of inulin on blood lipids in man.

This present study was carried out to investigate the effect of the daily ingestion of inulin (10 g) on fasting and postprandial lipids in healthy middle-aged men and women, who had plasma lipids in the upper end of the normal range. The study was a double-blind randomized placebo-controlled study in which fifty-four middle-aged men and women (aged between 35 and 70 years, BMI 20-32 kg/m<sup>2</sup>) received either 10 g (two 5 g sachets) of inulin (Rafiline HP Gel) or placebo (maltodextrin) for a period of 8 weeks, with a follow-up at 12 weeks. Fasting blood samples were collected before the supplementation period (baseline 1 and 2, separated by 1 week) and at weeks 4, 8 and 12. Detailed postprandial investigations were carried out in twenty-three volunteers at baseline and at week 8 using a two meal protocol. Following a 12 h overnight fast, volunteers were given test meals which contained 50 g fat at 08.30 hours (breakfast) and 30 g fat at 14.00 hours (lunch). Blood samples were collected at half-hourly intervals for 90 min following each meal and then hourly for 3 h after the breakfast and 1 h after the lunch. Plasma TAG (fasting and postprandial), TC and HDL-cholesterol (HDL-C) were measured by an automated enzymic method. Fasting LDL-cholesterol (LDL-C) was calculated using the Friedewald equation.

Fasting TAG, TC, HDL-C and LDL-C concentrations were not significantly different during the 8-week period for the inulin and placebo groups. There was a trend however, for fasting TAG concentrations to show a decrease, compared to baseline concentrations, between weeks 4 and 8 in the inulin group ( $P < 0.08$ ) which returned to baseline concentrations at week 12. Although there were no significant differences in baseline fasting TAG levels between the two groups, the week 8 fasting plasma TAG concentration was significantly lower in the inulin group compared with that for the placebo group ( $P < 0.03$ ). No significant differences were observed in the postprandial TAG responses between baseline and week 8 for either the inulin ( $n 13$ ) or the placebo ( $n 10$ ) groups.

We therefore conclude that the daily addition of 10 g inulin did not significantly affect TC, HDL-C and LDL-C during the 8-week test period. However, on comparison with the placebo group, significantly lower fasting TAG concentrations were observed at week 8 which supports findings from animal studies that inulin influences the formation and/or degradation of TAG-rich lipoprotein particles. The effects observed on fasting TAG in the present study were small, and the high doses and prolonged periods of feeding used in animal studies suggest that studies of longer duration, using higher doses of FOS, may be required to elicit important effects in human subjects.

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**Relationship between waist:hip ratio, waist circumference and postprandial insulin response in post-menopausal women.** By J.A. LOVEGROVE<sup>1</sup>, H. OSBORN<sup>1</sup>, J.W. WRIGHT<sup>2</sup>, V. MOHAMED-ALI<sup>3</sup> and C.M. WILLIAMS<sup>1</sup>. *<sup>1</sup>Hugh Sinclair Unit of Human Nutrition, The University of Reading, Reading RG6 6AP, <sup>2</sup>Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH, <sup>3</sup>Department of Medicine, Wittington Hospital, London N19 3UA*

Insulin insensitivity has been linked to the risk of a number of chronic diseases such as CHD, non-insulin-dependent diabetes mellitus (NIDDM) and post-menopausal breast cancer. Obesity, and more specifically central fat distribution, has been linked to a reduced insulin-sensitive state (Ley *et al.*, 1992). The present study investigated the relationship between body shape and postprandial insulin and glucose responses in post-menopausal women following the consumption of standard moderate fat-containing mixed meals, eaten at breakfast and lunch.

Healthy, normolipidaemic post-menopausal women were recruited from the University of Reading and the surrounding population. All fulfilled specific inclusion criteria and gave written informed consent. Volunteers were divided into two groups according to their waist:hip ratio (W:H). Those women with W:H >0.80 were classed as android ( $n 12$ ) and those with a W:H <0.78 as gynoid ( $n 16$ ). After a 12 h overnight fast a cannula was inserted and two basal blood samples were collected. The subjects were then given a breakfast (0 h) which provided 2469 KJ, 30 g fat, 11 g protein and 76 g carbohydrate, followed by a lunch (4.5 h) which provided 3138 KJ, 44 g fat, 11 g protein and 81 g carbohydrate 270 min later. No other food or drink, except water and sugar-free drinks, was consumed for the study period. Blood samples were taken every 30 min for the first 90 min after a meal, and hourly thereafter for 10 h. Plasma was prepared and stored at -20° for future analysis. Plasma glucose was measured using an enzymic method on an automated analyser (Monarch) and plasma insulin was measured using a specific insulin ELISA (Mohamed-Ali & Yudkin, 1992).

All volunteers successfully completed the study. The groups were closely matched for age: android, 62 (SD 9) years; gynoid, 61 (SD 6) years, and % body fat: android 43 (SD 3)% and gynoid 39 (SD 4)%. The W:H for the two groups were significantly different ( $P=0.0001$ ): android: 0.84 (SD 0.04) and gynoid 0.74 (SD 0.04). No significant differences were observed in fasting glucose and insulin levels between the two groups. However there was a significantly higher area under the postprandial curve (AUC) for insulin in the android compared with the gynoid group ( $P=0.02$ ), and a significantly higher insulin response to breakfast (peak 1) and lunch (peak 2) in the android compared with the gynoid group ( $P=0.03$  and  $P=0.04$  respectively) as shown in the Table. The insulin:glucose ratio (calculated from total postprandial response) was also found to be significantly higher ( $P=0.02$ ) in the android, 2.0 (SD 1.0) compared with the gynoid group, 1.2 (SD 0.3).

	AUC						Peak 2					
	Android		Gynoid		Android		Gynoid		Android		Gynoid	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Insulin (pmol/l)	136552*	68233	78864	19341	459*	237	295	136	338*	192	220	92
Glucose (mmol/l)	67717	4344	66523	5019	149	12	137	12	146	18	157	20

Mean values were significantly different from gynoid, \*  $P < 0.05$

The results of this study support the hypothesis that a central fat distribution is linked with reduced insulin sensitivity. In multiple regression analysis it was observed that the waist circumference accounted for 59% of the variability of the insulin:glucose ratio (whereas W:H only accounted for 38%), this suggests that waist circumference is a more sensitive proxy for insulin sensitivity than W:H. It is concluded that a central fat distribution is associated with reduced insulin sensitivity in post-menopausal women. This could help to explain why post-menopausal women, in whom fat accumulation in abdominal regions is common, have an increased risk of developing CHD and NIDDM. These results could also account for some of the discrepancies between studies which simply investigate size or adiposity as a variable.

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**The immunolocalization of apolipoprotein B-48 and B-100 in human tissues.** By NORHAIZAN MOHD ESA<sup>1</sup>, CHARLOTTE NICOLS<sup>1</sup>, MICHAEL J. DAVIES<sup>2</sup> AND BARRY J. GOULD<sup>1</sup>, <sup>1</sup>Centre for Nutrition Research, School of Biological Sciences, University of Surrey, Guildford GU2 5XH and <sup>2</sup>Cardiovascular Pathology Research Group, St George's Hospital Medical School, Tooting, London SW17 0RE

Apolipoprotein B-48 (apoB-48) is the specific transport protein of dietary fat which is packaged as chylomicrons in enterocytes. Most of the triacylglycerol (TAG) is removed from chylomicrons, by the action of the lipoprotein lipase (EC 3.1.1.34) of skeletal muscle and adipose tissue, with the formation of chylomicron remnants. These are taken up by the liver, and TAG is re-packaged in particles with the transport protein apoB-100 (apoB-48 is 48% of apoB-100). We have used our specific antiserum to apoB-48, which recognizes the C-terminal end of the protein, and a monoclonal antibody to the C-terminal end of apoB-100 to examine the tissue localization of these two proteins, of which the N-terminal 2152 amino acids are identical. The main aims of this research were, (i) to demonstrate the specificity of the antibodies by investigating human ileum and liver, and (ii) to see whether there was evidence for the direct involvement of chylomicron remnants in the formation of atherosclerotic plaque in human subjects.

The method was to take the tissue sections, cut from paraffin blocks, to inhibit the endogenous peroxidase activity before microwaving the section to allow access of antibodies to the cell contents. Non-specific binding was blocked with pig serum before incubation with the appropriate antibodies. The bound antibodies were detected with an anti-species antiserum and the signal was enhanced by use of an avidin-biotin-peroxidase complex which was visualized with diaminobenzidine and H<sub>2</sub>O<sub>2</sub>.

ApoB-48, which is synthesized in enterocytes, was clearly visible in the sections of ileum. However, apoB-100 was apparently absent from these same cells. These results were as expected and indicate that no, or very little, apoB-100 is formed in enterocytes. Hepatocytes were strongly stained for the presence of apoB-100, and there was also a low level of staining for apoB-48. Since liver is the site of synthesis of apoB-100 and the tissue where chylomicron remnants are taken up these results were also as expected.

Sections of atherosclerotic plaques of aorta were also examined, as were some 'fatty streaks'. The results showed that apoB-48 and apoB-100 were present in the aorta but only apoB-100 was apparent in fatty streak.

These results indicate that chylomicron remnants, in addition to liver-derived lipoprotein particles, contribute directly to the formation of atherosclerotic plaque of aorta.

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**Impact of triacylglycerol structure on early metabolic events in adipose tissue.** By LUCINDA K.M. SUMMERS<sup>1</sup>, BARBARA A. FIELDING<sup>1</sup>, VERA ILLIC<sup>1</sup>, MO L. CLARK<sup>1</sup>, PAUL T. QUINLAN<sup>2</sup> and KEITH N. FRAYN<sup>1</sup>, <sup>1</sup>Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE and <sup>2</sup>Unilever Research Colworth Laboratory, Sharnbrook, Bedford MK44 1LQ

There has been considerable interest in the idea that the molecular structure of dietary triacylglycerol (TG) might affect its absorption and subsequent metabolism. We and others have investigated this previously using structured TG with the predominant species POO/OOP and OPO (where P represents palmitoyl-, and O oleoyl-glycerol) (Zampelas *et al.* 1994; Summers *et al.* 1998), and found no metabolic differences in the early postprandial period. However, since palmitic and oleic acids are the predominant fatty acids in the plasma TG and non-esterified fatty acid (NEFA) pools, only major changes in specific fatty acid metabolism might be detected. Here we have studied the initial metabolic processing of chylomicron-TG following ingestion of structured TGs with the predominant molecular species StOO/OOSt and OSiO (where St represents stearoyl-glycerol).

Eight healthy females aged 40 – 65 years with BMI 21 – 53 kg/m<sup>2</sup> were each studied on two occasions after an overnight fast. Samples were taken from an arterialized hand vein (referred to as arterial below) and a vein draining the subcutaneous abdominal adipose tissue. After baseline samples subjects ate a meal (cornflakes and milkshake) providing 85 g carbohydrate and 60 g fat as either StOO or OSiO (using the nomenclature above, where StOO represents a mixture of *sn*-1- and *sn*-3-stearoyl di-oleoyl glycerol). Samples were taken for a further 6 h.

There was marked release of NEFA from subcutaneous adipose tissue as expected. The ratio of release of oleic to stearic acid was calculated. In the basal state this was about 10 reflecting normal release from adipocytes (Fig.). After the meal this value dropped rapidly towards 2, close to the value in the fat ingested (StOO, 1.85; OSiO, 1.75). This must reflect the generation of NEFA by the action of lipoprotein lipase (EC 3.1.1.34) on chylomicron-TG with concomitant suppression of intracellular lipolysis. The pattern of fatty acid release was indistinguishable following the two types of fat (Fig.).

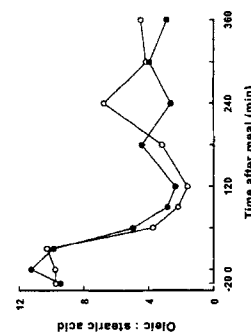


Fig. 1. Ratio oleic to stearic acid in the non-esterified fatty acids released from subcutaneous adipose tissue before and after meals containing 60 g StOO (open points) or OSiO (solid points). Median values shown; n 8.

Chylomicron-TG clearance in adipose tissue was also identical following the two fats.

We conclude that the molecular structure of TG is not a significant determinant of its early metabolic processing in subcutaneous adipose tissue. There appears to be no differential handling of the *sn*-2 fatty acid compared with the *sn*-1 or *sn*-3 position.

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**Fasting leptin responses to increases in fat balance induced by 50% fat and carbohydrate overfeeding in lean and obese women.** By REGINA M. McDEVITT<sup>1,2</sup>, SADAF FAROOQ<sup>3</sup>, STEPHEN O'RAHILLY<sup>3</sup> and ANDREW M. PRENTICE<sup>1</sup>, <sup>1</sup>MRC Dunn Clinical Nutrition Centre, Cambridge CB2 2DH, <sup>2</sup>Department of Biochemistry and Nutrition, SAC Auchincruive, KA9 5HW and <sup>3</sup>University Department of Medicine and Clinical Biochemistry, Cambridge CB2 2QQ.

Plasma leptin levels have been shown to respond to changes in energy intake (Jenkins *et al.* 1997) and to dietary composition (Schrauwen *et al.* 1997). As part of a study on *de novo* lipogenesis we measured fasting leptin responses to precisely quantified changes in energy, fat and glycogen stores induced by 50% overfeeding by fat or carbohydrate (CHO) over 4 d. The study was approved by the Ethics Committee of The Dunn Clinical Nutrition Centre and informed consent was obtained from each subject. Over five periods of 4 d in a whole-body calorimeter eight lean (BMI 24.7, SD 1.9 kg/m<sup>2</sup>) and five obese (BMI 32.4, SD 4.5 kg/m<sup>2</sup>) subjects were fed to energy balance (control) or were 50% overfed (OF) with fat or CHO as sucrose, glucose or fructose in random order. Fasting blood was drawn at the end of 96 h for each treatment (12 h after the last meal) and analysed for levels of leptin, insulin, glucose and triacylglycerol (TAG). Macronutrient balances were calculated as the cumulative sum of intake minus oxidation over 4 d, where oxidation was determined from gas exchange and urinary N excretion. As anticipated, leptin levels were positively correlated with BMI ( $P \leq 0.001$ ) and with percentage body fat ( $P \leq 0.001$ ). They were more than twice as high in obese subjects compared with lean ( $P \leq 0.001$ ) but treatment had no effect on leptin levels in either lean or obese subjects and body mass (BM) was not a significant covariate (see Table). Leptin levels were not significantly related to either glucose, insulin or TAG levels for any treatment. Leptin levels were not significantly related to fat balance in either lean or obese subjects. However, there was a significant positive relationship between leptin levels and CHO balance, though only in the obese subjects ( $P \leq 0.05$ ), and not in the lean subjects.

Lean (n 8)	Dietary treatment											
	Control		Sucrose OF		Glucose OF		Fructose OF		Fat OF			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Leptin (ng/ml)	21.1	4.2	22.4	5.2	21.5	5.8	22.4	4.3	19.7	4.6		
Fat balance (g/4 d)	-22.2	26.1	275.5	23.0	246.4	31.2	273.3	20.4	288.5	23.2		
CHO balance (g/4 d)	-46.9	39.5	102.3	31.2	159.5	31.6	115.5	25.1	55.8	22.3		
Obese (n 5)												
Leptin (ng/ml)	46.2	10.4	52.6	8.8	53.2	7.9	51.2	8.0	49.2	9.2		
Fat balance (g/4 d)	-33.9	35.6	250.3	27.2	300.4	25.2	305.7	22.3	328.9	30.7		
CHO balance (g/4 d)	14.7	51.5	200.2	100.4	96.5	60.7	112.2	61.7	101.7	42.4		

The lack of an increase in leptin levels after a 50% increase in energy intake is surprising, especially since leptin was shown to decrease in response to a short-term 50% decrease in energy in lean subjects after 4 d (Jenkins *et al.* 1997). In addition, the lack of a leptin response to overfeeding was the same whether the excess energy was given as fat or either of three carbohydrate sources. These data suggest that short-term overfeeding has no direct impact on leptin levels in lean and obese women. However, the consequences of the overfeeding, measured precisely as macronutrient balances, do indicate that in the obese subjects at least, leptin levels are related to CHO balance.

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**Effects of fish oil supplementation on *in vitro* oxidation of LDL in subjects with an atherogenic lipoprotein phenotype.** By E. LEIGH-FIRBANK<sup>1</sup>, A.M. MINIHANE<sup>1</sup>, D. LEAKE<sup>2</sup>, B.A. GRIFFIN<sup>3</sup>, M.C. MURPHY<sup>3</sup> and C.M. WILLIAMS<sup>1</sup>, <sup>1</sup>Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading RG6 6AP, <sup>2</sup>School of Animal and Microbial Sciences, University of Reading, Reading RG6 6AP, <sup>3</sup>Centre of Food Safety and Nutrition, University of Surrey, Guildford, Surrey GU2 5XH

Although some studies report increased susceptibility of LDL to *in vitro* oxidation in subjects supplemented with fish oils (Oostenburg *et al.* 1997; Tsai & Lu, 1997), other studies have found no effect on this *in vitro* marker (Bonanome *et al.* 1996; Mata *et al.* 1996). The atherogenic lipoprotein phenotype (ALP) is a common dyslipidaemia characterized by moderately raised triacylglycerols (TG), low HDL-cholesterol and an increased proportion of LDL in the small dense atherogenic form (LDL3). The atherogenicity of small dense LDL has, in part, been attributed to its lower  $\alpha$ -tocopherol content, conveying a greater susceptibility to oxidation *in vivo*. In the present study we have investigated the effect, on *in vitro* oxidative susceptibility of LDL, of a daily intake of 2.8 g eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in subjects with an ALP. This study forms part of a larger investigation which is evaluating the ability of fish-oil supplementation to ameliorate the lipid abnormalities of the ALP.

Twenty-two subjects with a blood lipid profile characteristic of an ALP (TG 1.5-3.0 mmol/l; HDL-C <1.1 mmol/l; LDL3 >40%) were recruited through the Royal Berkshire Hospital, Reading. The study design was a double-blind randomized cross-over trial of fish oil and placebo capsules with each treatment arm lasting 6 weeks and a 12 week washout in between. Fasting blood samples were collected at 0, 3 and 6 weeks on each treatment arm. *In vitro* oxidative susceptibility of LDL was monitored using the measurement of the lag phase following Cu-induced conjugated diene formation. Platelet phospholipid fatty acid composition was measured using capillary gc in phospholipids extracted from platelet-rich plasma. Plasma TG were measured using an enzymatic method on a Monarch autoanalyser.

On the fish oil arm LDL lag phase was reduced from 71 (SE 2.2) to 52 (SE 1.5) min at 0 and 6 weeks respectively ( $p < 0.0001$ ). On the fish-oil arm platelet phospholipid arachidonic acid content decreased from 23.7 (SE 0.78) to 21.4 (SE 0.29) g/100 g ( $p < 0.001$ ) and EPA increased from 0.33 (SE 0.15) to 3.19 (SE 0.14) g/100 g ( $p < 0.0001$ ) between 0 and 6 weeks. Fish-oil supplementation reduced fasting plasma TG from 2.65 (SE 0.17) mmol/l to 1.99 (SE 0.17) mmol/l ( $p < 0.001$ ), with a mean reduction of 29% between 0 and 6 weeks of treatment. There were no significant changes in TG or platelet phospholipid fatty acids on the placebo arm. A small increase in LDL lag phase occurred on the placebo arm ( $p < 0.01$ ).

Feeding 2.8 g/d of EPA/DHA to subjects with an ALP increases the oxidative susceptibility of their LDL *in vitro*. The consistency of this response in subjects of the present study may reflect the reportedly lower  $\alpha$ -tocopherol of small dense LDL, the atherogenic form of LDL which characterises the ALP. We plan to measure the  $\alpha$ -tocopherol content of the plasma and isolated LDL. However caution should be applied in interpreting the pathological significance of this finding in the light of questions raised regarding the *in vivo* relevance of this *in vitro* measure. Such caution may well be appropriate in the light of increasing evidence for cardioprotective effects of oily fish and fish-oil supplements reported from epidemiological studies.

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**The fatty acid composition of high fat diets affects energy intake and energy expenditure in laboratory rodents.** By C.E. DONNELLAN, F.A. WALLACE and P.C. CALDER, *Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

Feeding high-fat diets to laboratory rodents results in greater weight gain than feeding low-fat (LF) diets and much of the increased weight is due to adipose tissue deposition (Yaqoob *et al.* 1995). This study investigated whether the type of fat present in a high-fat diet affects energy balance and the development of adiposity in laboratory rodents.

Male C57Bl6 mice were fed *ad libitum* on a low-fat (25 g maize oil/kg; LF) diet or on one of four high-fat (200 g/kg) diets which used hydrogenated coconut oil (CO), olive oil (OO), safflower oil (SO) or fish oil (FO) as the source of fat. All diets contained identical levels of maize starch (200 g/kg), sucrose (295.8 g/kg), protein (200 g/kg) and vitamins and minerals; the high-fat diets also contained 10 g maize oil/kg to prevent essential fatty acid deficiency. The calculated total energy content of the LF diet was 12.26 kJ/g (with 7.5% energy from fat) while the total energy content of the high fat diets was 19.1 kJ/g (with 40.7% energy from fat). All mice weighed approximately 23 g and had been maintained on laboratory chow before being fed on the different diets. Food intake and animal weight were monitored weekly. At slaughter (after feeding for 7 weeks) epididymal fat pads were removed and weighed. Data are means with their standard errors for twelve (epididymal fat pad weight) or twenty-four (other measurements) mice fed on each diet.

Diet	Final weight (g)		Weight gain (g)		Food intake (g/7 weeks)		Energy intake (kJ/7 weeks)		Energy conversion (mg weight gain/kJ energy intake)		Epididymal fat pad weight (mg)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LF	25.3 <sup>a</sup>	0.2	2.5 <sup>a</sup>	0.7	214.2 <sup>a</sup>	1.8	2626 <sup>a</sup>	22	1.03 <sup>a</sup>	0.29	240 <sup>a</sup>	15
CO	27.3 <sup>b</sup>	0.5	3.9 <sup>a</sup>	0.4	158.9 <sup>b</sup>	1.7	3035 <sup>b</sup>	33	1.17 <sup>a</sup>	0.15	302 <sup>a</sup>	29
OO	31.9 <sup>c</sup>	0.7	8.8 <sup>b</sup>	0.6	169.4 <sup>c</sup>	2.5	3236 <sup>c</sup>	48	2.73 <sup>b</sup>	0.18	978 <sup>b</sup>	77
SO	30.5 <sup>c</sup>	0.8	7.5 <sup>b</sup>	0.7	166.6 <sup>c</sup>	1.8	3183 <sup>c</sup>	34	2.34 <sup>b</sup>	0.19	836 <sup>b</sup>	109
FO	25.3 <sup>a</sup>	0.8	2.4 <sup>a</sup>	0.6	181.3 <sup>d</sup>	1.6	3463 <sup>d</sup>	31	0.77 <sup>a</sup>	0.19	213 <sup>a</sup>	38

abcd Mean values within a column not sharing a common superscript letter were significantly different,  $P < 0.05$  (ANOVA)

Mice fed on the high-fat diets ate less food than those fed on the LF diet. However, because of the different energy densities of the diets, the high-fat-fed mice consumed more energy. Despite this, total weight gain was similar in mice fed on the LF, CO and FO diets and was less than in mice fed on the OO or SO diets. Energy conversion and adipose tissue deposition were not different among mice fed on the LF, CO and FO diets and were less than in mice fed on the OO or SO diets. Despite having the highest food and energy intakes among the high-fat-fed mice, the energy conversion in mice fed on the FO diet was the lowest, indicating that energy expenditure was increased in these animals.

Thus, the types of dietary fatty acids which are present in a high-fat diet appear to affect energy intake and energy conversion and so might affect energy expenditure. These effects might come about through influences of different fatty acids upon particular metabolic pathways and/or upon the production of, and sensitivity to, hormones and other mediators involved in the control of energy balance.

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**Within-individual variability in the gastrointestinal handling and metabolism of [1,1,1-<sup>13</sup>C]tripalmitin.** By J. BENNOSON, M.A. HUMAYUN, A.E. JONES, A. HOUNSLOW and S.A. WOOTTON, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

Measurements of within-individual variation during metabolic trials are generally not made. We have previously shown (Murphy *et al.* 1995) substantial within-individual variation in the gastrointestinal (GI) handling (Trial 1 = 14%, Trial 2 = 32% of administered dose) and metabolic disposal (Trial 1 = 25%, Trial 2 = 23% of absorbed dose) of [1-<sup>13</sup>C]palmitic acid in young women. However, this study was carried out with little control over the diet and activity of the subjects. The purpose of the present study was to employ similar tracer methodologies but with controlled experimental conditions to examine within-individual variability of the GI handling and metabolic disposal of [1,1,1-<sup>13</sup>C]tripalmitin.

On two occasions following a 3d identical feeding period (40% energy lipid, 45% energy carbohydrate, 15% energy protein) with limited exercise and ending in an overnight fast, six healthy, young men ingested [1,1,1-<sup>13</sup>C]tripalmitin within a lipid-casein-glucose-sucrose emulsion and test meal (3.2 MJ; 90.3 g carbohydrate; 35.7 g lipid; 27.2 g protein). Breath samples were collected before label administration then at hourly intervals for 10 h and again at 15 and 24 h. Whole-body CO<sub>2</sub> excretion was measured by indirect calorimetry (GEM, Europa Scientific Ltd, Crewe) at the same time points. A baseline stool sample and all stools passed over a 5 d period were collected. <sup>13</sup>C-enrichment of breath and stool samples was analysed by continuous flow-isotope ratio mass spectrometry (Europa Scientific Ltd.). The results are shown in the Table for excretion of <sup>13</sup>C-label in stool as a proportion of administered dose and on breath as <sup>13</sup>CO<sub>2</sub> as a proportion of absorbed dose. Total lipid oxidation over 10 h was estimated from indirect calorimetry (Frayn, 1983) and was used in conjunction with recovery of <sup>13</sup>C-label on breath to estimate exogenous lipid oxidation.

Trial 1 (T1)	Stool <sup>13</sup> C (% administered dose)		Breath <sup>13</sup> CO <sub>2</sub> (% absorbed dose)		Total lipid oxidation (g/10h)	
	Median	Range	Median	Range	Median	Range
Trial 1 (T1)	1.3	0.5 - 2.3	29.0	23.4 - 31.7	28.8	18.1-44.1
Trial 2 (T2)	0.9	0.7 - 2.5	27.8	18.4 - 33.7	35.4	15.4-43.6
Difference (T1-T2)	-0.4	-0.8 to +1.7	-1.2	-7.9 to +2.0	+6.6	-2.7 to +15.9

In general there was good agreement between the trials for the group as a whole, although one individual showed markedly more variation than the other subjects. Appearance of <sup>13</sup>C-label in stool indicated almost complete (98%) absorption of the [1,1,1-<sup>13</sup>C]tripalmitin. This suggests that when using this protocol stool collection is not essential, since differences in absorption are not contributing to variation in oxidation. Over the initial 10 h of the study, when indirect calorimetry was performed hourly, total lipid oxidation did not differ between the trials. Taken together with the oxidation of [1,1,1-<sup>13</sup>C]tripalmitin, it was estimated that exogenous lipid oxidation accounted for on average 50% (34 to 76%) of total lipid oxidation. These results would suggest that within-individual variation in the GI handling and metabolic disposal of dietary lipid can be minimized with the introduction of controlled experimental conditions.

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**Mononuclear cell fatty acid composition in healthy subjects supplementing their diets with evening primrose oil or fish oil.** By P. YAQOUB\*, E.A. NEWSHOLME and P.C. CALDER\*  
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To obtain further information about the effects of polyunsaturated fatty acids (PUFA) upon the human immune system, the fatty acid composition of peripheral blood mononuclear cells (PBMC) taken from healthy subjects supplementing their diet with encapsulated evening primrose oil (EPO) or fish oil (FO) was determined.

Healthy subjects (*n* = 8 per group) supplemented their diet with nine capsules per day for 12 weeks; each capsule contained 1 g placebo oil (an 80:20 mix of coconut and soyabean oils) or 1 g EPO (containing 11.8 g  $\gamma$ -linolenic acid (GLA)/100 g total fatty acids) or 1 g FO (containing 23.5 g eicosapentaenoic acid (EPA) and 13.3 g docosahexaenoic acid (DHA)/100 g total fatty acids). Blood was sampled every 4 weeks during supplementation and after 8 weeks of washout. PBMC were isolated and their fatty acid composition determined by GC.

**Table.** Fatty acid composition of human PBMC before, during and after supplementation with 9 g FO per d

Week	Fatty acid (g/100 g total fatty acids)							
	ARA		EPA		DHA		<i>n</i> -6/ <i>n</i> -3	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0 (Baseline)	22.3 <sup>a</sup>	0.8	0.8 <sup>a</sup>	0.4	1.9	0.5	12.4 <sup>a</sup>	1.7
4	18.2 <sup>b</sup>	1.0	2.5 <sup>b</sup>	0.3	3.2	0.2	5.1 <sup>b</sup>	0.3
8	18.8 <sup>b</sup>	1.5	2.8 <sup>b</sup>	0.2	3.3	0.3	5.3 <sup>b</sup>	0.6
12	18.4 <sup>b</sup>	1.3	3.3 <sup>b</sup>	0.3	3.7	0.6	4.1 <sup>b</sup>	1.2
Washout	21.8 <sup>a</sup>	1.0	1.0 <sup>a</sup>	0.3	3.0	0.4	10.5 <sup>a</sup>	1.6

<sup>a</sup>Mean values within a column not sharing a common superscript letter were significantly different, *P* < 0.05 (repeated measures ANOVA)

There was no appearance of GLA or significant increase in dihomo- $\gamma$ -linolenic acid in PBMC during supplementation with EPO (results not shown). The proportion of EPA was significantly higher (4-fold) in PBMC during FO supplementation compared with baseline or end of washout (see Table) and compared with the other supplements (results not shown). The proportion of EPA in PBMC from FO-supplemented subjects returned to baseline values by 8 weeks of washout. The proportion of DHA in PBMC followed the same trend as EPA, although this increase did not reach significance. FO supplementation significantly decreased the proportion of arachidonic acid (ARA) in PBMC lipids; changes in the proportions of the long chain *n*-6 and *n*-3 PUFAs, the ratio *n*-6/*n*-3 PUFA was significantly reduced during supplementation with FO and returned to baseline 8 weeks after completing supplementation.

It is concluded that supplementation of the diet with a large amount of EPO, which causes significant changes in plasma phospholipid fatty acid composition (results not shown), has little effect on PBMC fatty acid composition. In contrast, supplementation with FO causes marked changes in PBMC fatty acid composition which are readily induced and reversible.

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**Dietary fat influences the production of Th1- but not Th2-derived cytokines.** By F.A. WALLACE, P. YAQOUB, E.A. MILES and P.C. CALDER, Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX

It has previously been documented that dietary oils rich in *n*-3 polyunsaturated fatty acids, such as fish oil (FO), decrease the production of interleukin-2 (IL-2) by murine and human lymphocytes (see Calder, 1996). IL-2 and interferon- $\gamma$  (IFN- $\gamma$ ) are produced by the Th1 class of lymphocytes involved in cell-mediated immunity, macrophage and natural-killer-cell activation, transplant rejection and inflammation. There is little information about the effects of dietary fatty acids on the production of IFN- $\gamma$  or on the production of cytokines such as IL-4 which are produced by the Th2 class of lymphocytes involved in antibody-dependent responses and allergic reactions.

Male C57Bl/6 mice were fed for 6 weeks on diets containing 200 g/kg of either coconut oil (CO), olive oil (OO), safflower oil (SO) or fish oil (FO); each diet also contained 10 g maize oil/kg to prevent essential fatty acid deficiency. Spleen lymphocytes were prepared and cultured for 24 h in the presence of 5  $\mu$ g/ml concanavalin A (Con A). The concentrations of the cytokines IL-2, IFN- $\gamma$  and IL-4 (pg/ml) in the culture medium were measured by ELISA. Lymphocyte proliferation was assessed as Con A-stimulated thymidine incorporation over the final 18 h of a 66 h culture period (counts/min per well) (Yaqoob *et al.* 1994).

Diet	Thymidine incorporation			IL-2		IFN- $\gamma$		IL-4	
	Mean	SEM	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CO	34632 <sup>a</sup>	651	44	231 <sup>a</sup>	44	9.1 <sup>a</sup>	1.0	57	10
OO	12484 <sup>b</sup>	983	9	127 <sup>b</sup>	9	8.5 <sup>a</sup>	0.9	70	8
SO	16438 <sup>b</sup>	6795	8	106 <sup>b</sup>	8	3.1 <sup>b</sup>	1.3	67	12
FO	10402 <sup>b</sup>	1499	19	137 <sup>b</sup>	19	1.3 <sup>b</sup>	1.0	48	13

<sup>a</sup>Mean values within a column not sharing a common superscript letter were significantly different, *P* < 0.05 (ANOVA)

Each of the diets rich in unsaturated fatty acids (OO, SO, FO) decreased lymphocyte proliferation and IL-2 production compared with feeding the CO diet. IFN- $\gamma$  production was reduced by SO or FO feeding compared with feeding the CO- or OO-rich diets. IL-4 production was not significantly affected by the type of fat in the diet, although production was lowest by cells from FO-fed mice. It is concluded that, compared with saturated fatty acids, unsaturated fatty acids decrease Th1 lymphocyte responses and that dietary fatty acids do not influence *ex vivo* production of the Th2 cytokine, IL-4.

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**Effects of fish oil, olive oil, butter oil and maize oil on insulin resistance after turpentine-induced inflammation in the rat.** By S.S.IQBAL<sup>1</sup>, M.M.S.H. DUWAIHY<sup>2</sup>, R.A.AL-SHAGRAWI<sup>3</sup>, and D.J. MILLWARD<sup>4</sup>. <sup>1</sup>Department of Community Health Sciences, College of Applied Medical Sciences, <sup>2</sup>Department of Nutrition, King Abdul Aziz University Hospital, <sup>3</sup>Department of Food and Nutrition, College of Agriculture, King Saud University, P.O. Box 245 Riyadh 11411, Saudi Arabia, and <sup>4</sup>Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey Guildford GU2 5XH

Marked insulin resistance is a feature of the inflammatory response. Dietary fat can influence the inflammatory response and has been implicated in influencing insulin resistance. In the present study we have examined the influence of dietary fat on intravenous glucose tolerance and insulin responses and sensitivity in a rat inflammatory model involving a turpentine-induced sterile abscess.

48 Male wistar rats (250g) were given 100 g/kg fat diets based on maize oil (MO), olive oil (OO), butter oil (BO) or fish oil (FO, 50g fish oil:50g olive oil) for 5 weeks, achieving a mean body weight of 340g, before turpentine treatment. This involved a single subcutaneous injection (2 ml/kg BW) into 6 rats per diet group exactly 24 hrs prior to an intravenous glucose tolerance test. 6 non-injected rats served as controls. Rats were allowed food for 12 hrs after the turpentine then fasted overnight prior to an injection of 50 mg glucose/100g into the catheterised left carotid artery in a urethane-anaesthetised animal immediately following a baseline blood sample and a heparin injection (Davidson & Garvey, 1993). Further blood sampling occurred at 3, 6, 9, 12 and 15 minutes, and plasma was prepared and analysed for glucose and insulin concentrations. Rats were killed after 15 minutes and a number of metabolic and tissue compositional measurements made which will be reported elsewhere.

Fasting glucose levels were not significantly affected by either the diet or turpentine but mean values were higher in all turpentine groups. For control animals, FO and OO groups were the most glucose tolerant. Glucose disappearance rates (K%/min) were 3.44(SE 0.34), 3.96(SE 0.29), 2.97(SE 0.24) and 4.04(SE 0.41) for MO, OO, BO and FO groups respectively, BO<FO or OO (P<0.05). After turpentine, glucose tolerance worsened in all groups i.e. K values of 2.16 (SE 0.28), 2.84 (SE 0.41), 2.16 (SE 0.26) and 2.86 (SE 0.39) for MO, OO, BO and FO groups respectively, significant reductions for MO and OO groups (P<0.05). These differences in glucose disposal were associated with insulin resistance as indicated by basal and glucose stimulated insulin levels. Thus in the controls, for both fasting insulin levels and the insulin area under the curve, (AUC<sub>15</sub>), BO>FO, p<0.05 for fasting levels. After turpentine, compared with controls there were marked increases in both fasting insulin, (30-50%, P<0.05) and in the insulin AUC<sub>15</sub> (by 31%, 40%, 75% and 50% for MO, OO, BO and FO groups respectively P<0.05). Values for fasting insulin and AUC<sub>15</sub> were lowest in FO and highest for BO in each case. In the case of the insulinogenic index, the plasma insulin-glucose ratio (a crude measure of  $\beta$  cell response to glucose) both fasting and post glucose values were highest for BO and lowest for FO and OO. In the case of insulin sensitivity, (Kglucose/insulin AUC) both fasting and post glucose values were highest for FO and lowest for BO, significant differences for both measures in controls and after turpentine.

These results show that glucose tolerance and insulin sensitivity is better in rats fed PUFA or MUFA compared with SFA and that the impaired glucose tolerance and insulin resistance in inflammation is less marked with long chain n-3 PUFA or MUFA compared with SFA and n-6PUFA.

We thank Seven Seas Ltd for the Fish Oil.

**Dietary fish oil reduces the expression and function of the LDL scavenger receptor in mice.** By E.A. MILES, F.A. WALLACE and P.C. CALDER, *Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

During atherosclerosis macrophages within plaques take up oxidized LDL via the scavenger receptor to become lipid-laden foam cells. Dietary fish oil (FO) provides some protection against the development of atherosclerosis (Connor & Connor, 1990). We hypothesized that at least part of this beneficial effect might be due to a reduction in scavenger receptor expression on macrophages.

Male C57B16 mice were fed for 6 weeks on diets containing 200 g/kg of coconut oil (CO), olive oil (OO), safflower oil (SO) or fish oil (FO); each diet also contained 10 g maize oil/kg to prevent essential fatty acid deficiency. At 4 d before slaughter the mice were injected intraperitoneally with thioglycollate broth to elicit macrophage migration to the peritoneal cavity. At slaughter the peritoneal macrophages were isolated and purified. Flow cytometry was used to assess scavenger receptor expression (antibody staining) and function (uptake of acetylated-LDL (Ac-LDL)). Data are means and their standard errors for eight to ten mice fed on each diet and are either % positive cells or median fluorescence intensity (MFI).

Diet	Scavenger receptor expression				Ac-LDL uptake			
	% positive		MFI		% positive		MFI	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CO	79.3a	2.8	74.1	8.5	58.4	5.4	2389	492
OO	73.1a	3.5	83.3	17.3	59.4	4.3	2563	612
SO	73.6a	2.9	76.4	9.9	59.4	4.8	2373	408
FO	59.0b	4.6	54.0	1.9	51.5	4.8	1884	404

<sup>a,b</sup>Mean values within a column not sharing a common superscript letter were significantly different, P < 0.05 (ANOVA)

FO feeding significantly reduced the percentage of scavenger-receptor-positive macrophages compared with the other diets. This was mirrored by a trend towards a reduced number of macrophages able to take up Ac-LDL following FO feeding. The level of expression of the scavenger receptor on positive macrophages, as measured by MFI, was also reduced by FO feeding, but this did not reach statistical significance. Once again, this was mirrored by a reduced level of Ac-LDL uptake by those cells which were active.

We conclude that FO feeding reduces macrophage scavenger receptor expression and that this might contribute towards the protective effect of dietary FO towards atherosclerosis.

This research is supported by the BBSRC. FAW is supported by the Rank Prize Fund.

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**Comparison of human plasma, erythrocyte and adipose tissue fatty acid compositions and their use as biomarkers of habitual dietary fatty acid intake.** By CARINE BEYSEN and BARBARA A. FIELDING, *Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE*

To assess dietary fatty acid intake, dietary assessment methods are often used. Because of their subjectivity, the results are not always accurate and reproducible. Biomarkers may be used as an alternative to these conventional methods because of their objectivity. The purpose of the present study was to compare the fatty acid compositions of plasma, erythrocyte and adipose tissue with the fatty acid composition of the habitual diet and then to examine which of these different variables might be most suitable as a biomarker of habitual dietary intake of particular fatty acids.

The subjects were eleven women and ten men aged 21–56 years and with a BMI of 18–34 kg/m<sup>2</sup>. A blood sample was drawn from all subjects in the fasted state and an adipose tissue biopsy was obtained from nineteen of them. The fatty acid compositions of plasma non-esterified fatty acids (pl-NEFA), plasma triacylglycerols (pl-TAG), plasma cholesteryl esters (pl-CE), erythrocyte phospholipids (RBC-PL) and adipose tissue triacylglycerols (at-TAG) were analysed by GC to compare with the habitual dietary fatty acid intake. A 7 d food diary was completed to determine habitual intake of total energy, carbohydrate, protein, alcohol, total fat, polyunsaturated fat, monounsaturated fat, saturated fat and individual fatty acids. The results were analysed using Foodbase (copyright institute of brain chemistry and human nutrition)

The table shows that the mean fatty acid composition (g/100g total fatty acids) of the plasma, erythrocyte and adipose tissue fractions differed from each other. The main fatty acids were linoleic acid for pl-CE, saturated fatty acids (16:0, 18:0) for RBC-PL and oleic acid for at-TAG. In general, the fatty acid pattern of pl-NEFA of this group most closely resembled the dietary fatty acid pattern, even more than pl-TAG. Also, the dietary intake of the essential fatty acid  $\alpha$ -linolenic acid was reflected in pl-TAG ( $r$  0.49,  $P$  < 0.05,  $n$  15).

fatty acid...	16:0	18:0	18:1	18:2	18:3	20:4	SFA	MUFA	PUFA	P:S
diet	18.5	8.6	26.2	16.0	1.5	0.2	35.6	33.0	18.2	0.55
pl-NEFA	29.3	8.9	37.3	14.5	1.6	0.2	42.0	41.2	16.7	0.42
pl-TAG	29.0	2.9	36.2	21.1	1.4	0.8	34.6	40.8	24.1	0.73
pl-CE	13.1	0.5	18.0	57.2	0.9	4.6	14.4	21.0	63.9	4.50
RBC-PL	37.3	23.3	20.4	9.0	0.2	6.2	61.2	21.1	17.7	0.33
at-TAG	27.4	5.3	42.7	13.3	0.7	0.1	37.7	48.1	14.1	0.39

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; P:S, polyunsaturated:saturated fatty acid ratio.

These findings may indicate that because of the specific composition, not every variable is useful as a biomarker of the intake of a particular fatty acid. Dietary intake is not the only factor that determines the composition of the various tissue lipids. Homeostatic mechanisms, age, sex, unstable weight and endogenous synthesis also play a role in the fatty acid composition. A combination of biomarkers should be used to reflect the intake of different fatty acids or used as a complement to the conventional dietary assessment methods. Plasma NEFA may be the best choice to give an overall reflection of habitual dietary fatty acid intake.

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**Effect of dietary cholesterol on the expression of the microsomal triglyceride transfer protein gene.** By HELEN M. SIMS<sup>1</sup>, ALAN FORD<sup>2</sup>, ANDREW J. BENNETT<sup>2</sup>, DAVID A. WHITE<sup>2</sup>, MICHAEL A. BILLET<sup>2</sup> AND ANDREW M. SALTER<sup>1</sup>, <sup>1</sup>Division of Nutritional Biochemistry, School of Biological Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD and <sup>2</sup>School of Biomedical Sciences, University of Nottingham Medical School, Nottingham NG7 2UH

We have previously shown that cholesterol feeding increases plasma VLDL cholesterol and triacylglycerol concentrations in the Golden Syrian hamster (Sessions *et al.* 1993). This is associated with an increase in expression of the microsomal triglyceride transfer protein (MTP) gene (Bennett *et al.* 1996). MTP plays an essential role in the assembly of VLDL and may be regulatory in the secretion of the lipoprotein. MTP has previously been shown to be negatively regulated by insulin (Hagan *et al.* 1994), glucose (Lin *et al.* 1995), and ethanol (Lin *et al.* 1997). In addition, the MTP promoter has been shown to contain a modified sterol response element (SRE) (Lin *et al.* 1995) and we have hypothesized that it is through this that dietary cholesterol directly regulates gene transcription.

To test this hypothesis we have linked the human MTP promoter to a reporter firefly luciferase gene (Promega). Primary monolayer cultures of hamster hepatocytes were then prepared from animals fed on either normal chow or chow supplemented with 2.4g cholesterol/kg for 10 days. Cholesterol feeding was seen to result in the accumulation of cholesterol ester droplets within the cells. Cells were established overnight and then transfected with the MTP promoter-luciferase construct using "Primefect" (Flowgen). They were also simultaneously transfected with the Renilla luciferase gene driven by a promoter (CMV) which induces constitutive expression (requiring a different substrate, Dual-Luciferase™ Assay, Promega), thereby acting as a control for transfection efficiency. Cells were then kept for a further 48 h after which the activity of both luciferases was determined. No difference was seen in Renilla luciferase activity between hepatocytes isolated from control and cholesterol-fed animals suggesting no overall difference in transfection efficiency. Values for firefly luciferase were divided by Renilla luciferase activity to correct for differences in transfection efficiency between individual plates of hepatocytes.

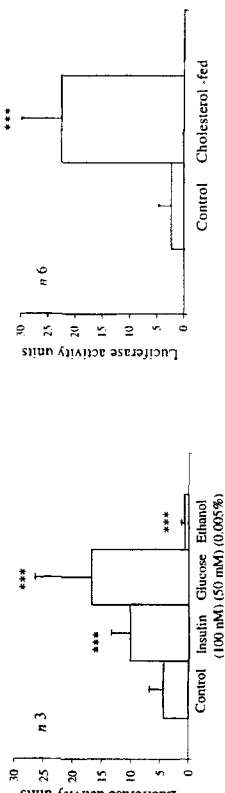


Figure 1  
\*\*\*Significantly different from control.

In control hepatocytes we confirm that ethanol significantly down regulates the MTP promoter, however not the negative response to insulin and glucose (Figure 1). Luciferase activity in the cells isolated from cholesterol-fed animals was considerably greater than in those from animals on the control diet (Figure 2). These results suggest the presence of positive and negative transcription factors which regulate expression of MTP by an, as yet, unknown mechanism but the response to cholesterol may possibly involve the modified SRE described here. Thus, the increase in hepatic MTP mRNA concentrations found on cholesterol feeding may be due to a direct effect of cholesterol on gene transcription. This work was supported by a project grant from the BBSRC.

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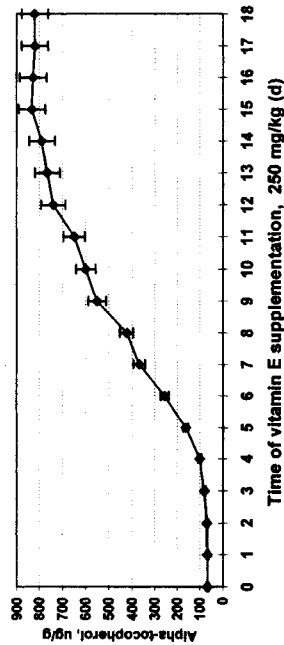
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**Vitamin E transfer from the feed into the egg yolk: effect of dietary supplementation.** By P.F. SURAI, R.M. McDEVITT, B.K. SPEAKE, R.C. NOBLE and N.H.C. SPARKS, Department of Biochemistry and Nutrition, SAC, Auchincruive, Ayr KA6 5HW

Vitamin E is the main natural chain-breaking fat-soluble antioxidant in biological membranes. It plays an important role in embryo development protecting developing tissues against lipid peroxidation (Surai *et al.* 1996). The purpose of the present study was to determine the time-course of vitamin E transfer from the diet into the egg yolk after dietary supplementation of the laying hen.

Laying hens were fed on a commercial wheat-barley-based diet containing 12.4 mg feed-derived  $\alpha$ -tocopherol/kg and supplemented by 10 mg synthetic  $\alpha$ -tocopheryl acetate/kg. At the age of 30 weeks, two experimental groups were formed: one group continued to be fed on the same diet and the second group was fed on this diet, but supplemented with 250 mg  $\alpha$ -tocopheryl acetate/kg. Eggs were collected every day and the  $\alpha$ -tocopherol concentration was determined by HPLC, using methanol-water (97:3, v/v) as a mobile phase. Concentrations of  $\alpha$ -tocopherol in the egg yolk are shown in the Fig.



The data indicate that vitamin E transfer from the feed into the egg yolk takes place very quickly and maximum accumulation of  $\alpha$ -tocopherol was found after 2 weeks of supplementation. In general, in the laying hen, the efficiency of vitamin E transfer to the egg yolk was very high. For example, in the control group efficiency of transfer comprised 44.4% and in the experimental group 27.2%. The high turnover of vitamin E in the liver can be demonstrated by calculation, indicating that in producing each egg, a hen releases an amount of vitamin E which is twice as high as all the vitamin E reserves in the liver. For vitamin A this calculation is completely different. The vitamin A reserves in the liver are enough to supply more than 100 eggs. After exclusion of vitamin E supplements from the experimental diet  $\alpha$ -tocopherol in the egg yolk decreased very quickly returning to its initial level after 10–14 d (results not shown).

The data demonstrate a comparatively high efficiency of vitamin E transfer from the feed into the egg yolk and confirm the importance of a correct dietary vitamin E supplementation to maintain a physiological level of this vitamin during embryo development.

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**Increasing stearoyl Co-A desaturase activity: a potential method to reduce the saturated fatty acid content of lamb meat.** By ANDREW M. SALTER<sup>1</sup>, SION E. RICHARDS<sup>1</sup>, RICHARD J. WARD<sup>1</sup>, MAUREEN T. TRAVERS<sup>2</sup>, MICHAEL C. BARBER<sup>2</sup>, RICHARD G. VERNON<sup>2</sup> and PETER J. BUTTERY<sup>1</sup>, <sup>1</sup>Division of Nutritional Biochemistry, School of Biological Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD and <sup>2</sup>Hannah Research Institute, Ayr, KA6 5HL

Lamb is often criticized for its high saturated fat content and the high proportion of stearic acid is said to be responsible for the poor organoleptic properties of cold cooked lamb. The fatty acid composition of ruminant adipose tissue is largely determined by *de novo* lipogenesis. The primary product of the fatty acid synthase (EC 2.3.1.85; FAS) reaction is palmitic acid but substantial amounts can be further elongated to stearic acid and then desaturated, by stearoyl CoA desaturase (EC 1.14.99.5; SCD), to produce oleic acid.

The fatty acid composition of three adipose tissue depots, omental (OM), perirenal (PR) and subcutaneous (SC) was determined in female Charollais x Mule sheep, fed *ad libitum* from weaning on finisher diet (metabolizable energy 12.4 MJ/kg, crude protein 13.40), aged 12 or 33 weeks (four per group). The oleic acid content increased with age in all depots. Both FAS and SCD activity increased with age. However, the relative increase in FAS (10-fold) was greater than that of SCD (3–4 -fold) and this may explain, in part, the relative decrease in the proportion of oleic acid in the older animals.

Fatty acid	Age (A) (weeks)	Depot (D)			Mean	ANOVA
		OM	PR	SC		
18:1 (%)	12	37.23	36.28	40.30	37.94	A 17 1.02 <0.001
	33	26.47	25.48	34.62	28.86	D 1.25 <0.001
	Mean	31.85	30.88	37.46	33.40	A x D 1.77 0.091
FAS	12	3.5	4.7	5.9	4.7	A 17 5.4 <0.001
	33	44.1	41.1	60.2	48.5	D 6.6 0.263
	Mean	23.8	22.9	33.1	26.6	A x D 9.4 0.390
SCD	12	17.3	18.6	12.8	16.2	A 17 12.3 0.003
	33	41.8	51.9	85.3	59.7	D 15.1 0.432
	Mean	29.5	35.3	49.1	38.0	A x D 21.4 0.267

FAS activity expressed as pmol NADH oxidized/min per 10<sup>6</sup> adipocytes and SCD activity expressed as (% oleic acid formed from <sup>14</sup>C-labelled stearic acid/min per 10<sup>6</sup> adipocytes) x 10<sup>3</sup> (based on method of Wahle, 1974).

We have shown that SCD mRNA is increased when ovine adipose tissue explants are incubated with insulin (Ward *et al.* 1998). We now show that incubation with insulin (I: 17.5 nM) also increases the relative proportion of oleic acid synthesized compared to control incubations (C). Dexamethasone (DM: 10 nM) has the opposite effect.

% Oleate	Treatment (T)	Depot (D)			Mean	ANOVA
		OM	PR	SC		
C	I	11.08	11.04	18.70	13.61	D 12 1.92 <0.001
	DM	20.06	21.88	37.50	26.48	T 12 2.12 <0.001
	I+DM	7.57	6.69	10.86	8.37	D x T 12 3.83 0.200
Mean		21.85	24.02	37.73	27.87	
		15.14	15.91	26.20	19.08	

The table shows the amount of oleate formed is expressed as a percentage of the total radio-labelled fatty acid synthesized from [<sup>14</sup>C]-acetate.

Thus, the potential exists for up-regulating SCD activity *in vivo* and this may be a method of increasing the relative proportions of unsaturated and saturated fatty acids in lamb meat.

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**Tissue-specific profiles of docosahexaenoic acid (DHA) in male chickens depends on the dietary provision.** By P.F. SURAI, R.M. McDEVITT, B.K. SPEAKE, R.C. NOBLE and N.H.C. SPARKS, Department of Biochemistry and Nutrition, SAC Auchincruive, Ayr K46 5HW

The lipid composition of the membrane is a major determinant of its structural and functional properties. In this respect DHA, with its six double bonds is of major importance (Watts, 1997), affecting membrane fluidity, flexibility and compressibility.

The purpose of the present study was to assess the distribution of DHA in various cockerel tissues following dietary DHA supplementation. Male chickens were allocated to two groups with twelve birds per group. The birds of the control group were fed on a commercial wheat-barley-based diet (CD), supplemented with 30 g maize oil/kg, whereas the test diet (TD) was supplemented with 30 g tuna orbital oil/kg. The CD contained 0.5 (SD 0.1) and the TD 14.5 (SD 0.4) g DHA/100 g. The birds were fed from 10 to 65 weeks of age. At the end of the experiment cockerels were killed, lipids were extracted from the various tissues using standard techniques and fatty acid composition was studied by capillary GC. The distribution of the main long-chain polyunsaturated fatty acids (PUFA) in the chicken tissue phospholipids (g/100 g) is shown in the Table (n 5).

Tissue	Control Diet			Test Diet		
	22.6n-3	22.4n-6	20.4n-6	22.6n-3	22.4n-6	20.4n-6
Liver	12.96	0.66	7.50	24.89c	0.36a	4.84b
Testes	2.47	14.06	15.80	5.99c	9.45b	11.73a
Heart	1.11	0.31	20.88	2.95c	0.20a	18.61
Muscle- breast	12.49	0.52	15.88	20.09c	0.38a	12.35a
Muscle- Thigh	8.46	0.45	13.16	15.94c	0.30a	10.46
Brain (cerebrum)	17.27	3.06	11.10	19.73a	1.55b	8.50b

Significance to control diet: \*P<0.05; †P<0.01; ‡P<0.001.

The data indicate that inclusion of DHA into the chick diet was associated with changes in the fatty acid composition of the tissue phospholipids. The level of DHA in the various tissues increased factorially as follows: liver 1.92, testes 2.43, heart 2.66, breast muscle 1.61, thigh muscle 1.88 and brain 1.14. It is clear from the data that heart is characterized by the highest response to the DHA feeding and the brain is the most resistant to the fatty acid changes.

An increase in the proportion of DHA (n-3) in the phospholipids of most of the tissues was associated with a decrease of n-6 (PUFA), particularly arachidonic acid (20:4n-6) and 22:4n-6 (testes) PUFA. However, the level of arachidonic acid in the heart was not changed after DHA feeding. The other lipid fractions of the tissues were also enriched by DHA as a result of the diet supplementation. For example, in the liver of the control chickens the level of DHA in the triacylglycerols increased from 2.21 to 11.72 % in the test group. Similarly in the breast muscle triacylglycerols there was an increase in DHA level from 0.70 to 7.4 % and in the thigh muscle triacylglycerols from 0.96 to 6.92 %.

The results clearly indicate tissue-specific features in DHA accumulation and distribution in chick tissues as a result of dietary supplementation with DHA.

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**Carotenoid distribution in tissues of the laying hen depends on dietary supplementation.** By P.F. SURAI, R.M. McDEVITT, B.K. SPEAKE and N.H.C. SPARKS, Department of Biochemistry and Nutrition, SAC Auchincruive, Ayr K46 5HW

Carotenoids play an important role in human and animal nutrition. They may have a beneficial effect in preventing some human diseases. In spite of a number of publications devoted to carotenoid metabolism and functions there is still lack of information in terms of species- and tissue-specific differences.

The purpose of the present study was to assess the distribution of carotenoids in various hen tissues following dietary carotenoid supplementation. Laying hens were fed on a commercial wheat-barley-based diet containing 8.5 mg lutein/kg and 6.5 mg citranaxanthin/kg. At the age of 30 weeks, two experimental groups were formed. One group was fed on a low-carotenoid diet (4.5 mg lutein/kg) and the second group was fed on the same diet supplemented with a carotenoid mixture, which provided: 24.5 mg lutein (Lt); 45.0 mg citranaxanthin (Ct); 3.0 mg canthaxanthin (Cn) and 24.0 mg ethyl ester of  $\beta$ -apo-8'-carotenoid acid per kg of feed respectively. After 40 d of feeding the experimental diets, the hens were killed and carotenoid levels in the tissues were determined by HPLC using acetonitrile-dichloromethane-methanol (7:2:1, by vol.) as a mobile phase. Concentrations of total carotenoids in the tissues of the laying hens (n5) were shown in the Table.

Diet	Low-carotenoid			High-carotenoid		
	Mean	SE	P value	Mean	SE	P value
Liver	6.17	0.54	<0.001	18.42	1.25	<0.001
Kidney	0.66	0.06	0.08	0.98	0.10	<0.01
Spleen	0.54	0.04	<0.01	1.24	0.13	<0.01
Abdominal fat	0.76	0.07	0.3	1.01	0.12	<0.01
Heart	0.46	0.03	<0.01	1.46	0.18	<0.01
Lung	0.38	0.05	<0.01	1.05	0.11	<0.01
Muscle	0.24	0.02	<0.05	0.46	0.06	<0.05
Blood plasma	0.73	0.09	<0.001	2.96	0.31	<0.001

total carotenoids in the tissues ( $\mu\text{g/g}$ ), in the plasma ( $\mu\text{g/ml}$ ).

In the tissues of the laying hens fed on low-carotenoid diet, the percentage of total carotenoids comprised by Lt was as follows; liver 82.6, kidney 86.4, spleen 88.9, fat 81.6, heart 82.6, lung 81.6%, muscle 83.3; and blood plasma 89.0. On the other hand, enrichment of the diet with the other carotenoids was associated with their accumulation in the tissues. In the hens fed on the high-carotenoid diet, the liver contained: Lt 28.9, Ct 30.3, carotenoid acid (Ca) 22.0 and Cn 3.5 % of total carotenoids. In the tissues, the distribution (%) of the carotenoids was as follows: kidney: Lt 65.3; Ct 18.4; Ca 10.2, spleen: Lt 49.2; Ct 21.8; Ca 18.6, heart: Lt 41.8; Ct 24.0; Cn 4.1; Ca 15.8 and plasma: Lt 38.9; Ct 22.6; Cn 3.5 and Ca 27.7 respectively.

The results showed a tissue-specific response and distribution of different carotenoids. For example, in spite of higher supplementation of the diet by Ct, in most of the tissues lutein remained the major carotenoid. The accumulation of Ct, Cn and Ca in the liver (as % of total carotenoids) was much higher compared with the other tissues. It is possible that all four carotenoids accumulated in the liver but that lutein is preferentially released into the blood in association with VLDL.

**Effect of dietary fatty acids on plasma and adipose tissue fatty acid proportions during pregnancy in the sow.** By JOHN A. ROOKE, *Animal Biology Division, SAC, Craibstone Estate, Aberdeen AB21 9YA*

A previous study (Rooke *et al.* 1998) has shown that feeding tuna oil to sows in late gestation increases docosahexaenoic acid (22:6n-3, DHA) proportions in newborn piglets. It is not known however whether feeding linolenic acid (18:3n-3, ALA), the precursor essential fatty acid, would achieve the same effect. Therefore, multiparous sows (ten per treatment) were fed on diets containing 17.5 g/kg maize oil (M), a mixture of linseed and maize oils (L) or tuna oil (F). The diets contained (g/kg total fatty acids) 23, 145 and 23 g ALA, 8, 1 and 101 g DHA and n-6:n-3 ratios of 15.3, 2.1 and 3.1 for diets M, L and F respectively. Sows were fed on the diets from 7 d post service. Samples of plasma and of subcutaneous adipose tissue were obtained from each sow on days 4, 35, 62 and 95 of gestation and analysed for their total fatty acid composition. Two piglets per sow were killed at birth and the fatty acid composition of their brains determined.

Diet... DHA*	Plasma			Adipose tissue		
	M	L	F	M	L	F
4 d	17	16	16	4	5	4
35 d	16	11	52	4	7	13
62 d	15	18	44	3	4	15
95 d	14	14	45	4	5	19
ALA*						
4 d	16	17	15	15	19	14
35 d	13	51	15	13	29	14
62 d	13	51	11	19	31	15
95 d	14	53	11	16	35	15

\* g ALA or DHA/kg total fatty acids.  
† SED for mean of ten observations.

The Table shows that feeding tuna oil increased DHA proportions in plasma by day 35 (quadratic effect,  $P < 0.001$ ) and storage of DHA in adipose tissue increased throughout gestation (linear effect,  $P < 0.001$ ) but had no effect on ALA proportions. Feeding ALA as linseed oil did not increase either plasma or adipose tissue DHA proportions throughout gestation when compared with sows fed on the maize oil diet only. ALA proportions in both plasma (quadratic effect,  $P < 0.001$ ) and adipose tissue (linear effect,  $P < 0.001$ ) were, however, increased by feeding ALA. Feeding tuna oil increased piglet brain long-chain n-3 fatty acid proportions (172 g/kg total fatty acids; SED 14.2) when compared with either maize (135) or linseed oil (139) diets.

The pregnant sow therefore does not readily elongate and desaturate ALA to DHA as found in other species (Gerster, 1995), nor does supplementation with ALA increase the deposition of long chain n-3 fatty acids in the brain of new-born piglets. Therefore feeding long-chain n-3 fatty acids to the sow is necessary to increase long-chain n-3 fatty acid proportions in piglet brain.

Gerster H (1995) *International Journal of Vitamin and Nutrition Research* 65, 3-20  
Rooke J A, Bland I M & Edwards S A (1998) *British Journal of Nutrition* (In the Press).

**Influence of diet type, potassium and animal species on the absorption of magnesium by ruminants.** By O. ADEDJI & N.F. SUTTLE, *Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ*

A recent extensive review of Mg 'bioavailability' noted that the fractional efficiency of apparent absorption of Mg ( $AA_{Mg}$ ) in ruminants could vary from -0.04 to +0.66 (Henry & Benz, 1995). Little attempt was made to identify sources of variation, other than those between inorganic Mg supplements. Earlier examination of the literature suggested that  $AA_{Mg}$  was twice as high for roughage/concentrate (R/C) mixtures as for fresh herbage (FH) and confirmed the marked inhibitory effect of dietary K (Suttle, 1987). Since grass is usually richer in K than a dry diet, differences between diet types in  $AA_{Mg}$  may be largely attributable to differences in K concentration. The objective of the present study was to identify systematic sources of variation in  $AA_{Mg}$  from published balance studies with sheep and cattle.

The database consisted of 113 published mean values for Mg balances in treatment groups of sheep and forty-two for cattle, most of them cited by Henry & Benz (1995). Sheep data were subdivided according to diet type (FH; R/C; conserved forage, CF). Within each sub-group, multiple linear regression techniques were used to assess the contributions of dietary K concentration to variation in  $AA_{Mg}$  (fraction of ingested Mg not recovered in faeces) after adjustment for differences in live weight. Data for R/C included experimental treatments involving the addition of inorganic Mg or K supplements. The sparse data for cattle were grouped into two diet types forage (FH + CF) and R/C and unadjusted data were analysed in the same way as those for sheep. The results are summarized in the Table which gives intercepts (c) and coefficients (a) for multiple linear regression equations for the relationships between fractional  $AA_{Mg}$  and dietary K (g/100 g DM) in sheep and cattle on different diets: values are means and standard deviations and  $R^2$  gives the variation accounted for.

Species	Diet	n	c		aK		R <sup>2</sup> (%)	P
			Mean	SD	Mean	SD		
Sheep	FH	57	0.67	0.058	-0.12	0.019	46.9	0.001
	CF	15	0.66	0.131	-0.16	0.054	35.2	0.002
	R/C	41	0.40	0.034	-0.04	0.012	20.1	0.002
Cattle	FH + CF	23	0.21	0.028	-0.016	0.0085	9.8	0.08
	R/C	19	0.28	0.041	-0.052	0.0158	35.6	0.004

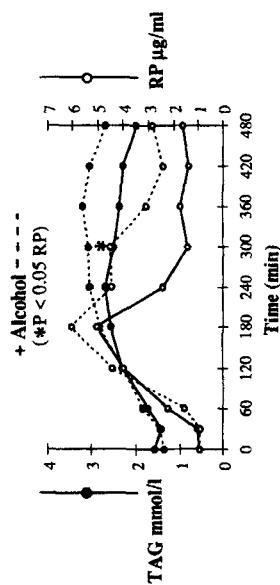
There were enormous differences between both intercepts and slopes for sheep and cattle, particularly where forages were concerned. When K was low (1 g/100 g DM), sheep absorbed forage Mg roughly three times more efficiently than cattle. In sheep, however,  $AA_{Mg}$  was much the more sensitive to K and when concentrations were high (5 g K/100 g DM) coefficients for forages in the two species were equally low (c 0.10). When mixed dry R/C diets low in K were fed,  $AA_{Mg}$  was again higher for sheep than cattle but the margin was lower and  $AA_{Mg}$  was equally sensitive to increases in K in the two species.

Systematic effects of diet type, dietary K and species on  $AA_{Mg}$  appeared to be subject to three-way interactions. K is known to inhibit Mg absorption from the rumen but not the small or large intestine and the contrasts probably reflect the importance of the rumen as a site for digestion and Mg absorption for a given species on a given diet and hence the scope for K x Mg antagonism. Since the major disorders of ruminants involving Mg are in dairy cows on spring pasture, grass should provide their 'meat' in any experiment addressing such disorders in the new millennium.

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Suttle N F (1987) *The Nutrition of Herbivores*, pp.333-361 [J H Ternouth and J B Hacker, editors]. Canberra: Academic Press.

**Acute effects of alcohol on postprandial lipid metabolism.** By ANNE J. HAWDON, NORHAIZAN MOHD ESA, JOHN W. WRIGHT, BARRY J. GOULD and BRUCE A. GRIFFIN, *Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

Enhanced postprandial lipaemia provides an important metabolic stimulus for the production of abnormalities in serum lipoproteins which increase the risk of CHD (Griffin, 1997). Alcohol elevates the postprandial rise in serum triacylglycerol (TAG) following the ingestion of dietary fat by promoting the production of TAG in the liver which is synthesized and secreted into the circulation as VLDL (Linarna et al. 1997). In contrast, very little is known about the effects of alcohol on TAG associated with dietary chylomicrons. A study was designed to examine the acute effect of alcohol on postprandial TAG-rich lipoproteins of intestinal and hepatic origin, and the activity of lipoprotein lipase (EC 3.1.1.34; LPL), the rate limiting determinant of TAG removal. Five healthy, male subjects (25-40 years), were given a test meal on two separate occasions with and without alcohol (0.6 g/kg body weight about 5 units). The meal contained 80 g mixed fat, and retinyl palmitate (RP) (170 000 IU), as a tracer of dietary chylomicrons. Blood samples were taken over 8 h for the determination of total plasma TAG, RP and the separation of 'TAG-rich' (TRL) and 'TAG-poor' (TPL) lipoprotein fractions. Post-heparin (7500 U) LPL activity (PHLA) was measured at 8 h. Apo B-48, as a marker of intestinally derived lipoproteins was measured by ELISA using a novel antiserum (Lovegrove et al. 1996).



All subjects showed an increase in the incremental area under the curve (IAUC) of TAG (366 (SD 161) v. 623 (SD 367) mmol/l.min, RP 716 (SD 566 (P<0.05)) v. 158 (SD 51) mmol/l.min), TRL-TAG (389 (SD 282) v. 877 (SD 460) mmol/l.min), and a decreased PHLA (281 (SD 63) v. 212 (SD 57) µmol fatty acids/min per ml) (P<0.05) after alcohol. Apo B-48 showed a biphasic response peaking at 3 and 5 h (control). On alcohol the peaks were delayed and prolonged peaking at between 2-4 h and 6-7 h. Alcohol also enhanced the TAG response in the late postprandial phase (>4 h) which is consistent with either an increase in the secretion or residence time of VLDL. Moreover, the significant elevation in RP (5 h) suggest that chylomicron remnants and intestinally derived lipoproteins respectively contributed to the alcohol-induced hypertriacylglycerolaemia. Although alcohol is not generally believed to influence LPL, an impaired removal of TAG by LPL may be implicated in this case. The overall impact of this phenomenon on lipid-mediated CHD risk is not clear but warrants further investigation, especially since the moderate consumption of alcohol confers protection against CHD.

Griffin BA (1997) *Proceedings of the Nutrition Society* 56, 693-702.

Linarna M, Heunskjelds ML, Kesäniemi YA and Savolainen MJ (1997) *Arteriosclerosis, Thrombosis & Vascular Biology* 17 (11), 2940-2947.

Lovegrove JA, Isherwood SG, Jackson KG, Williams CM and Gould BJ (1996) *Biochimica et Biophysica Acta-lipids and lipid metabolism* 1301, 221-229.

**Influence of a reduced saturated fat and isoenergetic increase in carbohydrate intake on postprandial lipaemia in middle-aged men.** By A.C. FEREDAY<sup>1</sup>, K.A. SLEVIN<sup>1</sup>, E. AH-SING<sup>1</sup>, J. WRIGHT<sup>1</sup>, C.M. WILLIAMS<sup>2</sup> and D.J. MILLWARD<sup>1</sup>, <sup>1</sup>Centre for Nutrition and Food Safety, School of Biological Science, University of Surrey, Guildford GU2 5XH, <sup>2</sup>Hugh Sinclair Unit of Human Nutrition, Department of Food Science and Technology, University of Reading, Reading RG6 6AP.

A high saturated fat (SFA) intake has been linked with insulin resistance and an increased risk of cardiovascular disease. We are investigating the effects of isoenergetic replacement of SFA with carbohydrate on the insulin resistance of macronutrient disposal and have shown an improved insulin sensitivity of glucose disposal (Slevin *et al.* 1998). We report here preliminary results of the influence of this dietary change on postprandial lipaemia (PPL), a risk factor for cardiovascular disease.

As described elsewhere (Slevin *et al.* 1998) the experimental design was a single intervention with initial habitual diet as control. Twelve non-smoking sedentary middle-aged men were selected on the basis of fasting insulin levels and an SFA intake typical of the current UK diet (16% of dietary energy). Intakes of SFA were reduced to 8% energy by means of individual dietary advice on replacing SFA isoenergetically with carbohydrate over a 10-week period. Compliance was assessed by 7 d food diaries and by personal interview. Measurements of erythrocyte phospholipid profiles showed significant decreases in total SFA and monounsaturated fatty acids at 4 and 10 weeks. Postprandial lipaemia was studied after a standardized high-fat breakfast providing 4.48 MJ (fat 82.7 g, protein 20.3 g, carbohydrate 72.5 g) given to subjects after an overnight fast following a standardized meal at 20.00 hours. Alcohol was restricted for 24 h before the study. Blood samples were taken every 30 min for 2 h and hourly for the following 7 h, for the measurement of triacylglycerols (TAG) and other variables. We report here preliminary measurements of overall postprandial lipaemia in terms of total and TAG-rich lipoprotein (TRL) TAG and non-esterified fatty acid (NEFA) levels.

There was a small reduction in weight (90.2 (SD 9.7) kg control v. 88.5 (SD 10.1) kg reduced SFA, P < 0.0005) while no changes were seen in total, HDL- or LDL-cholesterol as a result of the dietary intervention. In addition, in contrast with most previous studies, fasting TAG was unchanged by the dietary intervention (total TAG: 1.9 (SD 0.4) mmol/l control v. 2.0 (SD 0.5) mmol/l reduced SFA; TRL TAG: 0.5 (SD 0.3) mmol/l control v. 0.7 (SD 0.3) mmol/l reduced SFA). However the marked postprandial lipaemia observed in these subjects was worsened by the dietary change with an increase in TRL TAG (area under the curve (AUC) P = 0.028). This was most probably due to an increase in VLDL TAG as the TRL apolipoprotein B48 (a measure of chylomicron number) decreased (AUC P = 0.017).

A complex NEFA response was observed. Fasting NEFA was reduced by the dietary intervention (P < 0.0). NEFA suppression during the first 4 h after the meal was less marked (incremental AUC<sub>0-4 h</sub> P = 0.004) following the intervention which corresponded with a reduced peak insulin (P < 0.05). A subsequent increase above baseline NEFA levels after 4 h in these subjects was attenuated by the intervention (P = 0.004). Lipoprotein lipase activity was not changed.

From these results it appears that any benefit of a reduced insulin resistance of glucose disposal following replacement of dietary saturated fat with carbohydrate (Slevin *et al.* 1998) may be compromised by the adverse response of worsening dietary lipid tolerance.

This study was supported by the Ministry of Agriculture, Fisheries and Food.

Slevin KA, Fereday AC, Ah-Sing E, Wright J, Irvine A, Morgan LM, Williams CM & Millward DJ (1998) *Proceedings of the Nutrition Society* (In the Press).



**Postprandial lipaemic responses to hydrogenated soyabean oil and native soyabean oil in healthy volunteers.** By FARIDEH SHISHEBOR, HELEN M. ROCHE and MICHAEL J. GIBNEY, *Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity Health Sciences Centre, St James's Hospital, Dublin 8, Ireland*

Postprandial lipaemia refers to the acute period of dietary lipid absorption, transport and distribution, the magnitude of which has been associated with the process of atherosclerosis (Patsch *et al.* 1992). The effects of saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) on the postprandial lipaemic response have been examined in several studies (DeBruin *et al.* 1993; Zampelas *et al.* 1994), but the effects of *trans*-MUFA have not been investigated. The present study compared the postprandial lipid and lipoprotein responses to two test meals, one containing hydrogenated soyabean oil (HSO) and another containing native soyabean oil (NSO) in healthy volunteers (five males, four females). Subjects randomly consumed the fat-rich test meals (0.6 g fat/kg body weight) on two separate occasions. Fasting and postprandial samples were drawn every 2 h for 8 h. Samples were analysed for plasma triacylglycerol (TAG), TAG-rich lipoprotein (TRL) TAG, plasma cholesterol, plasma non-esterified fatty acids (NEFA) and HDL cholesterol concentrations. Following transformation of the data, statistical analysis was completed using repeated measures ANOVA.

There were no significant differences in the postprandial lipid and lipoprotein concentrations between meals. Postprandial TRL TAG concentrations (mmol/l) are presented in the Table. The magnitude of the TRL TAG incremental area under the curve (IAUC) was significantly lower after the HSO meal ( $P = 0.05$ ).

Time (h)	0	2	4	6	8	IAUC	
HSO	Mean	0.29	0.32	0.60	0.58	0.25	1.23
	sd	0.21	0.24	0.41	0.53	0.21	1.21
NSO	Mean	0.29	0.33	0.70	0.72	0.29	1.74*
	sd	0.22	0.23	0.42	0.56	0.23	1.25

Mean value was significantly different from that for HSO, \*  $P = 0.05$ .

The lower TRL TAG IAUC response may be due to slower intestinal digestion and absorption of *trans*-MUFA or to faster catabolism of TRL rich in *trans*-MUFA. In conclusion the acute effect of *trans*-MUFA on postprandial lipid metabolism is minor. However, further work is necessary to investigate the chronic effects of *trans*-MUFA on postprandial lipaemia and reverse cholesterol transport.

De Bruin TWA, Brouwer CB, van Linde-Sibenius M, Jansen H & Erkelens DW (1993) *American Journal of Clinical Nutrition* **58**, 477-483.

Patsch JR, Miesenbock G, Hopferwieser T, Muhlbirger V, Knapp E, Dunn JK, Gotto AM & Patsch W (1993) Relation of triglyceride metabolism and coronary artery disease. *Arteriosclerosis Thrombosis* **12**, 1336-45.

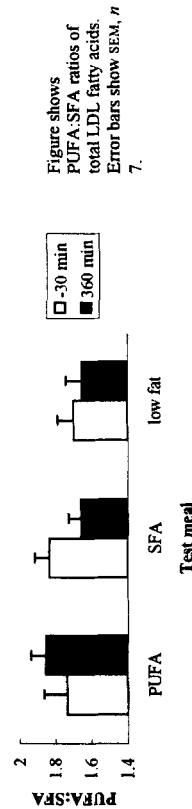
Zampelas A, Peel AS, Gould BJ, Wright J & Williams CM (1994) *European Journal of Clinical Nutrition* **48**, 842-848.

**Effect of meals of differing composition on postprandial fatty acid composition of LDL.** By JOANNE CALLOW, LUCINDA K.M. SUMMERS and KEITH N. FRAYN, *Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE*

The "oxidation hypothesis" of coronary heart disease is currently being extensively investigated. The oxidizability of LDL particles in particular is thought to be a key factor in their atherogenicity. LDL oxidizability is in turn influenced to a large extent by particle fatty acid composition. A number of studies have found that long-term changes in dietary fatty acid composition are reflected in that of LDL. The study presented here was designed to investigate acute changes in LDL in the postprandial period, a period in which many events implicated in atherogenesis occur.

Seven, healthy subjects (three male, aged 21–60 years, BMI 22.0–31.4 kg m<sup>-2</sup>) fasted overnight and then consumed a test meal on three occasions. The test meals consisted of a muesli-style breakfast cereal with milk or cream; one was high in polyunsaturated fatty acids (PUFA) (12.6% saturated fatty acids (SFA), 33.5% 18:1, 52.4% 18:2, 0.7% 18:3 where % refers to g/100 g total fatty acids), another was high in SFA (8.4% 12:0, 10.4% 14:0, 31.2% 16:0, 14.4% 18:0, 27.1% 18:1, 6.0% PUFA). Both of these meals contained 60 g fat; the third meal was a low-fat, isoenergetic control containing 5 g fat. Blood samples were taken into tubes containing EDTA from an arterialized hand vein 30 min before the consumption of the test meal and at 360 min after the meal. LDL was prepared from these samples by density gradient ultracentrifugation at 55 000 rev./min for 19 h at 23°. LDL lipid was extracted into chloroform-methanol (2:1, v/v) and its fatty acid composition measured by GC of fatty acid methyl esters.

The Figure shows the PUFA:SFA ratios of total LDL fatty acids at –30 and 360 min. Paired sample *t* tests showed that the decrease in the PUFA:SFA ratio after the SFA-rich meal was significant ( $P < 0.05$ ). Postprandial changes in PUFA:SFA ratio were calculated by subtracting the value of the ratio at –30 min from that at 360 min. There was a highly significant difference in postprandial change in PUFA:SFA ratio between the PUFA- and SFA-rich meals by paired sample *t* test ( $P < 0.01$ ).



These data show that LDL fatty acid composition changes acutely after the ingestion of a high-fat meal to reflect more closely the fatty acid composition of the meal. Our previous studies have shown enrichment of LDL by triacylglycerol after a high-fat meal, suggesting that LDL may gain triacylglycerol from chylomicrons, possibly in exchange for cholesterol ester. The results of this study provide further support for this lipid transfer. Changes in the fatty acid composition of LDL may influence its oxidizability and hence its atherogenicity.

J.C. holds a Medical Research Council Collaborative Studentship with Glaxo Wellcome.

**Paradoxical relationships between adiposity and lipaemia in individuals with an atherogenic lipoprotein phenotype (ALP).** By A.M. MINIHANE<sup>1</sup>, C. PATERSON<sup>1</sup>, C. CHAPMAN<sup>2</sup>, E.C. LEIGH-FIRBANK<sup>1</sup>, M.C. MURPHY<sup>2</sup>, J.W. WRIGHT<sup>2</sup>, B.A. GRIFFIN<sup>2</sup> and C.M. WILLIAMS<sup>1</sup>, <sup>1</sup>The Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading RG6 6AP; <sup>2</sup>Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH

Excess body weight, in particular a central distribution of fat in the abdominal area, has been implicated as a risk factor for CHD (Deprés *et al.* 1990). Hypertriglycerolaemia associated with hyperinsulinaemia and insulin resistance has been repeatedly observed in the centrally obese individual. In the present study the effect of total body mass and body fat distribution on fasting and postprandial triacylglycerol (TAG) responses was examined in individuals with the ALP. The ALP is characterized by an elevated fasting TAG level (1.5-3.0 mmol/l), low HDL-cholesterol concentration (<1.1 mmol/l) and a predominance of the small dense 'atherogenic' LDL-3 particle (>40% total LDL). Anthropometric measurements were made as part of an ongoing trial examining the ability of fish-oil fatty acids to ameliorate the combined dyslipidaemia of the ALP.

Fifty-three middle-aged men with the ALP lipid profile were recruited. Anthropometric assessments made included weight, height, waist and hip circumferences, and skinfold thickness at the triceps and subscapular sites. A fasting blood sample was collected from each individual. In addition postprandial TAG responses were determined following a breakfast (t=0 h, 50 g fat) and a lunch (t=5.5 h, 30 g fat), with blood samples collected at regular intervals throughout the day, up to 8 h after breakfast. The relationships between TAG responses, BMI (kg/m<sup>2</sup>) and measures of central adiposity which included waist, waist: hip ratio (W:H), waist: height ratio (W:Ht), and subscapular: triceps (Sc:Tr, centrality index) were determined. The TAG responses (mmol/l) are presented in the Table using a stratification system with the data divided into tertiles (F1 to F3 for fasting TAG; incremental area under the curve (IAUC) 1 to 3 for IAUC for TAG) for each adiposity index.

Tertile	BMI		W:H		Waist		W:Ht		Sc:Tr	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
F1	2.53*	0.26	2.58*	0.17	2.62*	0.26	2.71*	0.16	2.44*	0.15
F2	2.55*	0.16	2.54*	0.17	2.62*	0.16	2.51*	0.24	2.50*	0.21
F3	2.57*	0.21	2.54*	0.26	2.43*	0.19	2.46*	0.21	2.68*	0.25
IAUC1	654*	90	635*	59	671*	58	615*	59	571*	57
IAUC2	572*	59	627 <sup>ab</sup>	73	568 <sup>ab</sup>	73	574*	76	503*	52
IAUC3	540*	45	469 <sup>b</sup>	47	512 <sup>b</sup>	45	553*	45	679*	71

\*Mean values within a category not sharing a common superscript letter were significantly different,  $P < 0.05$ .

Tertiles: BMI (kg/m<sup>2</sup>) 1. <26.0, 2. 26.0-29.0, 3. >29.0; W:H 1. <0.92, 2. 0.92-0.95, 3. >0.95; Waist (cm) 1. <97, 2. 98-104, 3. ≥105; W:Ht 1. <0.552, 2. 0.552-0.590, 3. >0.590; Sc:Tr 1. <1.50, 2. 1.50-2.29, 3. >2.30.

Lower fasting TAG levels were observed in individuals with the highest waist, W:H and W:Ht ratios although the differences failed to reach significance at the 5% levels. Significantly lower postprandial TAG responses were observed in subjects in the highest waist ( $P=0.037$ ) and W:H ( $P=0.033$ ) tertiles compared with those in the lowest tertiles. The findings, which suggest that greater central adiposity is associated with reduced lipaemia in our ALP group, are unexpected. Further analysis such as insulin and genotyping for key proteins involved in lipoprotein metabolism will be carried out, in an attempt to explain this apparent paradox.

This research is supported by the BBSRC.

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**Inappropriate nocturnal postprandial responses amongst shift workers.** By DAVID RIBEIRO, SHELAGH HAMPTON, LINDA MORGAN and JOSEPHINE ARENDT, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH

We have postulated previously (Hampton *et al.* 1996) that the reported increased occurrence of CHD amongst shift workers might be due, at least in part, to inappropriate night-time postprandial carbohydrate and lipid intolerance. The present study was designed to compare the postprandial responses of subjects consuming a test meal (3330 kJ, % energy: 37 fat, 52 carbohydrate and 11 protein) at 13:30 hours with those of subjects consuming an identical meal on the first night of a simulated night shift, at 01:30 hours. The effect of altering the content of the pre-meal consumed before the test meal, and of exposing the subjects to bright light, was also investigated.

Twelve subjects were studied on three occasions. On the first occasion, subjects consumed a low-fat pre-meal (2008 kJ, % energy: 3 fat, 90 carbohydrate and 7 protein) at 08:00 hours, followed by the test meal at 13:30 hours, and then blood samples were taken at regular intervals for 9 h. On the second occasion, the same pre-meal was consumed at 20:00 hours, with the test meal at 01:30 hours, and then blood samples taken as before. On the third occasion, the night-time study was repeated, but the subjects were exposed to 1200 lux of full-spectrum light for four hours before, and four hours after, the test meal at 01:30 hours. The raw data was statistically assessed using a Repeated Measures ANOVA, followed by a Durcan's post hoc test. Postprandial plasma glucose and insulin levels following the night-time test meal, were similar to those following the daytime meal. Plasma triacylglycerol levels were significantly higher ( $P < 0.01$ ) after the test meal given at night compared with day, but this difference was removed when the subjects were exposed to bright light.

Ten further subjects followed the same protocol, with the exception that on each occasion they consumed a high-fat pre-meal (1243 kJ, % energy: 49 fat, 24 carbohydrate and 27 protein). In contrast to the high-carbohydrate pre-meal study, significantly higher glucose ( $P < 0.005$ ) and insulin ( $P < 0.05$ ) responses, as well as triacylglycerol responses ( $P < 0.01$ ) were observed after the night-time test meal, compared with the day-time test meal. The bright light exposure had no effect on the night-time responses.

These findings suggest that night-shift workers may show inappropriate nocturnal hormone and metabolic responses following a "lunch-type" meal at night, suggestive of insulin insensitivity and/or lipid intolerance at night, which could potentially be modified by exposure to bright light. The macronutrient content of meals eaten earlier in the day influences this diurnal variation of postprandial carbohydrate and lipid metabolism.

Hampton SM, Morgan LM, Lawrence N, Anastasiadou T, Norris F, Deacon S, Ribeiro D & Arendt J (1996) *Journal of Endocrinology* 151, 259-267.

**The effect of self-selected dietary supplements on micronutrient intakes in vegans.** By HELEN J. LIGHTOWLER and G. JILL DAVIES, *Nutrition Research Centre, South Bank University, 103 Borough Road, London SE1 0AA*

There is concern that some people consuming a vegan diet may not obtain an adequate intake of specific micronutrients, particularly vitamin B<sub>12</sub> and I. The need for dietary supplements in the vegan diet has previously been considered and it has been suggested that vegans should use appropriate dietary supplements to increase their intake of certain nutrients (Draper *et al.* 1993).

A cross-sectional study was carried out to assess the effects of self-selected dietary supplements taken by vegans on the reference nutrient intake (RNI) and lower reference nutrient intake (LRNI) (Department of Health, 1991) of selected micronutrients. Thirty-nine 'healthy' vegans were recruited to the study and asked to complete a 4 d weighed diet record diary. Micronutrient intakes were estimated using Comp-Eat 4.0 and intakes from dietary supplements were calculated according to the manufacturers' declarations on the packaging.

Six subjects withdrew from the study and a further three were excluded due to incomplete data. Of the remaining thirty subjects (eleven males and nineteen females; mean age 39 (SD 13) years), twelve (40%) were supplement users, although ten (33%) (five males and five females) took supplements at the time of the investigation. A total of twenty-one different dietary supplements were taken during the 4 d study period.

Micronutrient	Food sources			All sources							
	Daily intake Mean	% RNI SD	n < LRNI	Daily intake Mean	% RNI SD	n < LRNI					
Riboflavin (mg)	1.6	0.8	138	66	4	2.8	3.8	237	323	4	175
Vitamin B <sub>12</sub> (µg)	0.3	0.4	21	29	27	7.7	35.1	517	2337	20	2567
Vitamin C (mg)	133	69	333	173	0	2.19	4.11	862	2092	0	165
Retinol equivalents (µg)	517	382	82	64	6	734	924	117	156	4	142
Vitamin D (µg) <sup>†</sup>	0.5	0.7	-	-	-	1.1*	1.8	-	-	-	220
Calcium (mg)	495	230	71	33	10	541	355	77	50	10	107
Iron (mg)	15.8	6.7	148	89	1	16.4	7.0	153	93	1	104
Zinc (mg)	7.4	3.3	95	44	3	12.4	26.3	166	378	3	168
Selenium (µg)	51	40	79	63	17	64	94	99	156	17	125
Iodine (µg)	741	2789	530	1992	27	751*	2786	537	1990	24	101

Mean value was significantly higher than that from food sources, \*P < 0.05.

<sup>†</sup>No RNI.

The mean intakes of most micronutrients compared well with the respective RNI, especially with the use of dietary supplements. There was wide variation in intakes of some nutrients. Supplements increased intakes in a small number of vegans to excessive levels and, conversely, despite taking supplements, intakes in some subjects remained below the LRNI. Although the percentage increase in vitamin B<sub>12</sub> intake was high, intakes in two-thirds of subjects remained below the LRNI of 1.0 µg. Intakes of I in more than three-quarters of subjects and Se in over half were also less than the LRNI of 70 µg and 60-75 µg respectively. Furthermore, low intakes from diet and supplements of both Ca and vitamin D were found.

These findings highlight that vegans are not taking certain micronutrients from dietary supplements in amounts sufficient to raise intakes to the RNI. The use of supplements as a whole may be considered unsatisfactory due to the expense of these products and the uncertainty and variability of the particular micronutrient content of the supplement (Lee *et al.* 1994). A more satisfactory method of increasing micronutrient intakes, in particular vitamin B<sub>12</sub>, Se and I, in vegans to adequate levels needs to be considered.

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**Within-individual variability in postprandial lipaemia following oral ingestion of [1,1,1-<sup>13</sup>C]tripalmitin in healthy young subjects.** By M.A. HUMAYUN, J. BENNOSON, A.E. JONES, M. STOLINSKI, A. HOUNSLOW and S.A. WOOTTON, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

Postprandial lipaemia is a major determinant of circulating levels of lipoproteins and risk of developing cardiovascular disease. Whilst it has long been recognized that large inter-individual variability exists in postprandial lipaemia after an oral fat load (Cohn *et al.* 1988), the underlying mechanisms which account for the within-individual variability are not well understood, partly because of the difficulties in differentiating between endogenous and exogenous lipids. The purpose of present study was to examine the repeatability of the lipaemic responses to a lipid-rich meal labelled with [1,1,1-<sup>13</sup>C]tripalmitin. On two occasions, six healthy, young men ingested [1,1,1-<sup>13</sup>C]tripalmitin within a lipid-casein-glucose-sucrose emulsion and test meal (3.2 MJ; 90.3 g carbohydrate; 35.7 g lipid; 27.2 g protein) at 08.00 hours followed by a second identical unlabelled meal at 14.00 hours. The subjects consumed a prescribed diet (40% energy lipid, 45% energy carbohydrate, 15% energy protein) and refrained from volitional exercise for 3 d before each trial. On the evening before each trial, the subjects were admitted to the Clinical Nutrition & Metabolism Unit where they consumed a standardized meal at 19.00 hours and then fasted thereafter. Venous blood samples were collected from an indwelling cannula before and at hourly intervals for 10 h following label administration. A chylomicron-rich fraction (CRF Sf<sub>400</sub>) was separated by discontinuous-gradient ultracentrifugation, lipids extracted and the triacylglycerol (TAG) component isolated by TLC (Stolinski *et al.* 1997). The fatty acid profile of CRF-TAG and <sup>13</sup>C-enrichment of fatty acid methyl esters was determined by gas chromatography-isotope ratio mass spectrometry. In addition, total plasma TAG and non esterified fatty acids (NEFA) concentrations were determined enzymically. Postprandial areas under the curves over the 10 h study period were calculated for the concentrations of plasma TAG, the incremental increase in plasma TAG above baseline levels (Δ Plasma TAG), plasma NEFA and labelled palmitic acid isolated from CRF TAG (CRF-TAG <sup>13</sup>CPA).

	AUC Plasma-TAG (nmol/L.10h)		AUC Δ Plasma-TAG (nmol/L.10h)		AUC CRF-TAG <sup>13</sup> CPA (µg/ml plasma.10h)			
	Mean	SD	Mean	SD	Mean	SD		
Trial 1 (T1)	8.67	4.65	3.11	1.75	2.16	0.26	18.86	9.84
Trial 2 (T2)	8.77	3.27	2.72	1.84	3.25	2.31	18.48	8.96
Difference (T1-T2)	-0.10	1.54	0.40	1.01	-1.09	2.42	0.38	4.99

In general there was good agreement between trials for the group as a whole with the rank order within the group preserved for both the time-course and lipaemic responses of TAG, NEFA and labelled fatty acid suggesting that the within-individual variation in lipaemic response can be minimized with appropriate control of experimental conditions. Furthermore, a small transient increase in labelled fatty acid was observed in the initial hour following the second unlabelled meal which supports the view that circulating chylomicrons not only contain lipid from the most recent meal, but may also reflect the entry into the circulation of pre-formed chylomicrons containing lipid from previous meals (Peel *et al.* 1993).

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**Energy distribution and non-starch polysaccharide intake in vegans.** By HELEN J. LIGHTOWLER and G. JILL DAVIES, Nutrition Research Centre, South Bank University, 103 Borough Road, London SE1 0AA

Within the last decade, few studies have been carried out in the UK investigating the percentage of energy derived from macronutrients in the vegan diet (Sanders & Key, 1987; Thorogood *et al.* 1990; Draper *et al.* 1993) and none have investigated the intake of NSP. The aim of the current study was to assess the contribution to energy intake of protein, fat and carbohydrate in vegans consuming their habitual diet, and to estimate the intake of NSP.

Thirty-nine vegans, recruited through the Vegan Society, completed a 4 d weighed diet record diary. Nutrient intakes were estimated using Comp-Eat 4.0. The percentages of energy derived from macronutrients were compared with other recent UK vegan studies and NSP intakes were compared with data from the National Food Survey (Ministry of Agriculture, Fisheries and Food, 1997). Six subjects withdrew from the study and a further three were excluded due to incomplete data. Of the remaining thirty, eleven were male (mean age 40 (SD 8) years) and nineteen were female (mean age 38 (SD 15) years).

Nutrient	Present study				Other vegan studies	
	Male (n 11)		Female (n 19)		Male	Female
	Mean	SE	Range	Mean	SE	Range of means
Energy (kcal)	2445	178	1785 - 3832	1774***	103	1010 - 2823
Energy (MJ)	10.4	0.8	7.6 - 16.2	7.5***	0.4	4.3 - 11.8
% energy from:						
protein	12	0	10 - 15	12	0	8 - 15
fat	35	2	20 - 48	33	2	17 - 48
saturated fat	9	1	4 - 19	8	1	2 - 12
carbohydrate	50	3	31 - 61	53	2	41 - 70
P:S ratio	1.3	0.2	0.6 - 2.6	1.4	0.2	0.4 - 3.8
NSP (g)	27	3	6 - 42	21	2	7 - 43

P:S ratio, polyunsaturated:saturated fatty acid ratio. Mean values were significantly different from those for males, \*\*\**P* < 0.001.

The mean energy intakes in both male and female subjects were similar to those found in other studies. Mean intakes of protein, fat, saturated fat and carbohydrate, as a percentage of energy, were also similar, however there was wide variation in intakes of some nutrients. The upper end of the range in the percentage of energy from fat suggests that some vegans had intakes above the population average (Department of Health, 1991). The contribution of saturated fat to energy intake was in accord with the population average in most subjects and the high P:S ratio indicates that this low saturated fat intake was replaced by a higher intake of polyunsaturated fat. The mean NSP intake was significantly higher (*P* < 0.001) than the average UK intake of 12.4 g/d (Ministry of Agriculture, Fisheries and Food, 1997). There was wide variation in the NSP content of the diets with some individuals below the dietary reference value individual minimum of 12 g/d (Department of Health, 1991).

These findings highlight that the contributions of macronutrients to energy intake in the present subjects were comparable with those in previous vegan studies. The NSP intake was lower than anticipated, moreover low intakes in some individuals are of concern. Previous studies have overlooked the estimation of NSP intake in vegans, thus further work is needed to assess food sources and intakes of NSP in the current vegan diet.

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**Iodine intake and iodine status in vegans.** By HELEN J. LIGHTOWLER and G. JILL DAVIES, Nutrition Research Centre, South Bank University, 103 Borough Road, London SE1 0AA

I is an essential trace element required for normal mental and physical growth and development. Both deficient and excessive intakes of I can lead to thyroid dysfunction.

In the UK, the main sources of I in an omnivorous diet are milk and milk products, although fish is a rich natural source. Thus, there are certain groups of the population, such as vegans living on plant foods only, who may be at risk of low intakes of dietary I. Various potential nutrient deficiencies associated with the vegan diet have been thoroughly investigated, but limited research has been undertaken to assess the I intake of vegans. Therefore, the aim of the current work was to assess the I intake of adult vegans consuming their habitual diet and to measure concurrently their I status.

Thirty-nine 'healthy' vegans were recruited to this study. Duplicate portions and 24 h urine specimens were collected concurrently over four consecutive days and the I intake and status determined by chemical analysis. Six subjects withdrew from the study and a further three were excluded due to incomplete collections of duplicate diets and urine. Eleven males and nineteen females completed the study. The mean age of the subjects was 39 (SD 13) years and the mean proportion of life spent following a vegan diet was 24 (SD 14) %.

Subjects (n)	I intake (µg)		Median urinary I (µg/l)	IDD severity*
	Mean	SD		
Total (30)	169	291	16	20.2
Male (11)	137	149	4	16.8
Female (19)	187	346	12	20.5
Consuming seaweed (3)	866	523	0	37.8
Taking I supplements (5)				
- without supplements	97	77	2	---
- with supplements	151	76	1	36.9
Others† (22)	87	95	14	17.7

\* WHO criteria for the assessment of iodine deficiency disorders (IDD) severity (World Health Organisation, 1994).

† Subjects who did not consume seaweed or take I-containing dietary supplements.

There was a wide variation in I intake (the minimum intake was 25 µg and the maximum was 1467 µg) and the upper end of the range was attributed to the consumption of seaweed by a small number of subjects. The I intake of half of the vegans was below the lower reference nutrient intake (LRNI) of 70 µg (Department of Health, 1991); conversely, the intake of those who consumed seaweed approached the provisional maximum tolerable daily intake of 1000 µg. The I intake from dietary supplements was minimal, thus the use of supplements may be considered unsatisfactory due to the expense of the product and the variability of the I content (Lee *et al.* 1994). The urinary I excretion of the group was low and, according to the WHO criteria for the assessment of the severity of IDD, the IDD probability in 3 out of 5 groups was moderate and in 2 groups was severe.

These findings highlight that vegans are an 'at risk' group for both low and excessive intakes of I. The effects of such intakes on thyroid function in vegans requires further study, in particular the adaptation of the body to low I intakes and the effect on thyroid function of large infrequent intakes of this trace element. Encouragement to use iodised salt as a means of increasing I intake in the vegan diet may be inappropriate in the interest of healthy eating guidelines, thus a more satisfactory method of increasing I intake in vegans to adequate levels needs to be considered.

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**Variation in dietary fibre intake and bowel function in omnivores, vegetarians and vegans.** By AMANDA L. COOK, JOHN SYKES<sup>2</sup>, G. JILL DAVIES<sup>1</sup>, and PETER W. DETTMAR<sup>2</sup>, *Nutrition Research Centre, South Bank University, London SE1 0AA*, <sup>2</sup>Reckitt & Colman Products, Hull HU8 7DS

Variation in bowel function among people on different dietary regimens has been documented (Burkitt *et al.* 1972; Davies *et al.* 1986). Whether these differences result from the type of diet followed *per se* or other related factors is less clear. The aim of the present work was to re-analyse data from previous studies thereby determining whether differences in bowel function were attributable to dietary classification alone or other variable(s).

A meta-analysis of data obtained on seventy-eight individuals (55% female) was undertaken. Information was collated from previous studies reporting on dietary fibre intake and bowel function (Davies *et al.* 1986; Rees, 1995; Davies, unpublished results). This consisted of dietary classification (omnivore, vegetarian, vegan), dietary fibre intake, faecal form (Davies *et al.* 1986), defecation frequency, whole-gut transit time (Cummings *et al.* 1976), stool wet weight and sex.

Initial observations highlighted differences between the variables according to dietary group. This prompted further investigation into whether the bowel function differences could be explained by either dietary or sex factors.

Variable	Omnivore (n 31)		Vegetarian (n 29)		Vegan (n 18)		Overall (n 78)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dietary fibre intake (g/24 h)*	21.8	8.9	39.4	15.0	47.5	15.0	34.3	16.6
Faecal form (units 1-8)†	6.0	1.4	5.1	1.4	4.5	1.2	5.3	1.4
Defecation frequency (n/24 h)	1.2	0.4	1.3	0.4	1.6	1.0	1.3	0.6
Whole-gut transit time (h)	57.1	23.7	45.1	21.9	46.6	21.9	50.2	23.0
Stool wet weight (g/24 h)	136.2	67.7	199.0	76.3	223.1	89.1	179.6	84.7

\* Southgate (1969).  
† 1 = Loose, watery and runny - 8 = fragmented/segments; pellets.

Correlation analysis using Pearson's ( $r_p$ ) or Spearman's ( $r_s$ ) as appropriate revealed simple relationships between dietary fibre intake and bowel function parameters: faecal form  $r_p = -0.55$  ( $P < 0.001$ ); defecation frequency  $r_s = -0.21$  ( $P = 0.06$ ); WGT  $r_s = -0.35$  ( $P = 0.002$ ); and stool wet weight  $r_p = 0.78$  ( $P < 0.001$ ). More detailed analysis using two-way ANOVA with covariate (dietary fibre) and interaction (dietary classification and sex) terms was then performed. The within-group variance of defecation frequency was significantly different between the dietary groups, therefore this analysis was performed on ranked defecation frequency values.

The interaction of dietary classification and sex was not significant for any of the bowel variables and was removed from further analysis. Conversely dietary fibre intake was shown to account for a significant amount of variation for faecal form, ranked defecation frequency, whole-gut transit time and stool wet weight ( $P < 0.001$  for each case). After adjustment for dietary fibre intake there were significant differences between sexes for faecal form ( $P = 0.027$ ), ranked defecation frequency ( $P = 0.035$ ) and stool wet weight ( $P < 0.001$ ), but not for whole-gut transit time. However, there were no significant differences between the dietary classification groups over and above those attributable to dietary fibre intake and sex.

These findings confirm other studies showing that bowel function differences exist between individuals following omnivorous, vegetarian and vegan dietary regimens. However, it has also shown that these differences can largely be accounted for by dietary fibre intake and sex of individuals within the dietary groups, rather than by dietary classification alone. This study highlights the necessity of obtaining information on dietary fibre intakes and sex over that of dietary classification when assessing bowel function.

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**Dietary intake and lifestyle patterns amongst Surrey teenage girls: preliminary findings.** By JANET CATTERICK, JACKI A. BISHOP, CLAIRE L. RUGGLES, LYNNE S. SIGGERS and SUSAN A. NEW, *Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

Attainment of peak bone mass (PBM) is achieved during adolescence. Although genetically controlled, PBM is considered to be improved by good nutrition and load-bearing physical activity especially during early adolescence. Achievement of a high PBM will help to prevent osteoporosis in later life. Nutritional intake (Crawley, 1997) and physical activity (Armstrong *et al.* 1990) are considered to be declining in British adolescents. In order to develop methods of improving the lifestyle of adolescent girls it is necessary to ascertain the current position and their attitudes to healthy living.

We are currently collecting base line data on 400 Surrey girls aged 11-16 years. Subjects are asked to complete, concurrently, a 7 d estimated food diary and a 7 d physical activity diary (courtesy of Janet Gregory, Social Survey Division, Office for National Statistics, London) together with a confidential questionnaire on general health and lifestyle habits. Socio-economic information is collected from a questionnaire distributed to parents. An initial pilot study, involving thirteen girls aged 11 years and twenty girls aged 15 years, confirmed the importance of repeated visits during the recording period.

Preliminary data from a random sample of twenty one girls aged 12-13 years and twenty one girls aged 15-16 years are presented in the present abstract. The food diaries were analysed using the dietary package DIET5. Three subjects, whose diaries were poorly completed, were excluded. The mean energy intake:estimated BMR ratios for the two age groups were 1.39 (SD 0.28) and 1.36 (SD 0.19) respectively, demonstrating that the diaries had been completed satisfactorily.

	Girls aged 12-13 years (n 20)		Girls aged 15-16 years (n 19)	
	Mean	SD	Mean	SD
Energy (MJ)	7.58	1.21	7.72	8.21
Protein (%)	13.8	2.5	11.9	1.6
CHO (%)	49.5	3.6	47	49.2
Fat (%)	36.6	3.6	33	38.0
NSP (g)	10.8	3.5	18	9.5
Vitamin C (mg)	111	121	35	93
Calcium (mg)	748	183	800	703
Iron (mg)	10.1	3.5	14.8	8.8

EAR, estimated average requirements; RNI, reference nutrient intake (Department of Health, 1991)

The RNI for daily NSP intake (18 g) was not met by any of the subjects, with 35% of the younger subjects and 55% of the older subjects achieving intakes of less than 9 g/d. Only 30% of the younger subjects and 32% of the older subjects achieving the RNI for Ca. For Fe intake, even when supplements were included, was only achieved by 10% of subjects, with 18% having intakes of less than half the RNI. Physical activity levels tended to be moderate: only one subject was considered to be inactive with a Blair score of less than 37. Questionnaires revealed that 9% of the younger subjects and 63% of the older subjects had consumed alcohol during the previous 7 d, and that 36% of younger girls and 67% of older girls had tried smoking.

Further analysis of the study population is required to establish whether the patterns reported here are prevalent amongst Surrey adolescent girls.

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### Haemoglobin levels of first year female undergraduates. By SHEILA MAXWELL and ALLAN HACKETT, *IM Marsh Campus, Liverpool John Moores University, Liverpool L17 6BD*

Starting university can result in major upheaval to eating patterns due to living away from home, financial constraints and changes in social life and lifestyle. It is likely that for many students a detrimental change in dietary intake will occur and persist. One of the most likely consequences of such a change is a fall in Fe and vitamin C intakes which may increase the probability of anaemia. This report is of the first stage of a longitudinal study to investigate the influence of starting university on Fe status in female undergraduates.

At Liverpool John Moores University (LJMU) all new students take part in an induction programme which includes registration. Some 7305 new students registered, of whom 55% were female. By attending induction sessions for whole year groups in all the different schools at LJMU all female freshers were invited to take part; 798 subsequently gave finger prick blood samples for estimation of haemoglobin (Hb) content using portable calibrated Haemocue™ machines. All volunteers completed a short questionnaire concerning their academic status, dietary habits and programme of study.

The mean Hb concentration was 123.8 g/l (95% CI: 121.0, 126.5); 36.1% had levels below 120 g/l indicating cause for concern and 4.6% were below 100 g/l indicating the necessity for treatment. All subjects with low values (<105 g/l) were recommended to seek medical advice. The table shows Hb concentration according to age and according to eating habits.

Hb g/l	Age group (years)			Self-reported diet	
	18 - 24	25 - 35	> 35	Omnivorous	Vegetarian
Mean	123.2	127.1	124.7	123.1	126.0
95% CI:	122.1, 124.2	123.9, 130.4	120.9, 128.4	122.0, 124.2	122.5, 129.6
n	659	86	53	728	64

It can be seen that a significant number of these female undergraduates (who were generally very lively and healthy) could be classified as anaemic. The overall mean Hb level was low and below that reported for English adult women (130 g/l with only 14% of the sample below 120 g/l; White *et al.* 1993). Those following a self-declared vegan diet had on average the lowest mean values but the vegetarians had similar Hb levels to the omnivores. There was no trend with age. The next stage of this study will be to re-measure the Hb levels of as many of the subjects as possible with the expectation that the situation has deteriorated. This screening process was simple, relatively cheap and acceptable to large numbers of undergraduates and could form the basis of a routine monitoring scheme. Untreated anaemia is relatively common amongst female undergraduates and might compromise their studies; certainly anaemia can impair the physical performance of young women (Scholz *et al.* 1997).

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### The food and nutrient intake of students before commencing university and during the first semester. By PAULA MOYNIHAN, RACHEL BARTON, MELANIE KERBER and TIMOTHY BUTLER, *The Dental School, University of Newcastle, Newcastle upon Tyne NE2 4BW*

Students are reported to have erratic eating habits (Haung *et al.* 1994, Bellisle *et al.* 1995) and sub-optimum intakes of some nutrients (Troyer *et al.* 1990, Glore *et al.* 1993, Eves *et al.* 1994). The increased financial pressures which today's students face may cause a further decline in the nutritional quality of their diets. There are few up-to-date data on the food and nutrient intake by students in the UK and there is no information on the effect of going to university on student's diets. The aim of the present study was to investigate if the quality of the student's diets declined on leaving home and commencing university.

Seventy students on the 1997 admissions list for the undergraduate course in dentistry were invited to participate of which twenty-five (nine males and sixteen females) completed all aspects of the study. Students completed a 4 d estimated food diary during the month before starting university and again during the first semester, when they were resident in Halls. Mean daily intake of nutrients was determined using computerized food tables and purpose written programmes. Foods were categorized according to the food groups of *The Balance of Good Health* (Health Education Authority, 1994) and the weight of foods consumed in each of the groups for each subject was determined. Pre- and during-university differences for intake of nutrients and for weight of foods consumed was determined using paired t test. The results are presented in the Table. All energy intakes were > 1.3 X BMR.

	Before university		During university	
	Mean	SD	Mean	SD
Weights (g/24 h) of food types consumed:				
Breads and cereal foods	315	115	305	149
Fruits and vegetables	278	138	209**	124
Milk and dairy foods	171	120	172	146
Meat, fish and alternatives	185	84	176	79
Fatty sugary foods	297	215	309	190
Amounts of nutrients consumed:				
Energy (MJ)	9.3	2.1	9.7	3.7
Protein (g)	74	19	72	25
Energy from fat (%)	35	5.8	35	5.9
Energy from carbohydrate (%)	47	7.2	45	5.7
Total sugars (g)	123	38	111	44
NSP (g)	15	5	13	5
Calcium (mg)	854	291	951	373
Iron (mg)	12	5	12	5
Vitamin C (mg)	92	46	79	46
Folate (µg)	242	97	236	88
Thiamin (mg)	2.3	3.1	1.4*	0.4
Riboflavin (mg)	1.6	0.6	1.6	0.6

Mean values were significantly different from those before university: \*  $P < 0.05$ , \*\*  $P < 0.01$

Intake of fruit and vegetables was initially low, and further declined on commencing university. This is reflected in a trend towards reduced intakes of vitamin C and NSP. Fat intake was marginally above the dietary reference value of 33% energy intake and showed no increase on commencing university despite a trend towards increased intake of sugary fatty foods.

Despite the small sample size (due to low response rate and the short time interval between the admissions list becoming available and the start of the semester) this study has shown that the low intake of fruit and vegetables by students is further decreased when they commence university.

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**Changes in nutrient intake after changing to a self-selected vegetarian diet for 18 months.** By FRANCES ROBINSON and ALLAN F. HACKETT, *School of Education and Community Studies, Liverpool John Moores University, Barkhill Road, Liverpool L17 6BD*

The number of people in the UK claiming to be vegetarian is increasing. There is a considerable amount of evidence to suggest that vegetarians suffer less from several chronic diseases such as CHD (Thorogood *et al.* 1994). It is unclear, however, whether actually becoming vegetarian is of any benefit. Previous studies which have examined the effects on nutrient intake of changing to a vegetarian diet have all given detailed guidelines of what should and should not be eaten (Johansson *et al.* 1992; Delgado *et al.* 1996). We have previously reported the short-term effects (3 months) on diet of changing to a self-selected vegetarian diet (Robinson & Hackett, 1997). The present study shows a comparison of mean nutrient intake before becoming vegetarian and the mean intake over the subsequent 18 months as a vegetarian. The fourteen subjects were all adults (mean age 33 years), twelve female, who had made the decision to stop eating meat and they were offered no advice on changing their diets. Intake was assessed by 3 d estimated diary and analysed using Microdiet (University of Salford), once at baseline and 6 times over the next 18 months.

	Pre-vegetarian diet (n14)		Vegetarian diet (n14)	
	Mean	SE	Mean	SE
Energy (MJ)	8.74	0.55	7.27*	0.35
Carbohydrate (% energy)	43.6	1.45	49.7**	1.35
Fat (% energy)	37.3	1.51	33.4**	1.16
Protein (% energy)	13.8	0.92	12.4	0.58
P : S	0.46	0.07	0.67*	0.05
NSP (g)	12.2	1.38	15.4*	1.26
Iron (mg)	10.8	1.09	10.2	0.42

P:S, polyunsaturated: saturated fatty acid ratio. Mean values were significantly different from those for pre-vegetarian diet. \* P<0.05, \*\* P<0.01 (2-tail).

The results show a marked decline in energy intake and substantial changes in the proportions of energy supplied by carbohydrate and fat (similar changes were seen in the short term but were not significant at that point: Robinson & Hackett, 1997). The most dramatic changes were in the type of fat consumed (46% increase in P:S) and the increase in NSP intake (26% increase). There was no change in the total intake of Fe but all the haem Fe had disappeared and NSP had increased. Becoming vegetarian led to several beneficial changes in the diet of these subjects which developed and have been sustained.

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**Low meat eaters in north Glasgow: who are they and what else do they eat?** By W.L. WRIEDEN<sup>1</sup>, M.K. McCLUSKEY<sup>2</sup>, H. TUNSTALL-PEDOE<sup>2</sup> and C. BOLTON-SMITH<sup>1,3</sup>, *Centre for Applied Nutrition Research, University of Dundee, DDI 4HT, and <sup>2</sup>Cardiovascular Epidemiology Unit, Ninewells Hospital and Medical School, Dundee DDI 9SY*

There is now a consensus of opinion that diets rich in vegetables and fruit are advantageous to health. The assumption that low- or non-meat consumers have diets rich in fruit and vegetables has led to some confounding in identifying links between meat intakes and the risk of cancers and CHD. (Department of Health 1994, 1998) so there is a need to explain the relationship between meat consumption and fruit and vegetable consumption. The aim of the following analysis was to examine the health and dietary behaviours associated with no, or very occasional, meat consumption.

In north Glasgow four cross-sectional surveys were carried out as part of the WHO MONICA programme over a period of 10 years and diet assessed by a food frequency questionnaire (Bolton-Smith *et al.* 1991). The percentage of those claiming to eat no meat or meat less than once weekly ('seldom') was 2.5% in 1986 and 3.8% in 1995. In a combined analysis of the surveys of 1992 and 1995 it was found that there were significantly higher proportions of women, those under 35 years, non-smokers and those who had a experienced further or higher education in the 'seldom' group compared with the regular meat eaters ('regular').

	Seldom (%) (n 96)	Regular (%) (n 2838)	Significance of chi-squared test
Under 35 years	38	23	P<0.001
Non-smokers	71	56	P<0.01
Further or higher education	56	27	P<0.001
Semi-skimmed or skimmed milk	71	66	NS
Starchy carbohydrates five or more times/d	49	46	NS
Biscuits, cakes and sweets > once daily	35	39	NS
Fruit and vegetables four or more times/d	57	31	P<0.001
Fish ≥ twice weekly	32	60	P<0.001
Wholemeal or granary bread>50% of bread eaten	63	29	P<0.001

The 'seldom' group had a higher percentage of people eating fruit and vegetables (including fruit juice) four or more times a day and consuming wholemeal or brown bread as 50% of the bread eaten (in line with the Scottish Diet Behavioural targets for the year 2005 (Scottish Office, 1993)) but less than a third ate fish two or more times a week. Thus there is some evidence that the people who seldom eat meat do eat a diet relatively rich in fruit and vegetables. However over 40% of this group did not eat fruit and vegetables four or more times a day and there was no evidence that they ate more starchy carbohydrates or fewer confectionery items than the more frequent meat eaters. Clearly meat is still a staple part of the vast majority of this Scottish population but the situation may be different in more affluent areas.

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**The influence of diet on indicators of iron, copper and zinc status in children under 24 months Preliminary findings.** By EDWARD WALLIS-REDWORTH, EILEEN GOSSAGE, JANE MORGAN and ANDREW TAYLOR, *School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

A number of groups (Department of Health, 1994) have reported that Fe status in a high proportion of children aged between 8 and 24 months is marginal. Data on the association between biochemical markers of status and mineral supply are scarce for this age group. The present study aimed to determine whether Fe status (and hence growth and psychomotor performance) of infants and young children will benefit from the inclusion of red meat in the diet compared with that of infants whose diet contains white meat only or no meat. The ultimate aim of the project is to determine the best method of infant feeding which will result in a reduction in Fe-deficiency anaemia.

A longitudinal study of infants categorized into distinct feeding groups based on parental preference is being undertaken. Customary food intakes of the infants are being monitored on a 4-monthly basis by using the 7d weighed food intake method. In addition, serial blood samples are being undertaken to measure indicators of Fe status. Anthropometric measurements i.e. weight, length and head circumference are also being taken at 4-monthly intervals.

We report here the findings after the first year of the study and the mean intake values for a selection of the data.

Diet Group	Age (months)	n	Kj	Protein (mg)	Cu (mg)	Fe (mg)	Zn (mg)	Vitamin C (mg)	Length (cm)	Weight (kg)	OFC (cm)	Hb (g/l)	Hb (S.D.)
Red-meat eaters	4*	76	3,064	17.9	0.4	7.8	4.3	84.5	64.7	6.9	42	113.4	16.3
Red-meat eaters	12	44	3,731	33.6	0.42	7.9	4.5	92.2	76.3	10.1	46.7	122.3	17
Non-red-meat eaters	4*	46	2,602	14.5	0.4	5.5	3.6	75.8	65.2	7.1	42.4	113.9	11.6
Non-red-meat eaters	12	23	3,693	34.2	0.48	7.6	4.5	93	77.2	10.3	47	106.1	32.5

Hb, haemoglobin.

OFC, occipito-frontal circumference

\*Human milk intakes were estimated.

There were differences in the Fe intake among the groups at 4 months of age. There were no differences in growth at 4 months of age and at 12 months of age. Using a haemoglobin concentration of 110 g/l as a definition of Fe-deficiency anaemia, 36% of red-meat eaters and 38% of non-red-meat eaters were below this value at 4 months of age; this proportion remained the same at the 12 month visit for the non-red-meat group but fell to 15% within the red-meat group.

The preliminary results suggest that meat in the post weaning diet may have an influence on growth and Fe status in children under 24 months of age. We expect that further results from our study will help to elucidate this hypothesis.

This research was undertaken with a grant from the Meat and Livestock Commission, Department of Health. (1994) *Weaning and the Weaning Diet. Report on health and social subjects no. 45*. London: HMSO.

**A reassessment of the contribution of meat to the intake of fat by children.** By LYNN BURGESS, ALLAN HACKETT, SIMON KIRBY, SHEILA MAXWELL and INDIRA NATHAN, *IM Marsh Campus, Liverpool John Moores University, Liverpool L17 6BD*

Most dietary surveys use food tables to estimate nutrient intake but the range of foods is growing and their composition is dynamic. The publication of regular supplements is one way of trying to provide up-to-date information. We have recently published a comparison of the dietary intake of vegetarian and non-vegetarian children (Nathan *et al.* 1996) which concluded that intakes of fat were similar. It has been suggested that this conclusion was due to the use of out-of-date information on the composition of meat and meat products. The data have been reanalysed using relevant supplements to the food tables not available for the original study.

The present paper concerns only the fifty children who ate meat (mean age 9.4 (SD 1.4 years) years). Each child kept three, 3d diaries (over a period of 1 year) of all food and drinks consumed and was interviewed on the fourth day by one person to quantify the intake. The fifth edition of the food tables (Holland *et al.* 1991)(MW5) and all available supplements (r6) at that time were used to calculate nutrient intake. The diaries have been subsequently reanalysed using the *Meat Products and Dishes* (MMD) and the *Meat Poultry and Game* (MPG) supplements (alone and in combination). All calculations were done using Microdiet (University of Salford). This gave three estimates of nutrient intake (Table).

	1. MW5		2. MW5 + MPG		3. MW5 + MPG + MMD	
	Mean	SE	Mean	Change from 1 (%)	Mean	Change from 1. (%)
Energy (kJ)	8038	193	8024	-0.1	7496	-6.7*
Fat (g)	79	2.2	79	0.0	73	-7.6*
Fat (%)	36.4	0.5	36.4	0.0	36.0	0.0
CHO (g)	257	7.0	254	-1.2	237	-7.8*
CHO (%)	51.2	0.5	50.6	0.0	50.6	0.0
Protein (g)	59	1.5	61	+3.4	59	0.0
Protein (%)	12.5	0.2	12.9	0.0	13.4	0.0

Significantly different from 1.MW5, \* $P > 0.05 < 0.10$  (2 tail).

The first reanalysis using MPG alone showed no reduction in fat intakes. The reanalysis which used both of the recent supplements indicated a lower level of fat intake but energy and carbohydrate (CHO) intakes also fell. The contribution of fat to energy intake remained the same. These findings might be explained by the fact that MPG included relatively few meat products consumed by children e.g. chops, steaks. MPD however, contains many popular foods such as burgers and sausages. One key difference between MW5 and MPD is that the fat content of burgers is higher in MPD, apparently due to a lower rusk (CHO) content. The original survey showed the vegetarian children to have fat intakes of 75 g and 36%, these new analyses show this to be no lower (very marginally higher) than that of the meat eaters.

The impact of changes in animal husbandry, butchery and meat product composition does seem to have resulted in lower intakes of fat but has not affected the contribution of fat to energy intake in this sample of children.

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**Polyamines in preterm human milk and infant formulas.** By ELIZABETH L. HUGHES<sup>1</sup>, SUSAN BARDOCI<sup>2</sup> and BARBARA E. GOLDEN<sup>2</sup>. <sup>1</sup>Roswell Research Institute, Bucksburn, Aberdeen AB21 9SB, <sup>2</sup>Department of Child Health, University of Aberdeen, Aberdeen AB25 2ZD

The polyamines, putrescine, spermidine and spermine, are ubiquitous compounds known to be involved in cell growth and proliferation in many tissues. Spermidine and spermine supplementation induces precocious maturation of the rat intestinal mucosa (Dufour *et al.* 1988). Although polyamines are abundant in term human milk, they are absent or found in very low concentrations in many cow's-milk-based infant formulas (Pollack *et al.* 1992). Preterm infants, who often suffer from intestinal immaturity which contributes to their poor growth, may benefit from dietary spermidine or spermine supplementation. Therefore, the aim of the present pilot study was to compare the polyamine content of milk from mothers of preterm infants (<37 weeks gestation) with that of infant formula milks also used for preterm infants.

The polyamine concentration in forty-eight breastmilk samples, collected randomly, between the first week and the second month of lactation, from sixteen different mothers of preterm infants, was quantified by HPLC. Forty-three samples of the three preterm infant formula milks most frequently used in the Neonatal Unit at Aberdeen Maternity Hospital, Farley's Osterprem and Firstmilk and SMA Low Birth Weight milks, were also assayed for polyamine content.

	Putrescine (µmol/l)		Spermidine (µmol/l)		Spermine (µmol/l)	
	Median	Range	Median	Range	Median	Range
Breastmilk						
Term*	0.54	ND - 3.80	3.51	0.62 - 8.68	4.12	0.54 - 8.88
Preterm		ND - 4.40		0.52 - 9.56		0.45 - 11.6
Infant formulas	ND		ND		ND	

ND, not detected.  
\*Pollack *et al.* 1992.

The Table shows that, although great variation in maternal breastmilk polyamine levels was observed, the presence of significant quantities of polyamines was confirmed in almost all samples of milk from mothers of preterm infants. There appeared to be no obvious association between milk polyamine concentration and infant chronological or gestational age. However, polyamines were not detected in any of the preterm infant formula milks analysed.

These data indicate that human preterm breastmilk provides substantial quantities of polyamines, similar to those in term breastmilk, which could potentially influence intestinal maturation. The absence of polyamines in infant formula milks may have developmental implications for formula-milk-fed infants, especially if born preterm.

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**Iron intake in young children in relation to body weight and energy intake.** By ABDULAZIZ M. AL-OTHMAN<sup>1</sup>, NEVILLE R. BELTON<sup>1</sup> and TERRY R. KIRK<sup>2</sup>. <sup>1</sup>Department of Child Life and Health, University of Edinburgh, Edinburgh, EH9 1UW and <sup>2</sup>Centre for Nutrition and Food Research, Department of Dietetics and Nutrition, Queen Margaret College, Edinburgh, EH12 8TS

The report, *Weaning and the Weaning Diet* (Department of Health, 1994) recommended that more information on patterns of nutrition and diet during the first 2 years of life should be available and that the natural history of Fe deficiency in infants in this country should be determined.

The reference nutrient intake (RNI) for Fe is 7.8 mg up to the age of 1 year and 6.9 mg for children aged 1-3 years (Department of Health, 1991). Despite this apparently lower requirement, Fe deficiency is more common in the second year of life than the first.

We have measured the intake of Fe and related it to energy intake and body weight by performing 4d weighed intakes in 189 children in Riyadh, Saudi Arabia and sixty-one children in Edinburgh, all between 8 months and 3 years of age. The results are shown in the Table.

Daily iron intake	Boys				Girls			
	Mean	Med	Min	Max	Mean	Med	Min	Max
Riyadh								
8-14 months n 74 (M 37, F 37)	6.55	6.61	2.92	11.57	6.46	6.24	2.96	13.28
mg	8.62	8.70	3.48	14.87	9.05	8.05	4.56	18.95
mg/4184 KJ	0.71	0.69	0.34	1.40	0.74	0.63	0.41	1.98
mg/kg body wt								
15-22 months n 75 (M 37, F 38)	4.50	4.07	2.09	9.51	4.25	4.00	1.61	8.89
mg	4.96	4.84	1.40	7.88	5.05	4.66	1.55	9.72
mg/4184 KJ	0.41	0.35	0.18	0.83	0.39	0.37	0.15	0.87
mg/kg body wt								
23-36 months n 40 (M 23, F 17)	4.96	4.74	1.27	7.51	5.39	5.40	1.57	8.56
mg	4.52	4.15	1.16	7.95	4.52	4.18	1.31	8.51
mg/4184 KJ	0.39	0.39	0.10	0.63	0.45	0.42	0.17	0.81
mg/kg body wt								
Edinburgh								
8-14 months n 13 (M 7, F 6)	7.12	6.86	3.24	11.62	5.64	5.01	2.61	10.63
mg	7.82	8.52	2.92	12.24	7.28	6.84	3.31	13.22
mg/4184 KJ	0.68	0.70	0.30	1.14	0.58	0.50	0.25	1.07
mg/kg body wt								
15-22 months n 23 (M 15, F 8)	4.71	4.03	2.51	9.16	4.50	4.80	1.73	6.26
mg	5.39	5.27	2.31	10.81	4.54	5.04	1.58	6.41
mg/4184 KJ	0.40	0.32	0.22	0.84	0.41	0.45	0.16	0.59
mg/kg body wt								
23-36 months n 25 (M 15, F 10)	5.00	4.47	1.84	8.75	4.84	4.33	2.43	11.02
mg	4.91	4.60	1.49	8.04	5.32	4.66	1.93	9.53
mg/4184 KJ	0.34	0.30	0.11	0.58	0.36	0.35	0.15	0.72
mg/kg body wt								

Although Fe-deficiency anaemia (low haemoglobin and ferritin), is more common in Saudi Arabia than in the UK, 24% in the Riyadh population studied compared with 12% of 1½-2½ years olds in the UK study of 1992-3 (Gregory *et al.* 1995), these results indicate that Fe intake in Riyadh is not very different from that in Edinburgh even when expressed per 4184 KJ or per kg body weight. The Fe intake values were higher in the age group 8-14 months in both Riyadh and Edinburgh but in the two older groups were similar to Fe intakes of the 1½-2½ year olds in the UK study (Gregory *et al.* 1995).

These results confirm that factors other than the total amounts of Fe in the diet must be of importance in determining the prevalence of Fe-deficiency anaemia. Other data from this study (Al-Othman *et al.*, 1998) suggests that constituents in the diet such as haem Fe and breakfast cereals may be significant. Non-nutrient influences such as parasites, infection and blood loss may also be important.

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**The effect of phyto-oestrogen-rich foods on urinary output of phyto-oestrogen metabolites: a pilot study.** By JAYNE V. WOODSIDE<sup>1</sup>, MIKE MORTON<sup>2</sup> and ANTHONY LEATHERM<sup>1</sup>, <sup>1</sup>Department of Surgery, UCL, 67-73 Riding House Street, London W1P 7LD and <sup>2</sup>Tenovus Cancer Research Centre, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XX

There is growing interest in the reported health benefits of the isoflavonoid and lignan phyto-oestrogens, which are oestrogen-like plant compounds. Epidemiological, *in vitro* and animal studies provide evidence that phyto-oestrogens may reduce cancer risk. Soyabean products are rich in isoflavonoid phyto-oestrogens, but it is not known how phyto-oestrogen intake can be affected by small alterations to a typical Western diet. The lignans are found in a number of fruits, vegetables and grains. We have carried out a small study to assess to what extent urinary phyto-oestrogen output can be affected by a short-term isoflavonoid- and lignan-rich diet. Seven healthy volunteers, three men and four women, followed a special diet over a period of 3 d. Each was given 100 g soya chunks, 150 g lentils and 250 g kidney beans to be consumed daily. Urine was collected for 2 d before initiation of the diet, during the 3 d of intervention, and for 2 d after the diet had returned to normal. Subjects filled in food diaries to assess baseline diet and compliance with the test diet. The 24 h urine samples were analysed by gas chromatography-mass spectrometry according to Morton *et al.* (1994) for the isoflavones daidzein and equol and the lignan enterolactone.

The Table shows the mean levels of daidzein, equol and enterolactone over the 7 d period. Daidzein levels increased over the 7 d period, reaching over 4000 ng/ml on days 4-5. However, equol levels did not show a similar rise, indicating that at least some of these subjects were unable to produce equol from daidzein. It is known that there is considerable individual variability in metabolic response to a known dose of isoflavone-rich food, and this may be due to variation in bacterial enzymes in gut microflora (Cassidy *et al.* 1994). Enterolactone levels did not change significantly over the 7 d period, although an increase may have been noticed if urine collections had been carried out for a longer period post-intervention.

Day	Daidzein (n 7)		Equol (n 7)		Enterolactone (n 7)	
	Mean	SE	Mean	SE	Mean	SE
1 (baseline)	321	73	6.09	1.07	862	329
2 (baseline)	592	261	6.26	1.02	1094	365
3 (intervention)	2608	530	5.63	1.61	751	239
4 (intervention)	4151	692	7.71	2.69	910	277
5 (intervention)	4138	1284	4.57	0.40	774	328
6 (post-intervention)	2461	777	4.92	1.23	838	232
7 (post-intervention)	490	157	7.09	1.47	699	310

This study, in a small number of subjects, shows that phyto-oestrogen intake can be affected in the short term by changes to the Western diet. This finding may have implications for the primary and secondary prevention of hormonally-dependent cancers.

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**Is developmental dyslexia a fatty acid deficiency syndrome?** By ALEXANDRA J. RICHARDSON<sup>1</sup>, TERESE EASTON<sup>1</sup>, ANNA C. CORRIE<sup>2</sup>, CHRISTINE CLISBY<sup>2</sup> and B. JACQUELINE STORDY<sup>3</sup>, <sup>1</sup>Division of Neurosciences and Psychological Medicine, Imperial College School of Medicine, St Dunstan's Road, London W6 8RP, <sup>2</sup>University Lab. of Physiology, Oxford, <sup>3</sup>Efamol Research Institute, Guildford

Recent evidence suggests that certain long-chain polyunsaturated fatty acids (LCP) may be important in developmental dyslexia. These fatty acids are essential for normal brain development, and deficiencies have been found in related conditions such as attention-deficit/hyperactivity disorder (AD/HD) (Stevens *et al.* 1995). In dyslexia, brain imaging has revealed abnormalities of membrane lipid turnover consistent with fatty acid deficiency (Richardson *et al.* 1997); and Stordy (1995) found visual deficits in dyslexic adults that normalized following supplementation with n-3 fatty acids. This suggests a mild abnormality of fatty acid metabolism in dyslexia that may be amenable to correction through diet.

To investigate this, two large-scale double-blind placebo-controlled trials of treatment with LCP (in the form of Efalex™) are in progress. Here the primary question of whether dyslexia is associated with fatty acid deficiency is addressed using data from baseline assessments. These include interview / checklist ratings of clinical signs found to relate to blood biochemical measures of LCP deficiency in children with AD/HD (Stevens *et al.* 1995). In ninety-four adults (sixty-one dyslexic, thirty-three control, matched for age, sex and general ability) and sixty-six children (all dyslexic, aged 8-12 years) these fatty acid deficiency ratings were examined in relation to (1) dyslexic status (adults only) (2) psychometric measures of reading and related cognitive skills and (3) self-report ratings of other symptoms and traits associated with dyslexia.

	Adults		Children		Reading T-score*	
	n	Mean	n	Mean	Mean	SD
		SD		SD		
Control	33	3.6	33	22.7	39.8	5.0
Dyslexic	61	5.4 <sup>a</sup>	33	30.4 <sup>b</sup>	37.0 <sup>b</sup>	4.9

\* total from 7 items (excessive thirst, frequent urination, dry skin, dry hair, brittle nails, dandruff and follicular keratosis. Each FADS item is scored for severity on a scale of 0-3). <sup>a</sup> Age-standardized reading score with mean = 50, sd = 10. <sup>b</sup> dyslexic vs control group:  $P < 0.02$ , Mann-Whitney (2-tail). <sup>c</sup> High vs Low FADS groups:  $P < 0.03$ , t-test (2-tail).

As the Table shows, fatty acid deficiency signs (FADS) were higher in dyslexic than non-dyslexic adults, while in dyslexic children high scores on FADS were associated with more severe reading deficits. FADS did not relate to other psychometric test scores in either adults or children, suggesting that the relationships found are not confined to a particular subgroup. With respect to self-report measures, high FADS in children were related to more visual symptoms when reading ( $r_s$  0.34,  $P < 0.02$ ). In adults, FADS were associated with global scores on a standard dyslexia screening checklist ( $r_s$  0.32,  $P < 0.003$ ) and with a wide range of measures of dyslexic symptomatology, including not only visual problems but also auditory-linguistic and motor co-ordination difficulties ( $P < 0.006$  in all cases).

These results are consistent with fatty acid deficiency in dyslexia, and with other evidence that a high intake of LCP can be beneficial in this condition (Stordy, 1995). As meat and fish provide the main dietary sources of preformed LCP, these findings suggest that diets low or lacking in these foods may provide less than optimal requirements for individuals with dyslexia and related conditions. Further investigation is warranted into nutritional influences and the effects of LCP supplementation in dyslexia.

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**Inhibition of mutagen formation by organosulfur compounds.** By VENETIA TROMPETA and JOHN O'BRIEN, *Food Safety Research Group, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

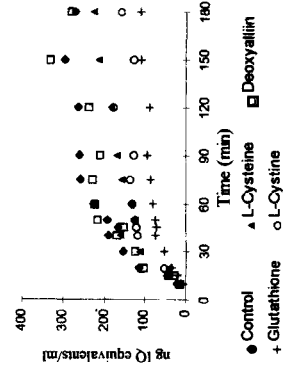
Recent reports suggest that there is an association between colon cancer and consumption of red meat (Department of Health, 1998). It has been postulated that heterocyclic amines (HA) could be a contributing factor, since they are mutagenic activity in the Ames test and are carcinogenic to animals models (Snyderwine, 1994). HA are produced during cooking of muscle meat as a result of Maillard reactions in the presence of creatin(in)e (Pearson *et al.*, 1992). Organosulfur compounds, especially, the S amino acids and their derivatives, appear to be particularly effective in inhibiting nonenzymic browning reactions (Friedman, 1996).

The present study examined the effect of the organosulfur compounds, L-cysteine, L-cystine, reduced glutathione, deoxyalliin, and N-acetyl-L-cysteine, on mutagen formation in a meat model system. The model system consisted of glycose, glycine and creatinine in a diethylene glycol system, heated at 150° in the presence or absence of organosulfur compound. The degree of browning and the mutagenicity of samples were determined at appropriate time intervals.

Mutagen formation was described by zero-order kinetics with plateau formation. Glutathione, L-cysteine, L-cystine and deoxyalliin inhibited the formation of mutagens. However, although N-acetyl-L-cysteine inhibited colour formation, it had no effect on mutagen formation. Similarly, L-cystine had no effect on browning but was a potent inhibitor of mutagen formation. These results suggest that the mechanism of inhibition of nonenzymic browning by organosulfur compounds is different from the mechanism of inhibition of mutagen formation. Assays of extracted mutagens confirmed that the decrease in mutagenicity was a consequence of a decrease in mutagen formation and not due to interference with the Ames assay.

**Table.** Rate constants (k, ng IQ<sup>1</sup> eq/ml per min) of mutagen formation in heated meat model systems.

System	Rate constant	95% CI
Control	3.397	1.046
L-Cysteine	0.888**	0.375
N-Acetyl-L-cysteine	4.354	1.509
Deoxyalliin	1.482**	0.566
Glutathione	1.039**	0.351
L-Cystine	2.060**	0.812
2-Amino-3-methyl-3H-imidazo[4,5-f]quinoline		
	<b>P&lt;0.01, **P&lt;0.001</b>	



Since glutathione is a natural constituent of fresh meat, it may act as a natural inhibitor of mutagen formation during cooking. The results suggest that manipulation of the composition of meats and bouillions offers a tangible means of reducing dietary mutagen burden.

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**Dose response effect with dietary meat on faecal N-nitrosocompound and nitrogen excretion in subjects.** By R. HUGHES<sup>1</sup>, J.R.A. POLLOCK<sup>2</sup> and S. BINGHAM<sup>1</sup>, <sup>1</sup>Dunn Clinical Nutrition Centre, Medical Research Council, Hills Road, Cambridge CB2 2DH; <sup>2</sup>Pollock and Pool Ltd, Ladbroke Close, Reading RG5 4DX

The endogenous formation of N-nitrosocompounds (NOC) may be one explanation for the positive epidemiological relationship between high red meat consumption and colon cancer incidence. Many NOC are known carcinogens and can be formed endogenously under both acidic and neutral conditions. The colonic lumen is rich in nitrogenous residues, the levels of which are known to be affected by diet; many of these residues act as potent substrates for bacterial nitrosation. Recent studies have revealed an increase in faecal apparent total NOC (ATNC) excretion following an increase in meat consumption (Bingham *et al.* 1996; Silvester *et al.* 1997).

We studied the effects of different levels of dietary meat on eight healthy male volunteers. The volunteers were maintained in a metabolic suite and given a randomized sequence of diets over four 10d dietary periods (0, 60, 240 or 420 g meat). The diets were isoenergetic and constant in fat and NSP. Deionized water was used for cooking and drinking throughout the study to minimize variations in nitrate intake. Faecal samples were collected on days 8 and 10 of each dietary period and analysed for ATNC by thermal energy analysis (Pignatelli *et al.* 1987). Faecal samples collected on days 6 and 7 were freeze-dried, milled and analysed for total N by the Kjeldahl method.

The dietary intervention had no effect on mean faecal weight or mean transit time ( $P=0.51$ ;  $P=0.86$  respectively). However, faecal ATNC and N concentrations increased with increased meat consumption as shown in the Table.

Diet	ATNC (µg/d)		Nitrogen (µg/d dry)		Nitrogen (g/d dry)	
	Mean	SE	Mean	SE	Mean	SE
0 g meat	107.16	15.8	53.69	7.2	53.24	2.0
60 g meat	87.781	14.9	51.06	10.7	51.27	3.6
240 g meat	348.44	93.2	159.21	33.1	56.48	3.0
420 g meat	498.03	156.8	198.79	36.4	59.06	2.8

\* $P<0.001$ , \*\* $P<0.0001$ , † $P=0.059$  for dietary effects using Two-Way ANOVA with diet and subject as factors.

Daily faecal ATNC excretion increased with increased dietary meat in a dose-dependent manner ( $P<0.0001$ ) despite a between person CV ranging from 40-90%. There were no significant changes in daily faecal N excretion ( $P=0.276$ ). Duplicate diets were analysed for ATNC and a mean level of 1.3 µg/d was detected for the high meat diets so the ATNC levels detected in the faecal samples must have originated endogenously. Faecal ATNC concentration was positively correlated with mean transit time ( $r=0.58$ ,  $P=0.001$ ) and negatively correlated with mean faecal weight ( $r=-0.48$ ,  $P=0.005$ ). Hence, increased faecal bulk and a fast transit time are associated with reduced contact of these compounds with the colonic mucosa. Further work is being carried out to look at the effects of the diets on biomarkers of NOC exposure.

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**Effects of antioxidant vitamin supplementation on markers of DNA damage and plasma antioxidants.** By EMILY R. BEATTY<sup>1</sup>, TIMOTHY G. ENGLAND<sup>2</sup>, CATHERINE A. GEISSLER<sup>1</sup>, OKEZIE I. ARUOMA<sup>2</sup> and BARRY HALLIWELL<sup>2</sup>, <sup>1</sup>Department of Nutrition and Dietetics, King's College London, Campden Hill Road, London W8 7AH, <sup>2</sup>International Antioxidant Research Centre, Pharmacology Group, King's College London, Manresa Road, London SW3 6LX

High intakes of fruit and vegetables are associated with reduced incidence of many cancers (Block *et al.* 1992). It has been suggested that the antioxidants they contain decrease oxidative damage to DNA thus reducing mutagenic changes in cells. The present study aimed to investigate if antioxidant vitamin supplementation affected damage to DNA as measured by GC-mass spectroscopy (GC-MS).

Fifty-five healthy, non-smoking, non-supplement taking volunteers aged 20-40 years were randomly assigned into two groups, receiving either placebo, or 130 mg d- $\alpha$ -tocopheryl succinate, 100 mg vitamin C and 7 mg  $\beta$ -carotene (Henkel, USA), three times per d for 8 weeks. DNA was extracted from whole blood, purified, hydrolysed and derivatized (Jenner *et al.* 1998) and damaged bases measured by GC-MS (Dizdaroğlu, 1991). Fat-soluble plasma antioxidants were measured by HPLC (Thurnham *et al.* 1988) and vitamin C by COBAS BIO automated centrifugal analyser (Vuilleumier & Keck, 1989).

Base product (nmol/mg DNA)	Day 0		Day 28		Day 56		
	Mean	SE	Mean	SE	Mean	SE	
2-OH adenine,	Control	0.195	0.030	0.149	0.022	0.140	0.023
	Supplemented	0.167	0.020	0.102 <sup>†</sup>	0.021	0.086* <sup>†</sup>	0.011
FAPy adenine,	Control	0.107	0.032	0.164	0.038	0.139	0.034
	Supplemented	0.091	0.019	0.260* <sup>†</sup>	0.053	0.090	0.018
5-OH hydantoin,	Control	0.107	0.013	0.113	0.019	0.099	0.013
	Supplemented	0.136	0.023	0.247* <sup>††</sup>	0.036	0.114	0.018
5-(OH,Me) hydantoin,	Control	0.165	0.027	0.187	0.027	0.162	0.021
	Supplemented	0.182	0.029	0.363* <sup>†</sup>	0.061	0.198	0.034
Total damaged bases,	Control	2.63	0.31	1.94	0.17	1.82 <sup>†</sup>	0.18
	Supplemented	1.76	0.13	2.63* <sup>†</sup>	0.26	1.67	0.17

Mean values significantly different from control group on same day. <sup>†</sup>P < 0.05, <sup>††</sup>P < 0.01. Mean values significantly different from day 0 of same treatment group, <sup>\*</sup>P < 0.05, <sup>††</sup>P < 0.01, (repeated measures ANOVA, Bonferroni corrected).

FAPy adenine = 4,6-diamino-5-formamidopyrimidine.

Of the twelve markers of DNA damage measured, those for which the effect of supplementation was significant are shown in the Table. Supplementation decreased 2-hydroxy adenine over the course of the study. FAPy adenine, 5-hydroxy hydantoin, 5-(hydroxy methyl) hydantoin, and total products of DNA base damage increased after 28 d supplementation, returning to baseline levels by day 56. When initial concentrations of plasma antioxidants were considered as covariates, low initial plasma  $\beta$ -carotene and vitamin C concentrations were significantly associated with increases in 5-hydroxy hydantoin ( $P < 0.001$ ,  $P = 0.029$ ), and low initial plasma vitamin C concentration with increases in total base products ( $P = 0.004$ ).

These results suggest that supplementation with antioxidant nutrients does not decrease steady state levels of oxidative DNA damage *in vivo*. In fact, as seen with vitamin C alone (Podmore *et al.* 1998), supplementation may initially increase damage to DNA, and this effect may be modulated by initial plasma antioxidant concentration. This may be a direct pro-oxidant effect or due to down regulation of DNA repair or endogenous antioxidant defence systems in response to this supplement.

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**The effects of vitamin C supplementation on dynamic lung function: a double-blind cross-over trial.** By A. KEATING and A. TYLER, *Anglo European College of Chiropractic, Parkwood Road, Bournemouth BH5 2DF*

Studies published within the past 7 years appear to indicate a positive correlation between fresh fruit consumption, vitamin C status, and enhanced dynamic lung function (Strachan, 1991; Ness *et al.* 1996). The present study seeks to establish whether vitamin C supplementation causes a similar effect.

The study involved eighty-eight male students between the ages of 18 and 27 years from the Anglo European College of Chiropractic. A group of forty-five randomly assigned subjects (Cvit $\rightarrow$ Plac) were supplemented with 500 mg vitamin C/d in a sustained release formulation tablet. The remaining subjects (Plac $\rightarrow$ Cvit) took a daily placebo tablet of identical appearance. The two groups were matched as closely as possible for age, weight, body type, smoking habit and physical activity. Informed consent to participate was obtained from all subjects in the trial, and ethical committee approval was gained before the experimental procedure.

Initial dynamic lung function data were obtained by forced exhalation into a Vitalograph Spirometer (Model R) for subsequent graphical analysis. Further data were obtained every 2 weeks until the end of the trial at 8 weeks. Halfway through the trial, from the end of the fourth week, the vitamin C and placebo supplements to the two groups were crossed over without the knowledge of either of the groups, or of the experimenter.

Data for peak expiratory flow rate (PEFR) and forced expiratory volume in 1 s (FEV<sub>1</sub>) were normalized, and expressed as proportions of the initial readings. Data for initial readings, and readings at week 4 (cross-over point) and week 8 appear in the Table. Data were analysed within groups by a paired *t* test with reference to the initial readings, and between groups for each corresponding time epoch with an unpaired *t* test.

Variable	Group	0 Weeks		4 Weeks		8 Weeks	
		Mean	SE	Mean	SE	Mean	SE
FEV <sub>1</sub>	Cvit $\rightarrow$ Plac	1	0	1.053	0.028	1.054* <sup>††</sup>	0.018
FEV <sub>1</sub>	Plac $\rightarrow$ Cvit	1	0	0.997	0.014	0.994	0.018
PEFR	Cvit $\rightarrow$ Plac	1	0	1.162* <sup>††</sup>	0.055	1.109* <sup>††</sup>	0.042
PEFR	Plac $\rightarrow$ Cvit	1	0	1.01	0.03	1.065	0.036

Mean values were significantly different from initial reading, <sup>\*\*</sup>P < 0.01

Mean values were significantly different between vitamin C and placebo groups, <sup>††</sup>P = 0.02

The Table shows a significant increase in FEV<sub>1</sub> after 8 weeks and in PEFR after 4 weeks in the Cvit $\rightarrow$ Plac group. Significant differences were apparent between the two groups at 4 weeks for PEFR and 8 weeks for FEV<sub>1</sub>. It is apparent that PEFR responds more quickly, and with a bigger relative effect in response to supplementation, than does FEV<sub>1</sub>.

We conclude that vitamin C supplementation has a significant effect on PEFR and FEV<sub>1</sub> at the 1% level in young healthy male volunteers, and that PEFR is a more sensitive index of changes in dynamic lung function than FEV<sub>1</sub>.

The authors gratefully acknowledge Larkhall Green Farm Ltd for the gifts of vitamin C and placebo.

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**Diet and gallstones in Saudi Women** By A.M.AL-KUDSI<sup>1</sup> AND D. J. MILLWARD<sup>2</sup>. *Department of Nutrition, King Khalid University Hospital, King Saud University, P.O. Box 245 Riyadh 11411, Saudi Arabia, and 2 Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH.*

Cholesterol-based gallstones are mainly associated with obesity, and a possible genetic predisposition, with few studies indicating clear cut dietary differences. We report here a study of diet and gallstones in a relatively homogeneous population of women in Saudi Arabia. Subjects were selected from women who attended a primary health care outpatients clinic at King Khalid University Hospital Riyadh, Saudi Arabia for minor acute problems. Those without any chronic diseases or acute symptoms likely to be associated with gallbladder disease were invited to have an ultrasound scan for gallstones. Those who agreed completed a demographic questionnaire, underwent measurements of anthropometry and fasted blood biochemistry, a 24hr food intake recall and a food frequency questionnaire. Subjects who completed all procedures were entered into the study. Ultrasonography involved a high-resolution real-time scanner with a 3.5-MHz linear assay transducer in overnight fasted subjects studied in three positions with gallstones identified by standard criteria and with quality assurance performed by repeat examination of randomly selected cases and controls. The 70-item, nine frequency category food frequency questionnaire was updated and extended from a previously validated 61-item version with additions of local traditional foods of known composition, with use of standard portion sizes to estimate quantities, with a write-in section for foods not listed, for brand names of foods and for total daily quantity of fruits and vegetables eaten. The analysis concentrated on energy and macronutrients, including source of carbohydrates, fat type, and total non-starch polysaccharides (NSP). Validation was limited to comparison with the 24 hr recall and inspection of total energy intakes as a multiple of BMR estimated from the Schofield equations. Subjects with intakes  $<1.3 \times \text{BMR}$  were rejected so that of 308 women with complete data sets 287 were included in the final analysis.

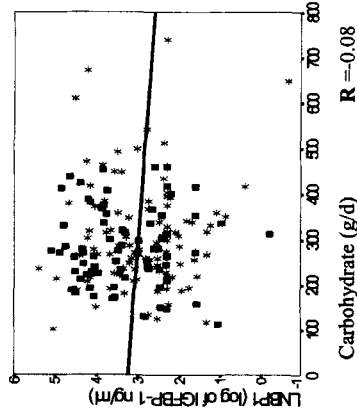
60 women were found to have gallstones. Mean intakes of energy and macronutrients assessed by FFQ values were higher than 24hr values differences ranging from 3.6% for carbohydrate to 9% for fibre. Individual intakes by the two methods were highly correlated but crosstabulations of quantities of macronutrient and energy intakes showed poor agreement (from 43% for energy to only 22% for protein). However crosstabulation  $\pm$  one centile indicated  $>92\%$  of subjects correctly classified. Overall energy intakes by FFQ were  $1.51 \times \text{BMR}$  with BMI = 26.5 (SD 4.3). The BMI of cases was higher than controls (29.2 cf 25.8) with 42%  $>30$  compared with 7% controls. Clear dietary differences were observed between cases and controls by both measures of food intakes. Cases consumed significantly more total energy, 2207 (SD125) cf 2048 (SD 107) Kcals/day, carbohydrate, 293 (SE 13.1) cf 276 (SE 20.7) g/day, and fat, 75.9 (SE 7.7) cf 59.4 (SE 7.2) g/day, and significantly less protein, 88 (SD 8.7) cf 102 (SD 6.5) g/day and NSP 17.1 (SD 0.9) cf 20.8 (SD 3.4) g/day,  $P < 0.001$  in each case. The FFQ showed that higher fat intakes (total and % energy, 31% cf 26%), resulted from higher saturated fat (18.4% cf 12.6% energy) and lower PUFA (3.2 cf 5.7g/d, 1.3 cf 2.5 % energy), with no difference in MUFA as a % energy (9.4 cf 9.7% energy). These data clearly indicate the importance of diet in the aetiology of gallstones with total energy, carbohydrate and fat intakes positively and protein and fibre intakes negatively associated with gallstone risks.

**Insulin-like growth factor binding protein-1: a potential marker for carbohydrate intake?** By A. GREENHALGH<sup>1</sup>, J.E. CADE<sup>1</sup>, S. SHARMA<sup>1</sup>, A.H. HEALD<sup>2</sup>, J. SAMPAYO<sup>1,2</sup>, M. GIBSON<sup>2</sup>, A. WHITE<sup>2</sup> and J.K. CRUICKSHANK<sup>1</sup>. *1 Clinical Epidemiology Unit, and 2 Endocrine Science Research Group, Medical School, University of Manchester, Manchester M13 9PT*

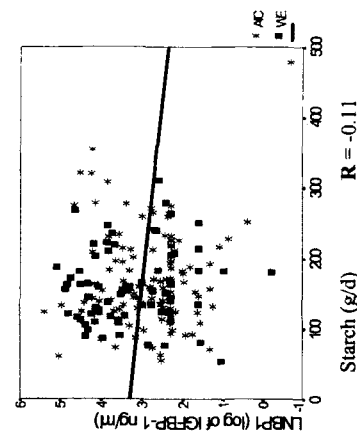
There are currently no appropriate biomarkers for carbohydrate intake (Margetts & Nelson, 1997). The present study aimed to explore the relationship of carbohydrate intake to insulin-like growth factor binding protein-1 (IGFBP-1) in people having 75 g glucose tolerance tests. IGFBP-1 offers potential as a biomarker, as it is inversely regulated by portal insulin, but being synthesized in and secreted from the liver, unlike insulin is not affected by variable hepatic "first pass" uptake (Conover *et al.* 1992). Population samples were randomly selected from general practitioner registers for local people of white European (WE) and African Caribbean (AfC) descent. Carbohydrate intake was assessed using a specifically designed food-frequency questionnaire (FFQ) for each ethnic group (Sharma *et al.* 1996; Greenhalgh, unpublished results).

In those with dietary intake data fasting plasma IGFBP-1 levels, measured by radioimmunoassay, were significantly higher in 73 WE than 112 AfC (geometric mean 26.5 (95% CI 20.5–34.2) ng/ml v. 16.9 (14.0–20.4) ng/ml  $P=0.0001$ ). Mean carbohydrate intakes were 309 g/d in AfC and 286 g/d in WE (mean difference 23, 95% CI -54 to + 8 g,  $P=0.144$ ) but 173 and 158 g/d respectively for starch.

#### IGFBP-1 and carbohydrate intake



#### IGFBP-1 and starch intake



In a univariate analysis, IGFBP-1 was negatively correlated with habitual daily starch intake ( $P=0.03$ ). However, in a multivariate regression model adjusting for age, BMI, sex, fasting glucose and ethnicity IGFBP-1 was no longer statistically significantly associated with starch intake.

These initial data suggest a possible weak negative relationship between habitual starch consumption and IGFBP-1 concentrations, a result we are examining further in a larger sample, and using alternative methods of estimation of starch and carbohydrate intake.

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**Suitability and acceptability of a dietary interview-survey designed to identify barriers to food selection, by denture and non-denture wearers: a pilot study.** By CLAIRE L. MOODY and PAULA J. MOYNIHAN, *The Dental School, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4BW*

Studies investigating the diets of denture-wearers have shown substantial differences in the consumption of fruit, vegetables and fibre-rich foods, between edentulous and dentate persons (Moynihan *et al.* 1994; Touger-Decker *et al.* 1996), emphasizing the importance of dietary intervention for this group of the population. Compromised masticatory function may be an additional barrier to dietary change to the others already considered for the general population (Cox *et al.* 1995). In view of this a dietary interview-survey (DIS) was designed to identify the perceived barriers to consuming fruit, vegetables and fibre-rich foods for denture and non-denture wearers. The DIS was designed with bi-polar-response grid and anecdotal response questions addressing: income and accessibility to food; preparation skills and storage facilities available; importance of diet in relation to health and masticatory efficiency. A suitable coding scheme based upon work by Raats *et al.* (1995) was used to process the results. The aim of the present pilot study was to assess additional implications; cost, time and patient acceptability of the DIS, with the objective of judging its suitability for use in a larger scale study.

Ten edentulous (average age 58 years) and ten dentate (average age 57 years) volunteers recruited from one dental practice were visited by a nutritionist at home to complete the DIS. Feedback from the volunteers about the acceptability of the DIS was obtained by a postal questionnaire. Recruitment and DIS were completed within 35 d, the DIS took 48 (SD 15) min to complete and the total time spent and cost per visit was 134 (SD 27) min and £1.31 (SD £0.44) respectively. Eighteen (90%) volunteers returned the feedback questionnaire, of whom all thought the DIS-questions were clear, that the study was fully explained and that any questions asked were answered appropriately. Key results from both questionnaires are summarized in the Table.

Examples of responses to questions from each section of the DIS		Denture	Non-denture
Q1) Consider fruit, vegetables and fibre-rich foods to be affordable for family income		10	10
Q2) Consider it easy to cook with fruit, vegetables and fibre-rich foods		8	9
Q3) Have a perceived need to increase their consumption of one or more of the target foods		6	9
Q4) Rate overall intake of fruit, vegetables and fibre-rich foods as low		5	1
Q5) Describe denture as comfortable		4	N/A
Q6) Describe chewing efficiency as worse compared to natural teeth		6	N/A
Q7) Eat fruit, vegetables and fibre-rich foods at least once per day (food frequency question)		2	7
Volunteer feedback on the DIS (the answer choices provided are given in parentheses)		% giving positive responses	
What did you think about the length? (too long, too short, just right)		78 (n 18)	
What did you think about the style? (poor, fair, good)		78 (n 14)	
Did you find the questions interesting? (yes, no, no opinion)		89 (n 16)	

The DIS provided useful information concerning the barriers to dietary change, but owing to the small sample size it is inappropriate to draw any conclusions; however, the findings of this pilot study show that the DIS was well accepted by the target population and that despite the length, it is suitable for use in a larger scale study.

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**The relative validity of practice nurse dietary assessment in general practice.** By R.L. THOMPSON<sup>1</sup>, P.S. LITTLE<sup>2</sup>, J. BARNETT<sup>3</sup> and B.M. MARGRETT<sup>1</sup>, <sup>1</sup>*Institute of Human Nutrition and* <sup>2</sup>*Primary Medical Care, University of Southampton, SO16 6YD*

The role of the practice nurse has increased in recent years. Nurses now have an important role in giving lifestyle advice to patients at risk of cardiovascular disease (Calnan *et al.* 1994). In order to be cost-effective brief methods of dietary assessment are required. The objective of the present study was to validate a range of dietary assessment instruments administered by a practice nurse in general practice. The subjects included patients with risk factors for cardiovascular disease (n 61) and an age- and sex-stratified general population group (n 50). Using a randomized block design, brief assessment instruments (short food-frequency questionnaires) and more complex conventional dietary assessment tools (24 h recall, 7 d checklist and food-frequency questionnaire) were compared with an accepted 'relative' standard, a 7 d weighed record. The standard was checked by performing test-retest reliability in additional subjects (n 29). In the general practice setting what is clinically important is to assess accurately whether a subject has a high-saturated-fat diet (cut-off >10% energy from saturated fat) or low-fibre diet (<18 g/d) so that appropriate dietary advice can be given. In this report agreement has been assessed in terms of sensitivity and specificity.

The weighed record was re-tested in a sample of twenty-nine subjects and showed a sensitivity of 83% and a specificity of 50% for percentage energy from saturated fat and 94%, 60% respectively for NSP. The Table shows the results for the other dietary assessment methods.

Dietary assessment tools	% Energy from saturated fat		NSP (g/d)	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Checklist (7 d)	84	41	80	92
Food-frequency questionnaire	84	63	64	64
24 h recall	67	63	88	56
HEA1* (number of specified portions of foods per d)	81	56	80	44
HEA2 (number of specified portions of foods per week)	82	74	76	64
HEA3 (As HEA2 but frequency and portion size reported separately)	85	31	91	24
DINE (Roe <i>et al.</i> 1994)	72	47	93	22

\* HEA, Health Education Authority

For percentage energy from saturated fat sensitivity was greater than 80% for all tools except for the 24 h recall and DINE questionnaire. Specificity tended to vary a lot more between the methods but was highest for HEA2. For NSP sensitivity was over 90% for DINE and HEA3. Specificity was low for DINE and high for HEA2.

The brief methods performed equally well compared with the more complex methods. High sensitivity and high specificity are required to ensure that those subjects with high-fat or low-fibre diets can be targeted but it is also important that subjects are not incorrectly labelled as requiring dietary advice when it is not required. In terms of sensitivity and specificity of the brief tools HEA2 performed the best for percentage energy from saturated fat and NSP.

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**Accuracy of estimates of energy intake using the multiple-pass 24 h recall method in young children and adult women: a doubly-labelled water study.** By RACHEL K. JOHNSON<sup>1</sup>, REBECCA P. SOULTANAKIS<sup>2</sup>, MICHAEL I. GORAN<sup>3</sup> and DWIGHT E. MATTHEWS<sup>4</sup>. <sup>1</sup>Dunn Nutrition Centre, Cambridge CB2 2DH, <sup>2</sup>Department of Nutrition and Food Sciences, University of Vermont, USA, <sup>3</sup>Department of Nutrition Science, University of Alabama at Birmingham, USA, <sup>4</sup>Department of Medicine, University of Vermont, USA

Underreporting of food intake is pervasive in dietary surveys. The magnitude of underreporting varies depending on the age, sex, and body composition of the sample and the dietary intake method. Novel dietary intake tools are being developed in an attempt to minimize the problem of energy intake (EI) underreporting. One innovative example is the multiple-pass 24 h recall (MP24R), designed by the U.S. Department of Agriculture (USDA) to provide respondents with multiple cues and opportunities to report their food intake. The aim of the present study was to determine the accuracy of EI obtained using the MP24R in young children and adult women. Doubly-labelled water (DLW) measurements of total energy expenditure (TEE) were used for validation. In addition, the association of body composition with EI misreporting (EI - TEE) was determined.

Twenty-four children (twelve boys, twelve girls, age range 4-7 years) and thirty-five women (age range 19-46 years) participated in the study. The MP24R was used to estimate EI. With this method, the interviewer uses three distinct passes to gather information about a subject's food intake during the preceding 24 h (Johnson *et al.* 1996). The passes include (1) the quick list, the respondent is asked to recall everything eaten the previous day using any recall strategy they choose, (2) the detailed description; the respondent is asked to clarify any foods mentioned in the quick list, and (3) the review; the interviewer reviews the list of foods mentioned, probes for additional eating occasions, and clarifies food portion sizes using two-dimensional food models. Three MP24R were obtained from the children's mothers (collected in conjunction with their child, two in person, one by telephone) and four MP24R were obtained from the women (two in person, two by telephone) over a 14 d period. TEE was measured over the same 14 d period under free-living conditions using DLW. Body composition was measured using total body water determined from the DLW measurement in the children and dual photon absorptiometry in the women.

Using a paired t test, no difference was found between the 3 d mean EI (6.5 (SD 1.82) MJ) and TEE in the children (6.7 (SD 2.06) MJ) ( $t$  2.07,  $P=0.05$ ). In the women, the 4 d mean EI (9.2 (SD 2.54) MJ) was significantly lower than TEE (11.1 (SD 2.10) MJ) ( $t$  3.78,  $P=0.001$ ). Percentage body fat was negatively associated with EI misreporting ( $r$  -0.46,  $P=0.06$ ) in the women.

In the children, the MP24R provided a valid, unbiased group estimate of EI. However, when the identical dietary intake methodology was used with adult women, it did not generate a group measure of EI that was either accurate or unbiased. Underreporting was widespread among the women and strongly associated with increased body fatness. Even when a dietary intake method specifically designed to minimize the problem of EI underreporting was used with adult women, we were unable to obtain results that were free from underreporting in the women.

The effect of age on the accuracy of EI reporting is complex and probably affected by a number of factors including the stability of habitual eating patterns and societal pressures to report a "good" diet devoid of "sin" foods. More research is needed on the factors (both physical and psychological) associated with underreporting in various samples. A better understanding of these variables could lead to the development of dietary intake methods aimed at improving the accuracy of data collection as well as analytical techniques for handling biased data.

Johnson RK, Driscoll P & Goran MI (1996) *Journal of the American Dietetic Association* **96**, 1140-1144.

**Adequacy of revised UK reference data for nutritional assessment of 7-8 year olds.** By SHIRLEY-ANNE H. SAVAGE<sup>1</sup>, JOHN J. REILLY<sup>1</sup>, CARRIE RUXTON<sup>2</sup> and TERRY R. KIRK<sup>3</sup>. <sup>1</sup>University of Glasgow, Department of Human Nutrition, Yorkhill Hospitals, Glasgow G3 8SJ, <sup>2</sup>Sugar Bureau, Duncan House, Dolphin Square, London SW1V 3PW and <sup>3</sup>Queen Margaret College, Clerwood Terrace, Edinburgh EH12 8TS

Objective anthropometric assessment is recommended for the identification of over- or undernutrition (Cross *et al.* 1995) and comparisons with appropriate reference data are essential to this process (Reilly, 1997). Reference data for height, weight and body mass index (BMI) have been available since 1995 (Cole *et al.* 1995; Freeman *et al.* 1995). These have not yet been tested in clinical practice and contain biases in infancy and early childhood (Savage *et al.* 1996). The aim of the present study was to test the adequacy of these reference data by comparing them with the height, weight and BMI of a representative sample of 7-8-year-old children living in Edinburgh ( $n$  255: 123 girls; 132 boys; mean age 7.5 (SD 0.4) years).

Anthropometric measurements were made by a single trained observer and expressed as standard deviation (SD) scores relative to reference data for height, weight, and BMI. Adequacy of reference data was assessed in two ways, first by calculating mean SD scores and 95% confidence intervals (CI) for each variable. Second, to assess agreement at the extremes of the distribution, we compared the observed and expected frequencies <10th and >90th centiles for each variable.

For each variable, and in both sexes, mean SD scores were close to zero. The table gives mean SD score (and 95% CI) for height, weight and BMI. Percentages of the sample falling below the 10th and above the 90th centile did not differ significantly from expected (chi squared goodness of fit,  $P>0.05$ ), except for weight in girls with an excess of girls below the 10th centile (observed 17%; expected 10%,  $P<0.05$ ).

	Height SD Score		Weight SD Score		BMI SD Score	
	Mean	CI	Mean	CI	Mean	CI
Boys	0.03	-0.14 to 0.19	0.07	-0.10 to 0.25	0.10	-0.08 to 0.28
Girls	-0.09	-0.25 to 0.08	-0.18	-0.36 to -0.01	-0.19	-0.37 to -0.01

The present study supports the use of the currently recommended reference data for height, weight and BMI in 7-8-year-old children. The significant difference for body weight in girls may be of minor clinical significance. We conclude that, on the basis of this evidence, estimating the prevalence of over- or undernutrition in childhood, or screening for over- or undernutrition, can now be carried out with greater confidence using the 1990 reference data for BMI.

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Freeman JV, Cole TJ, Chinn S, Jones PRM, White EM & Preece MA (1995) *Archives of Disease in Childhood* **73**, 17-24.

Reilly JJ (1997) Nutritional assessment. In *Foyfar and Arnells Textbook of Pediatrics*, 5th ed, pp. 1186-1187 [AM Campbell and N McIntosh, editors]. Churchill Livingstone: New York.

Savage SAH, Reilly JJ & Durmin JVGA (1996) *Proceedings of the Nutrition Society* **55**, 81A.



**Refinements used in accurate assessment of dietary fatty acid intake using a standard nutrient database.** By COLETTE N.M. KELLY<sup>1</sup>, RUTH D. SMITH<sup>1</sup>, ALLAN G. STEPHENS<sup>2</sup> and CHRISTINE M. WILLIAMS<sup>1</sup>, <sup>1</sup>The Hugh Sinclair Unit of Human Nutrition, University of Reading, RG6 6AP, <sup>2</sup>School of Animal and Microbial Sciences, University of Reading, RG6 6AJ

There is a need for further development of methodologies for use in dietary intervention studies investigating effects of specific fatty acids. Accurate estimation of habitual or experimental dietary fatty acid intake is restricted by the limited information available on the fatty acid compositions of foods in current nutrient databases. Dietary records also frequently provide insufficient information on the types of oils and fats used in food preparation and on the fatty acid composition of pre-prepared recipe meals.

The aim of the present study was to compare data obtained for fatty acid intakes assessed from nineteen diet records, with and without the use of additional details of foods, recipes, oils and spreads. Nineteen students (eleven males, eight females) residing in a fully catered Hall of Residence at the University of Reading were carefully instructed to complete a 7 d weighed intake diary. Dietary records were analysed using two approaches: (A) using the standard information available in the 'Foodbase' nutrition database, Institute of Brain Chemistry and Human Nutrition, and (B) using refinements and additions to the database. In the case of assessment method B the complete fatty acid profiles of all fats and oils used in the residence kitchens (obtained directly from the manufacturers), were manually entered into Foodbase. Nutritional information provided by manufacturers of pre-prepared foods was also included in the database in addition to all recipes used in the Hall kitchens. Moreover, direct analysis of the total fat content of uncooked and cooked foods provided accurate information regarding fat uptake on cooking.

Intake/d (% energy)	A		B		Intake/d (% energy)	A		B	
	Mean	SE	Mean	SE		Mean	SE	Mean	SE
Energy (kJ)	10686	572	10433**	555	Total-MUFA	12.82	0.31	14.39***	0.36
Protein	13.84	0.38	12.65***	0.39	cis-PUFA	5.54	0.20	5.30*	0.19
Carbohydrate	47.04	1.09	47.56	1.02	trans-PUFA	0.38	0.04	0.21***	0.04
Fat	35.06	0.88	35.98*	0.82	Total-PUFA	5.93	0.19	5.51***	0.18
SFA	11.60	0.52	12.14*	0.50	Total trans	1.53	0.09	2.08***	0.10
cis-MUFA	11.67	0.29	12.52***	0.33	Total fatty acids	30.54	0.80	32.19***	0.78
trans-MUFA	1.14	0.06	1.87***	0.08					

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.  
Mean values were significantly different from analysis A: \*  $P < 0.05$ , \*\*  $P < 0.001$ , \*\*\*  $P < 0.0001$ .

The results demonstrate that methods A and B provide significantly different values for all the nutrient classes assessed apart from % energy from carbohydrate. Whilst the differences are small, and may have little importance in routine assessment of habitual dietary fatty acid intakes, the refinements used are important in intervention studies where small changes in individual fatty acids or fatty acid classes are to be made. Further information on the accuracy of assessment method B will be obtained by comparing the values calculated from a 7 d diet record with those obtained by direct fatty acid analysis of the recorded foods.

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**Minimizing errors associated with coding 24 h dietary recalls for INTERMAP in the UK.** By R. DRAKE<sup>1</sup>, N. SERES<sup>1</sup>, S. BLACKWELL<sup>2</sup>, B. DENNIS<sup>2</sup>, J. STAMLER<sup>3</sup> and P. ELLIOTT<sup>1</sup>, <sup>1</sup>Department of Epidemiology and Public Health, Imperial College School of Medicine at St Mary's, London W2 1PG, <sup>2</sup>Department of Biostatistics, University of North Carolina, USA, <sup>3</sup>Department of Preventive Medicine, Northwestern University Medical School, Illinois, USA

There is increasing interest in the validity of dietary data gathered. Errors arising from underreporting and biased reporting by study participants have received much attention (Goldberg *et al.* 1991) but errors arising during coding and errors associated with food tables have been neglected. Inaccuracies may arise during the coding or calculating stage due either to mistakes or difficulties in interpretation (Bingham, 1987). The potential error arising during coding was estimated in a study cited by Whiting & Leverton (1960) in which a 10% minimum to maximum range in estimated energy intakes was found and a 24% minimum to maximum range for protein, as obtained from individual diet differences from the mean value.

For the INTERMAP study of diet and blood pressure quality control procedures adapted from the National Heart Lung and Blood Institute (NHLBI) system are being employed to minimize coding errors (Dennis *et al.* 1980). Coders undergo a rigorous training session and quality control standards are set for the maximum number of line errors allowed in the coding of a 24 h recall. Any discrepancies between food codes or weights assigned to an item by a coder and those assigned by a Site Nutritionist, which result in editing of the recall, are counted as line errors. To facilitate standardized coding a Code Book is being developed which provides information on matching participants descriptions of foods to food codes and information for assigning weights of portion sizes.

To assess the potential error arising when different coders code the same 24 h recall data from the initial stages of training new INTERMAP staff were examined. Four trainees including two nutritionists and two nutrition undergraduate students were each asked to code four set recalls using the Code Book. When codes assigned by trainees were compared with those assigned by a trained coder using the Code Book a mean 14% of the lines of code in a recall were found to contain errors, ranging from 0 to 43%. These line errors were associated with errors in the estimation of energy of up to 1674 KJ and of protein of up to 32 g.

As trainees coded subsequent recalls and received additional training in the use of the Code Book fewer line errors arose. For the final stage of training each of the trainees collected and coded five 24 h recalls with fewer than 6 % line errors. To ensure this high standard of coding is being maintained throughout the course of INTERMAP field work quality control procedures are in place. For every batch of ten recalls coded one is re-coded blind by a Site Nutritionist and if there are more than 6 % line errors the entire batch is re-coded. This procedure continues until the batch passes. To date the first 250 recalls collected for INTERMAP in the UK have met this quality control standard and the Code Book is currently being expanded to allow a larger number of foods to be coded.

Initial work for INTERMAP in the UK suggests that with standardized training, use of a Code Book and on-going quality control, as is being achieved in INTERMAP, it is possible to reduce the number of coding errors to acceptable levels.

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**Evaluation of a bar-code system for nutrient analysis in dietary surveys.** By SUSAN ELEY, ANNIE S. ANDERSON\*, LINDA MAHER and MICHAEL E.J. LEAN, *Department of Human Nutrition, University of Glasgow, Glasgow Royal Infirmary, Glasgow G31 2ER.*

Dietary assessment procedures are labour intensive and therefore expensive. New technologies may offer time and cost benefits to large-scale dietary surveys. A novel system for nutrient analysis, Foodmeter UK has been developed and tested over 5 years. Its key features are a nutrient database of 600 commonly eaten foods (95% of foods eaten in 7 d surveys), a booklet identifying each food with a bar-code, bar-codes for gram weight and for portions sizes (small, medium, large) and a bar-code reader with dietary analysis software for personal computer.

In the present study the bar-code system has been evaluated by comparison with a commonly used manual entry nutrient analysis software package COMP-EAT. A dietary survey (using the 7 d weighed inventory approach) of 160 adults aged 18–65 years was conducted within the Glasgow City District to collect appropriate data for the evaluation. Almost 36% of the sample reported energy intakes less than 1.2 times BMR.

	Bar-code system		Manual system		Paired differences		Spearman correlation coefficient between two methods
	Mean	sem	Mean	sem	Mean	sem	
Total energy (MJ)	8.2	0.2	8.2	0.2	0.01	0.04	0.25
Total protein (g)	79.0	2.6	73.0	1.9	6.1	2.2	2.72**
Total fat (g)	82.4	2.1	80.9	2.2	1.6	0.7	2.11*
Saturated fat (g)	30.4	0.9	30.5	1.0	-0.1	0.4	-0.35
Total carbohydrate (g)	233.5	5.5	227.0	5.4	6.6	1.2	5.29***
Starch (g)	132.9	2.9	126.9	2.9	6.0	0.9	6.93***
Sugars (g)	98.6	3.4	94.5	3.5	4.1	0.9	4.42**
NSP (g)	11.7	0.4	11.3	0.4	0.4	0.1	3.59***
Energy from protein (%)	16.3	0.5	15.0	0.2	1.4	0.5	2.77**
Energy from fat (%)	37.0	0.4	37.0	0.4	0.02	0.2	0.09
Energy from saturated fat (%)	13.6	0.2	13.9	0.3	-0.3	0.1	-2.03*
Energy from carbohydrate (%)	44.1	0.5	43.7	0.5	0.4	0.2	2.30*
Retinol (µg)	666.9	86.9	672.8	92.8	-5.9	19.7	-0.30
Vitamin C (mg)	63.7	4.4	64.4	4.5	-0.7	1.7	-0.41
Calcium (mg)	837.5	27.3	829.4	29.6	8.1	8.1	1.00
Iron (mg)	11.3	0.3	11.4	0.3	-0.1	0.09	-1.33
Folate (mg)	204.5	7.2	207.6	7.5	-3.1	2.5	-1.24

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

The Table shows comparisons of nutrient intakes estimated by each system. From further analysis using the Bland & Altman (1986) method, the bias between the two methods, for each absolute nutrient intake estimate, was small ranging from 0.93 to 1.03.

The bar-code system took significantly less professional time in data entry and nutrient analysis than the widely used manual system (29 v. 47 min/7 d diary,  $P < 0.001$ ).

It is suggested that the bar-code system offers greater speed with a saving of professional time needed for nutrient analysis of dietary surveys. This system is commended for maintaining accuracy while promoting economy.

This work was funded by the Ministry of Agriculture, Fisheries and Food (UK).

Bland JM & Altman DG (1986) *Lancet* **1**, 307–310.

\* Current address: Centre for Applied Nutrition Research, University of Dundee DD1 4HT.

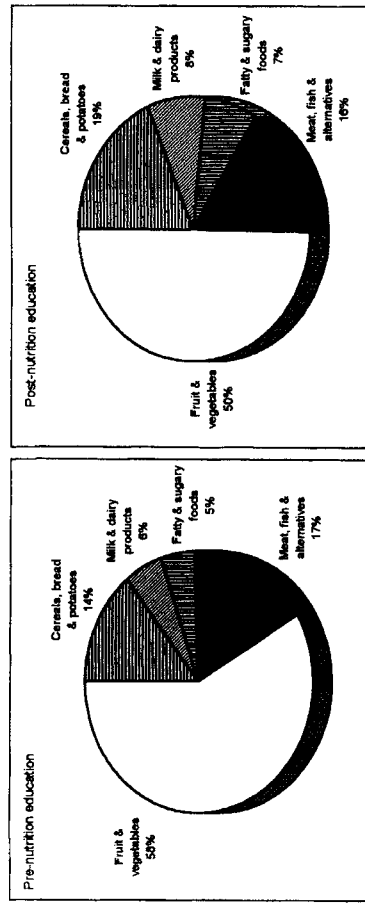
**Assessing nutritional knowledge in prepubescent children using 'draw and tell' methodology.** By K.I. PIRE, *The Wessex Institute for Health Research & Development, University of Southampton, Southampton SO16 6YD*

Many life-long dietary habits are established at about puberty. Nutrition education programmes targeted to this age group need to be evaluated to ensure that they are effective.

Perception of a healthy balanced diet was assessed using 'draw and tell' sessions before and after a 2-week nutrition education programme in fifty-five 9–10-year-olds. The advantages of the 'draw and tell' technique are that it is quick and easy to carry out, and children tend to include more detail in their drawings than they express with the labels, for example, they will draw a carton of 'semi-skimmed' milk but label it simply 'milk'. Also, many children in this age group have difficulty spelling and without the aid of the drawings some of the foods descriptors are unintelligible. The nutrition education programme used material based on the *Balance of Good Health* food-based guidelines (Health Education Authority, 1994).

The children's drawings were analysed by food group using the *Balance of Good Health* guidelines. Any item of food drawn was taken as one 'portion' and no account was taken of actual portion size.

The post-education drawings showed an increased proportion of foods in the cereals, milk products and fatty and sugary food groups, and a decreased proportion in the fruit and vegetables and meat groups.



However, as can be seen from the Table, there was an overall increase in the number of items drawn rather than a large decrease in the number of fruits and vegetables that altered the overall proportions.

Food group	Foods items drawn before education		Foods items drawn after education		Change	
	n	%	n	%	n	%
Cereals, bread and potatoes	49	14	72	19	23	+5
Fruit and vegetables	200	58	192	50	-8	-8
Milk and milk products	19	6	31	8	12	+2
Meat, fish and alternatives	59	17	64	16	5	-1
Fatty and sugary foods	16	5	28	7	12	+2
Total	343	100	387	100		

The drawings showed an apparent change in knowledge after nutrition education with an emphasis on more carbohydrate and protein foods rather than in a decrease in the amount of fruit and vegetables.

The over-representation of fruit and vegetables and under-representation of milk and cereal products may be due, in part, to the fact that the drawings depicted a single meal. Future work will look at the effect on the balance of the food groups of extending the drawings from a single meal to a whole day.

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The calcium and phosphorus intakes of Shenyang women in the north-east of the People's Republic of China. By LIYA YAN<sup>1</sup>, XIAOHONG WANG<sup>1</sup>, ANN PRENTICE<sup>2</sup> and MICHAEL H GOLDEN<sup>3</sup>. <sup>1</sup>Department of Preventive of Medicine, Shenyang Medical College, Shenyang 110031, P R China, <sup>2</sup>MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ, <sup>3</sup>Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen

Ca and P are important mineral constituents of the skeleton. There have been some concerns that a low Ca intake and a low ratio of Ca:P in the diet may have adverse effects on bone health. The aim of the present study was to evaluate the intakes and sources of Ca and P in Chinese women from Shenyang, a large industrial city in the north-east of China. The subjects were volunteers in a cross-sectional study of bone metabolism. Sixty women who were representatives of workers from a large factory area were selected for dietary assessment, thirty young women aged 25-38 years and thirty old women aged 63-75 years. Current nutrient intakes were determined by 5 day weighed food records and the intakes of Ca and P were calculated according to Chinese food composition tables (The Institute of Nutrition and Food Hygiene 1992).

Mean Ca and P intakes were 422 (SD 106) mg and 1045 (SD 274) mg for young women and 387 (SD 128) mg and 972 (SD 339) mg for old. The patterns of food sources for the two nutrients in the two age groups were similar. The combined results are shown in the Table. The mean intake of Ca was just

	Calcium		Phosphorus	
	Amount (mg)	% of total	Amount (mg)	% of total
Dairy	41	10	30	3
Meat, fish, eggs	65	16	323	32
Soya bean products	85	21	111	11
Cereal	53	13	413	41
Vegetables	117	29	101	10
Others	44	11	30	3
Total	405	100	1008	100

over 400 mg/d which was about half of the Ca intake of British adult women (Gregory *et al.* 1990). Most of the Ca intake was from vegetables and soybean products (bean curd and dried bean curd). Dairy produce contributed only 10% of total Ca intake. This compares with 23% for Chinese women in Hong Kong (Lau & Woo, 1994) and 50% for British women (Gregory *et al.* 1990). The mean intake of P was, however, over 1000 mg/d which almost reached the intake level of British women. The main sources of P were grains and animal protein. The Ca:P in the diet was 1:2.5. The influence of the low Ca intake and low Ca:P ratio and also other factors on bone health in the population whose hip fracture incidence is low (Yan *et al.* 1996) is under investigation.

This work was supported in part by the Sandoz Foundation for Gerontological Research and the Nestle Foundation.

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Yan L, Zhou B, Hou J, & Prentice A. (1996) *Chinese Journal of Osteoporosis* 2, 69-71.

Evidence-based practice: its relevance to nutritional intervention programmes. By FATEMEH RABIEE, School of Health and Policy Studies, University of Central England in Birmingham, Perry Barr, Birmingham B42 2SU

Many of the health and nutrition education initiatives in the UK are professionally-led and some of them have been criticized for giving little attention to lay people's concerns and priorities (Davison *et al.* 1991). The WHO Healthy Cities initiatives (World Health Organization, 1990) and the Health of the Nation strategy (Department of Health, 1992) strongly recommend community participation and interagency collaboration.

The present project was set up in part to respond to the request from a group of health professionals to provide a health and nutrition education programme for low income women living in an outer-city deprived area of Birmingham. The health professionals believed that due to lack of nutritional knowledge and cooking skills, women were heavily dependent on fast food and consumed very little fresh fruit and vegetables. They were also concerned about the high prevalence of smoking in the area.

It is well established that nutritional knowledge and cooking skills alone are not sufficient for nutritional behavioural change; access to healthy, cheap food is an equally important factor (Department of Health, 1996). In the absence of any evidence-based information (Katz & Peberdy, 1997) and in line with *Listening to Local Voices* (Sykes *et al.* 1993), it was decided to undertake a needs assessment exercise before starting any intervention programme.

A co-operative inquiry approach (Reason, 1992) was used and data were collected on professionals (*n* 15) and the public's (*n* 19) concerns about health, as well as dietary practices and access to healthy foods amongst the public (*n* 41). The methods of data collection were: observation, semi-structured/structured individual interviews and focus group interviews.

Data from the interviews and food-frequency questionnaires suggested that contrary to the views of the professionals, the consumption of fast food was not high amongst the public; between 12 and 22% of them consumed meat pies, burgers, fish and chips  $\geq 4$  times per week. The low consumption of fruit and vegetables (46% less than two helpings per day) and the high prevalence of smoking (68%), however, confirm the health professionals' concerns about health. Access to healthy, cheap foods was generally good, and women were managing their household budgets competently by shopping at local markets. In addition, 73% of women cooked every day and only two of these (5%) interviewed were interested in a nutritional education programme and developing their cooking skills.

In conclusion, food and nutrition was not an issue, the environment, housing, parenting and coping skills were the main health concerns for the public. The findings demonstrate the importance of evidence-based information and argue that the aims and goals which provide the framework for any public health nutrition intervention programme, should reflect not only the normative needs of the professionals, but also take into account the expressed and felt needs of the target population (Hawe *et al.* 1992). Further research is required to identify whether social class differences in the responses to health information campaigns (Turrell, 1998) are in part due to the gap between professionals' and the public's priorities about health. Findings from this study suggest the use of the co-operative inquiry approach as a model for closing the gap and working together to promote the public's health.

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**15-Hydroxy-prostaglandin dehydrogenase activity is altered in a rat model of the fetal origins of adult hypertension.** By RACHEL C. SHERMAN<sup>1</sup>, ALAN A. JACKSON<sup>1</sup>, and SIMON C. LANGLEY-EVANS<sup>2</sup>, <sup>1</sup>Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, <sup>2</sup>Department of University Medicine, Level D, South Block, Southampton General Hospital, Southampton SO16 6YD

There is now a considerable amount of evidence from epidemiological studies, which suggests that intrauterine growth retardation is associated with an increased risk of hypertension in adult life (Barker, 1994). These studies have led to the suggestion that the nutritional profile of the mother may exert powerful effects upon the metabolic functions of the fetus ("programming"). Experiments in animals support this hypothesis (Sherman & Langley-Evans, 1998). Rats exposed to a moderately low-protein diet *in utero* have significantly elevated blood pressures in later life compared with control animals (Langley-Evans *et al.* 1996). This rat model of maternal diet-induced hypertension is being used to investigate the possible mechanisms underlying the intrauterine programming of adult disease.

It has previously been demonstrated that the adult offspring of rats exposed to a maternal low-protein diet (90 g casein/kg) exhibit significantly elevated urinary prostaglandin E<sub>2</sub> excretion, compared with rats exposed to a control (180 g casein/kg) diet *in utero* (Sherman & Langley-Evans, 1996). In order to investigate whether this increased excretion is related to an impairment of the breakdown of the prostaglandins, 15-hydroxy-prostaglandin dehydrogenase (PGDH) activity was assessed in tissues from rats exposed to a control diet or maternal low-protein diet during fetal life. Pregnant Wistar rats were fed on either a 90 g casein/kg diet or a 180 g casein/kg diet throughout pregnancy. All rats were fed on the same standard 189 g protein/kg laboratory rat diet from littering. The offspring of these animals thus differed only in their prenatal dietary exposure. Blood pressure measurements were performed on these offspring at 4 weeks of age, after which time the animals were culled and tissue samples collected.

Maternal diet	PGDH activity (pmol product formed/min per mg protein)					
	Kidney		Liver		Spleen	
	Mean (n)	SEM	Mean (n)	SEM	Mean (n)	SEM
180 g casein/kg	101 (11)	2	19 (6)	2	31 (6)	1
90 g casein/kg	53*** (7)	1	25 (6)	2	37 (6)	1

Significantly different from the control group, \*\*\**P*<0.001.

Systolic blood pressures of the offspring of mothers fed on a low-protein diet during pregnancy were significantly elevated relative to the pressures of control animals (10 mmHg, *P*<0.01). Renal PGDH activity was higher than activity in spleen or liver. The renal PGDH activity was significantly lower in the low-protein exposed offspring than in control offspring (*P*<0.001), whilst activity in other organs tended (*P*<0.09) to be increased (Table).

These results indicate that the metabolism of prostaglandins is altered in rats exposed to a maternal low-protein diet during fetal life. This may, in part, explain the increased urinary excretion of prostaglandin E<sub>2</sub> observed in such animals. It is possible that synthesis of prostaglandins is also increased, as demonstrated in spontaneously hypertensive rat (SHR). Although prostaglandin E<sub>2</sub> is a vasodilator, increased concentrations are associated with an up-regulated renin-angiotensin system and elevated blood pressure. The SHR strain also exhibits increased prostaglandin E<sub>2</sub> excretion and decreased renal PGDH activity and thus has commonality with the observed effects of maternal undernutrition.

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**Impairment of fetal kidney development induced by maternal protein restriction in the rat.** By SIMON C. LANGLEY-EVANS, and SIMON JM. WELHAM, Department of University Medicine, University of Southampton, Southampton General Hospital, Tremona Road, Southampton SO16 6YD

Hypertension and CHD are programmed *in utero* by exposure of the fetus to maternal undernutrition. Low weight at birth and other indices of fetal growth retardation correlate well with adult blood pressure. These observations are reproducible when pregnant rats are fed on low-protein diets. It has been proposed that prenatal undernutrition may determine later blood pressure through impairment of renal nephrogenesis.

Fifty-four virgin female Wistar rats (200-225 g) were mated and on the day of conception were allocated to either a 180 g casein/kg (control) or a 90 g casein/kg diet (low protein). Twelve rats were fed on 180 g casein/kg throughout pregnancy. The remaining rats were fed on the low-protein diet during the periods day 0-7 (*n* 10), day 8-14 (*n* 10), day 15-22 (*n* 10) or day 0-22 (*n* 12) gestation (full term is 22 d). Whilst not consuming the low-protein diet, these rats were fed with the control diet. Five rats from each group were killed on day 20 gestation and five at full term. The fetuses and neonates from these pregnancies were killed by decapitation and the kidneys carefully removed, weighed and fixed in formalin for histological examination. All of the remaining animals were fed on the same non-purified laboratory rat diet (18.9 g protein/kg) at littering and their offspring were retained until 4 weeks old. The blood pressures of these animals were then determined using a tail cuff method. The rats were killed and kidneys removed for histological analysis.

At day 20 gestation kidney weight was significantly lower in fetuses exposed to the low-protein diet over days 0-20 than in control fetuses. Similarly at full-term gestation, neonates exposed to low-protein diets over the periods day 8-14, day 15-22 and day 0-22 had smaller kidneys than control animals. In 4-week-old rats kidney weights were similar in rats exposed to control and low-protein diets *in utero*. Blood pressures of low-protein-exposed offspring were significantly elevated relative to the pressures of control animals (control *n* 18: mean 132 (se 4) mmHg, low protein *n* 20: mean 151 (se 4) mmHg, *P*<0.001). Total nephron number was determined by sectioning and staining kidneys from fetuses, neonates and 4-week-old offspring (Table). At day 20 gestation nephron number was higher in animals exposed to low-protein diets throughout gestation, or from day 8 onwards, than in control rats. In full-term neonates, low protein exposure over the periods day 8-14 or day 15-22 significantly attenuated nephrogenesis, such that these animals were born with fewer nephrons than control rats. At 4 weeks of age, kidneys from hypertensive rats exposed to low-protein diets throughout fetal development contained significantly fewer nephrons than kidneys from control rats.

Maternal diet (g casein/kg)	Period of feeding (gestation days)	Total nephron number								
		Fetal	Neonatal	4 weeks						
		Mean	se	Mean	n	se				
180	0-22	336	16	41	1210	20	135	15514	7	818
90	0-7	288*	10	11	1221†	9	205			
90	8-14	517**	11	43	713*	17	51			
90	15-22	1433*	6	189	868*	12	104			
90	0-22	542**	12	71	1101†	14	99	13074*	8	409

Mean values were significantly different from 180g casein/kg control group. \* *P*<0.05. Mean values were significantly different from 90 g casein/kg day 15-22 group. † *P*<0.05. Mean values were significantly different from 90 g casein/kg day 8-14 group. ‡ *P*<0.05.

These data are consistent with the hypothesis that an adverse maternal nutritional environment in fetal life may impair renal development. If persistent into adult life, a deficit of nephrons may affect renal function and provide the basis for intrauterine programming of hypertension and hence cardiovascular disease.

**Body fatness and circulating triacylglycerol concentration in relation to pre- and postnatal dietary experience in the rat.** By CLAIRE L. PICKARD<sup>1</sup>, MARIA H. HURLEY<sup>1</sup> and H. DAVID MCCARTHY<sup>2</sup>, <sup>1</sup>School of Life Sciences, University of North London, London N7 8DB and <sup>2</sup>Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX

The importance of prenatal dietary experience and subsequent birth weight for risk of non-communicable diseases in adult life is gaining increasing acknowledgement (Barker, 1994). Additionally, adult diet could interact with early diet to further influence risk for adult diseases such as obesity, hypertension and non-insulin-dependent diabetes mellitus (Jackson *et al.* 1996). In this context, animal models can assist in understanding the metabolic and morphological perturbations which can result from interaction between early and adult diet and hence influence adult disease. We have previously demonstrated that hypertension induced *in utero* is critically dependent upon adult diet (Pickard & McCarthy, 1997). In the present study, we have extended our investigations into this rat model of the fetal origins of adult disease by examining the effects of pre- and postnatal diets and their interaction upon body fatness and circulating triacylglycerol (TG) concentrations.

Female rats were fed on a diet containing 180 or 90 g casein/kg for 2 weeks before mating and throughout pregnancy. Standard laboratory chow (SLC) was then fed to all rat dams throughout lactation and litters were standardized to eight per group (four male, four female). Upon weaning, offspring (*n* 8 per group) were fed on composite diets of either a high carbohydrate (HC, 62% C, 20% P, 10% F), high protein (HP, 32% C, 49% P, 10% F) or high fat (HF, 32% C, 20% P, 40% F) content or SLC. At age 12 weeks rats were fasted overnight then killed; fasting plasma TG concentration was measured enzymically and body composition chemically analysed.

Final body weight did not differ significantly between pre- and postnatal groups in females whereas in males there was a tendency for body weight to be increased as a result of HF feeding in the 180 g casein/kg group ( $P = 0.06$ ). In males, prenatal diet had no significant impact upon body fatness whereas postnatal HF feeding significantly increased percentage body fat compared with HC and HP feeding ( $P < 0.05$ ) but not compared with SLC. In females, percentage body fat was significantly higher as a result of HF feeding compared with all other postnatal dietary groups ( $P < 0.01$  v. HC and SLC;  $P < 0.02$  v. HP). In male and female offspring, prenatal diet had no significant effect upon circulating TG whereas TG concentrations were significantly different between postnatal dietary groups. In males, HF or HP feeding increased plasma TG concentrations compared with HC and SLC feeding ( $P < 0.02$ ). In females, HF or HP feeding increased TG levels compared with HC feeding ( $P < 0.03$ ) whereas there were no significant differences between HF, HP or SLC feeding.

In conclusion, these findings suggest that in this rat model, a marginal protein intake during pregnancy does not appear to have an impact on adult fatness nor affect fasting plasma TG concentrations in the offspring. Furthermore, there does not appear to be any interaction between pre- and postnatal diets upon these variables unlike upon systolic blood pressure where postnatal HF or HP feeding reverses the hypertension induced *in utero* (Pickard & McCarthy, 1997). Only postnatal diet appears to affect adult fatness and circulating TG concentration in this model. However, HF feeding does not increase body fatness to the extent where the animals can be considered to be in an 'obese-like' state. This work was funded by the British Heart Foundation.

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**Influence of pre- and postnatal diet upon skeletal muscle fibre composition in the rat.** By CLAIRE L. PICKARD<sup>1</sup> and H. DAVID MCCARTHY<sup>2</sup>, <sup>1</sup>Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX, <sup>2</sup>School of Life Sciences, University of North London, London N7 8DB

Poor growth *in utero* and early postnatally has been related to increased risk of hypertension and non-insulin-dependent diabetes mellitus (NIDDM) in later life (Barker, 1994). Furthermore, skeletal muscle fibre type appears to be altered in these disease states and in obesity (Karlsson *et al.* 1991; Abou Mrad *et al.* 1992). We have previously demonstrated that a low-protein diet fed to pregnant rat dams induces hypertension and altered insulin sensitivity in the offspring and that the hypertension is critically dependent upon the postnatal diet composition (Pickard *et al.* 1996; Pickard & McCarthy, 1997). This has led us now to examine the effect of a low-protein diet during pregnancy upon skeletal muscle fibre composition in the offspring and to examine whether postnatal diet further modifies muscle fibre type.

Thirty-two female offspring were studied from dams previously maintained on 180 or 90 g casein/kg diet for 2 weeks before and throughout pregnancy. Groups of eight rats from each prenatal dietary group were fed on one of four postnatal diets: standard laboratory chow (SLC), high carbohydrate (HC, 62% C, 20% P, 10% F), high protein (HP, 32% C, 49% P, 10% F) or high fat (HF, 32% C, 20% P, 40% F). At age 12 weeks the rats were killed and the extensor digitorum longus (EDL) and soleus (SOL) muscles were dissected, weighed and immediately frozen in liquid N<sub>2</sub>. Sections (15 µm) were cut on a cryostat and stained to demonstrate ATPase activity (Brook & Kaiser, 1970). Percentage type I and type II fibres for each muscle are shown in the Table below.

Muscle	Fibre type	EDL				SOL					
		Type I		Type II		Type I		Type II			
		Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Postnatal diet	Maternal diet										
	90 g/kg	92.9	1.1	7.1	1.1	79.7	1.6	20.3	1.6		
SLC	180 g/kg	91.9	1.5	8.1	1.5	79.5	1.1	20.5	1.1		
	90 g/kg	90.3	1.0	9.7	1.0	78.4	1.9	21.6	1.9		
HC	180 g/kg	92.6	1.0	7.4	1.0	84.4	2.7	15.6	2.7		
	90 g/kg	88.0*	1.4	12.0*	1.4	82.5	2.7	17.6	2.7		
HP	180 g/kg	93.5	1.1	6.5	1.1	80.4	3.2	19.6	3.2		
	90 g/kg	89.0*	0.8	11.1*	0.8	83.9	2.5	16.1	2.5		
HF	180 g/kg	87.9	2.4	12.1	2.4	82.4	2.7	17.6	2.7		
	90 g/kg	89.0*	0.8	11.1*	0.8	83.9	2.5	16.1	2.5		

Mean values were significantly different from 180 g/kg, \* $P < 0.05$ . Mean values were significantly different from SLC,  $P < 0.05$ .

Percentage fibre-types were unaffected by any diet in the SOL whereas in the EDL there was a significant move towards an increase in the proportion of type II fibres as a result of HP and HF feeding combined with a low-protein diet *in utero*.

These findings suggest that fibre composition can be influenced in specific skeletal muscles as a result of interaction between pre- and postnatal diet. Whether this can influence metabolism or pathology such as obesity, hypertension and NIDDM is unclear at this time but merits further investigation. This work was funded by the British Heart Foundation.

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**Early maternal dietary protein manipulation and adult offspring cholesterol metabolism.** By M. MORTAZI<sup>1</sup>, T. J. COLE<sup>2</sup> and A. LUCAS<sup>3</sup>. <sup>1</sup>Department of Nutrition and Diabetics, King's College London, London W8 7AH, <sup>2</sup>MRC Dunn Nutrition Unit, Cambridge CB4 1XJ, <sup>3</sup>Institute of Child Health, 30 Guilford Street, London WC1N 1EH

Previous animal studies have shown that manipulation of postnatal diet has an important influence on the regulation of cholesterol homeostasis later in life (Hahn, 1984; Mott *et al.* 1990). In addition we showed that plasma lipids of adult rats were programmed by a maternal low-protein diet given prenatally as well as postnatally (Lucas *et al.* 1996). To investigate the mechanism further we have studied the effect of maternal low-protein diet, introduced during gestation and/or lactation, on HMG CoA reductase (EC 1.1.1.34) (rate limiting enzyme of cholesterol synthesis) and plasma lipids in adult offspring.

Adult virgin female Wistar rats (250–270 g) were mated and, after pregnancy was confirmed, assigned to one of two synthetic diets; either a normal protein control diet containing 200 g casein/kg or an isoenergetic low-protein diet containing 80 g casein/kg. The low-protein diet was made isoenergetic by adding 130 g glucose/kg. The dams remained on the same diet throughout pregnancy and lactation. Litters were standardized to eight after 3 d postpartum, and randomized to four groups. The Control group consisted of pups from dams that received the control diet. The Low protein group consisted of pups from dams on the low-protein diet. The Postnatal low protein group were pups born to dams fed on the control diet and then suckled by dams fed on the low-protein diet. The Prenatal low-protein group were pups born to dams fed on the low protein diet and then suckled by dams fed on the control diet. All pups were weaned on day 21 to normal lab chow diet, RM1, and were killed on day 180 when blood and liver samples were collected. HMG CoA reductase was measured by a radiochemical method (Goodwin & Margolis 1976) and total and HDL-cholesterol and triacylglycerols in plasma were measured by an enzymic colorimetric test using a standard kit assay (Roche).

	Control		Low protein		Postnatal low protein		Prenatal low protein	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total cholesterol (mmol/l) (n 123)	3.24	0.28	2.29***	0.13	2.21***	0.08	2.01***	0.10
HDL-cholesterol (mmol/l) (n 117)	2.46	0.24	1.60***	0.10	1.68***	0.07	1.53***	0.07
Triacylglycerols (mmol/l) (n 124)	4.26	0.58	4.02	0.23	3.57	0.21	3.78	0.19
Total HMG CoA reductase (pmol/min per mg protein) (n 70)	786	128	224***	31	801	61	711	94
Active HMG CoA reductase (pmol/min per mg protein) (n 70)	91	15	51*	12	145	40	94	18

n, no. of pups.  
Mean values were significantly different from those for control: \*P<0.05, \*\*\*P<0.001 (ANOVA)

The Table shows that the maternal low-protein diet during gestation, lactation or both periods decreased mean total cholesterol and HDL-cholesterol concentrations in the adult offspring. In contrast total and active HMG CoA reductase levels were reduced only in adult offspring whose dams were fed on the low-protein diet during both periods.

These results further support the hypothesis that maternal protein malnutrition during gestation and lactation has a long-term effect on the offspring's cholesterol metabolism in adulthood. However we suggest that plasma cholesterol and HMG CoA reductase may in part be independently programmed during these periods.

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**Women's perceptions of diet, folic acid and pregnancy.** By MONIQUE M. RAAFS, LUCY THORPE and CAROLINE HURREN. *Health Education Authority, Trevelyan House, 30 Great Peter Street, London SW1P 2HW*

In 1995 the Department of Health commissioned a survey to assess the awareness, in the general female population (aged 16 years and over, nationally representative, random location sampling in 272 regions), of the Government's recommendations on the intake of folic acid for the prevention of first occurrence of neural tube defects (Department of Health, unpublished results). Between autumn 1995 and spring 1998 the Health Education Authority (HEA) ran the first national integrated campaign aimed at increasing the average intake of folates and folic acid in women who may become pregnant by at least 400 µg from foods containing natural folate, foods fortified with folic acid, and folic acid supplements. After the launch, the HEA commissioned three further nationally representative surveys of women aged 16–45 years in 1996 (random quota sampling in 9 regions, street interview), 1997 (random location sampling in 52 regions, interview conducted at home) and 1998 (random location sampling in 40 regions, interview conducted at home) (Health Education Authority, unpublished results). Statistical comparisons between surveys cannot be made, due to differences in sampling techniques.

Women were asked what they believed pregnant women, or those who may become pregnant, should eat or take more of; spontaneous responses were collected. The most common response was fresh or green vegetables, with the mention of folic acid showing a steady increase over the years. When asked what they believed pregnant women, or women who may become pregnant, should eat or take less of, women were most likely to mention alcohol, smoking, fatty and/or greasy foods and cream and/or soft cheese. When directly asked whether they had heard of the words 'folic acid' or 'folates' in connection with pregnancy, many more women claimed they had and this value has also shown a steady increase over the years.

Eat or take more of... (spontaneous mentions)	1995 (n 2070)				1996 (n 508)				1997 (n 617)				1998 (n 473)					
	29%	27%	26%	19%	20%	19%	23%	9%	4%	47%	43%	25%	12%	7%	6%	5%	3%	51%
Fresh/green/leafy vegetables	33%	33%	42%	28%	31%	35%	10%	27%	9%	75%	69%	34%	22%	15%	66%	53%	8%	66%
Vitamins																		
Fruit																		
Milk/milk products																		
Iron tablets/iron rich food																		
Healthy food generally																		
Folic acid																		
Fish																		
Eat or take less of... (spontaneous mentions)																		
Alcohol																		
Smoking																		
Fatty/greasy foods																		
Cream/soft cheese																		
Pate																		
Liver																		
Eggs																		
Raw/uncooked eggs																		
Prompted awareness of folic acid																		

Increases in knowledge about the importance of folic acid in relation to pregnancy are apparent. Further evidence of measurable impact of the HEA's campaign will be reflected in changes in actual behaviour with regard to folic acid intake before and during early pregnancy.



**Influence of maternal vitamin C intake on collagen concentration during fetal development in the guinea-pig.** By SHAMPA DAS and HILARY J. POWERS, *Sheffield University Division of Child Health, Sheffield Childrens Hospital, Western Bank, Sheffield S10 2TH*

Vitamin C plays an important role in collagen synthesis, in that it is essential for activity of prolyl hydroxylase (EC 1.4.1.2) which catalyses the conversion of proline residues to hydroxyproline (Hpr) in collagen polypeptides. Hpr is abundant in collagen and confers stability upon the molecule. Low vitamin C status during pregnancy can be associated with premature rupture of amniotic membranes (Casaneuva *et al.* 1993), an effect likely to be linked to collagen synthesis. Work from our group has shown that maternal vitamin C intake in guinea-pigs can influence fetal plasma and tissue vitamin C concentrations during pregnancy (Das & Powers, 1998). The aim of the present study was to determine whether this influence is also exerted over collagen synthesis in amniotic sac and fetal femur. Twenty four female time-mated Dunkin-Hartley guinea-pigs were fed *ad libitum* on a moderate (group A) or high (group B) vitamin C diet. Diet A contained 300 mg vitamin C/kg, with 100 mg vitamin C/l drinking water and diet B contained 1050 mg vitamin C/kg, with 1 g vitamin C/l water. On days 49, 63, 66 and term of gestation, three animals from each group were killed. Amniotic sacs were collected from the preterm fetuses and one femur was taken from each fetus, and stored at -20° until analysis. Tissues were hydrolysed by boiling in 6 M-HCl for 24 h and then analysed for Hpr content using an automated version of Erlich's reaction (Ho & Pang, 1989).

Results are shown in Table 1, with Hpr content being expressed both as a concentration and total tissue Hpr. Effects of diet and gestational age on Hpr in amniotic sac or femur were investigated by two-way ANOVA, followed by post-hoc analysis.

	Gestational age (d)													
	49				63				66				Term	
	Hpr/g	Hpr total	Hpr/g	Hpr total	Hpr/g	Hpr total	Hpr/g	Hpr total	Hpr/g	Hpr total	Hpr/g	Hpr total	Mean	SD
Diet A	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Femur	8.53 <sup>a</sup>	1.1	0.58	0.2	9.54 <sup>b</sup>	1.3	1.77	0.4	10.4 <sup>c</sup>	0.9	1.70	0.1	8.65 <sup>d</sup>	0.8
Amniotic sac	16	7	7	7	10	10	10	10	10	10	10	10	5	n/a
Diet B	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Femur	7.38 <sup>a</sup>	1.9	0.67	0.3	9.29 <sup>b</sup>	1.0	1.17	0.4	10.8 <sup>c</sup>	0.6	1.40	0.7	9.14 <sup>d</sup>	1.5
Amniotic sac	3.25	2.1	1.16 <sup>xy</sup>	0.7	2.87	0.6	1.54 <sup>b</sup>	0.5	1.87	0.8	1.38 <sup>b</sup>	0.3	n/a	n/a

a,b,c,x,y Mean values within a tissue category not sharing a common superscript letter were significantly different, P<0.05

Femur Hpr concentration increased progressively up to 66 d and decreased at term. Total femur Hpr showed an increase with gestational age up to term, associated with an increase in femur weight. This would suggest that between 66 d and term Hpr synthesis is not able to keep up with femur growth. There was no effect of diet on either Hpr concentration or total Hpr content. In the amniotic sacs, both groups exhibited an increase in total Hpr from 49 to 63 d, which was sustained at 66 d reflecting the pattern of weight gain of amniotic sac. At 49 d group B showed higher Hpr concentration than group A, but this was not significant. At the same time point total Hpr content was significantly higher in group B than group A, an effect which multiple regression analysis showed to be independent of tissue weight (P<0.01).

The progression of gestation generated clear tissue-specific changes in Hpr concentration. The results suggest that even a moderate vitamin C intake during pregnancy may limit collagen deposition in the amniotic sac early in gestation.

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**Dietary restraint and weight gain during pregnancy.** By RANA DRAKE<sup>1</sup>, S. REDDY<sup>2</sup> and J. DAVIES<sup>3</sup>, *Department of Epidemiology and Public Health, Imperial College School of Medicine at St Mary's, London W2 1PG, <sup>2</sup>Nutrition Unit, Department of Health, London SE1 6LW, <sup>3</sup>Nutrition Research Centre, South Bank University, London SE1 0AA*

Weight gain during pregnancy is highly variable and the magnitude of the gain has health implications for both the mother and baby. Several large studies have shown linear associations between maternal weight gain and birth weight and it appears that as pregnancy weight increases the importance of weight gain during pregnancy diminishes (Institute of Medicine, 1990). Whilst low weight gains, therefore, pose health risks for the infant, large gains may increase the risks of complications during pregnancy and the mother's risk of becoming obese in the future. Attitudes favouring slimmness have been found to be associated with lower weight gains during pregnancy in one study (Palmer *et al.* 1985) and another study found that some women with a history of dieting were less concerned about their weight during pregnancy resulting in excessive weight gains (Fairburn & Welch, 1990).

In the present study sixty three Caucasian women expecting their first or second baby were recruited from a London hospital. In early pregnancy (approximately 12 weeks gestation) participants reported their prepregnancy weight and completed the Herman & Polivy (1980) dietary restraint questionnaire and questions about past weight-control practices. Later (approximately 30 weeks gestation) they completed a pregnancy and weight gain attitude questionnaire (Palmer *et al.* 1985) and after delivery they reported their last recorded weight before the birth. Participants were divided into two groups according to their level of dietary restraint, those with scores above the median were classified as restrained eaters and those below the median as unrestrained eaters. The restrained eaters had a higher mean prepregnancy BMI (22.0 v 20.7, P=0.033), reported having used a greater number of methods to control their weight in the past (4 v 2, P<0.001) and had lower scores on the pregnancy and weight gain attitude score (3.0 v 3.5, P=0.004) indicating more negative attitudes to weight gain during pregnancy. In addition restrained and unrestrained eaters were found to experience different patterns of weight gain during pregnancy.

Weight gain compared with recommended range*	Restrained eaters (n 27)		Unrestrained eaters (n 23)		P value
	%	n	%	n	
Below	22	6	4	1	0.09
Within	30	8	61	14	0.02
Above	48	13	35	8	0.02

\*Institute of Medicine (1990).

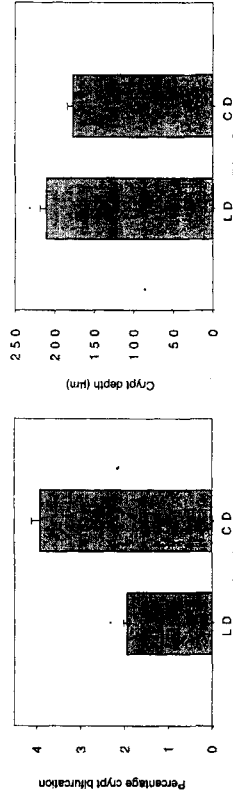
The results suggest that dietary restraint is associated with negative attitudes to weight gain during pregnancy and with weight gains either below or above recommended ranges according to prepregnancy BMI. This could have long-term health implications for both mother and infant and therefore warrants further attention.

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**Luminal riboflavin is essential for normal morphological development of the gastrointestinal tract.** By CATHERINE A. YATES and HILARY J. POWERS. *Division of Child Health, University of Sheffield, Sheffield S10 2TH*

The absence of luminal nutrition during the critical weaning period has been shown to alter development of the gastrointestinal tract. This occurs despite the maintenance of adequate nutrition by intravenous means (Castillo *et al.* 1990). We have previously found that feeding a riboflavin-deficient diet from weaning results in a rapid alteration in the morphological development of the intestine. The present study set out to determine whether the presence of riboflavin in the lumen is essential for appropriate development of the tract or if the maintenance of systemic flavin levels will allow development to proceed normally.

Sixteen weaning female Wistar rats were weight-matched and allocated to one of two groups to receive a riboflavin-deficient diet (LD) or a control diet (CD) *ad libitum*. To maintain systemic flavin levels each animal allocated to the deficient diet received an intraperitoneal injection of FMN (50 µl of an 8 mg/ml solution) daily. At the same time animals in the control group received 50 µl isotonic saline. After 141 h on the diet all animals were killed. At kill, blood was removed by cardiac puncture for the measurement of riboflavin status as erythrocyte glutathione reductase activation coefficient (EGRAC) and the liver was removed for flavin analysis. The intestine was removed and a 10 mm segment was cut from the proximal end. This was fixed in 40 ml/l formaldehyde before processing for paraffin wax embedding. Transverse sections 5 µm thick were cut and stained using haematoxylin and eosin. Crypt depth was measured using an image analysis system. Percentage bifurcation was counted using a light microscope. Measurements were made in four sections from each animal.



\* Significantly different from control, P < 0.01.

There was no difference in EGRAC values or liver FAD levels between the lumenally deficient diet group and the control diet group (1.40 (sem 0.028) v. 1.41 (sem 0.064) and 23.7 (sem 0.59) v. 23.6 (sem 0.68) respectively). These data indicate that the flavin injection regimen was sufficient to maintain adequate riboflavin status. Despite this we found a failure to increase percentage crypt bifurcation in the lumenally deficient animals relative to the controls (Fig. 1) (Mann Whitney U, P=0.008). This failure was accompanied by crypt hypertrophy (Fig. 2) (Mann Whitney U, P=0.01). These data strongly suggest a mechanism for recognizing the presence of riboflavin in the lumen which permits a programme of normal gastrointestinal development.

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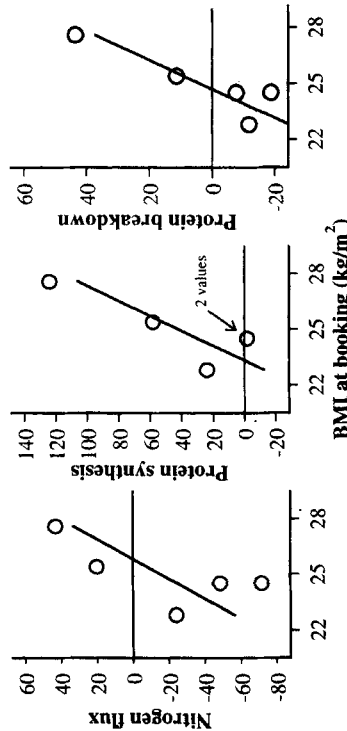
**Whole-body protein turnover in pregnant English women of different body mass index.** By S.L. DUGGLEBY and A.A. JACKSON. *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

During pregnancy, there are both increased demands for protein and a change in pattern of amino acids required by the fetus. There is little evidence to suggest that maternal dietary intake of protein is increased during pregnancy. Therefore, alterations to amino acid metabolism by the mother might be one way in which these increased demands are met. The extent to which this is achieved might be influenced by maternal body composition. Body composition influences protein metabolism in non-pregnant individuals: obese individuals obtain less of their energy from protein breakdown during starvation than thin individuals (Henry, 1992).

Five pregnant women were recruited from antenatal clinics when they attended the midwives' booking clinics at 12-16 weeks. They took part in studies in mid (17-20 weeks) and late (30-32 weeks) pregnancy. Whole-body protein turnover was measured by the end-product method using [<sup>15</sup>N]glycine. A single oral dose of 125 mg [<sup>15</sup>N]glycine was administered between 08.00 and 09.30 hours. Urine was collected for the following 24 h (Grove & Jackson, 1995). <sup>15</sup>N enrichment in urinary urea and NH<sub>3</sub> was measured by mass spectrometry. Rates of turnover were calculated from the arithmetic average of the two end-products (Fern *et al.* 1985).

**Change in protein turnover from mid to late pregnancy (mg N/kg per d)**

$r = 0.69, P = 0.20$        $r = 0.82, P = 0.09$        $r = 0.89, P = 0.04$



As BMI increased, there was a tendency for the change in rates of protein turnover to become more positive. Rates of protein synthesis and breakdown tended to increase in women whose BMI was greater than the median of 25 kg/m<sup>2</sup>. The relation of BMI with the change in protein breakdown reached statistical significance. These trends were similar when rates of protein metabolism were expressed in absolute terms (g N/d).

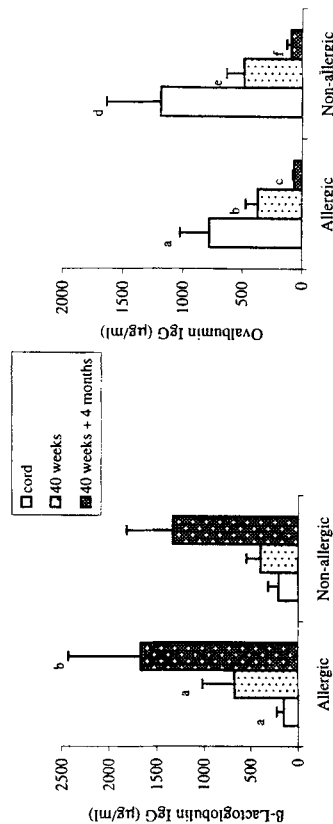
Changes in protein turnover during pregnancy differ between women of different BMI. These data suggest that women of different BMI vary in their metabolic response to pregnancy, which may influence their ability to meet increased demands for protein and amino acids through amino acid delivery to the fetus. This may affect fetal growth and development and the individual's capacity to function later in life.

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**Food specific immunoglobulin (IgG) levels and allergic outcome in preterm infants.** By F.J. NORRIS<sup>1</sup>, M. LARKIN<sup>1,2</sup>, C.M. WILLIAMS<sup>1,2</sup>, S. DEACOCK<sup>3</sup>, J.B. MORGAN<sup>1</sup> and S.M. HAMPTON<sup>1</sup>, <sup>1</sup>School of Biological Sciences, University of Surrey, Guildford, GU2 5XH, Departments of <sup>2</sup>Midwifery, and <sup>3</sup>Immunology, Royal Surrey County Hospital, Guildford, GU1 1AA

The aim of the present study was to investigate specific ovalbumin IgG and β-lactoglobulin IgG levels in the cord blood and serum of preterm infants with and without a subsequent clinically diagnosed allergic response.

Blood samples were obtained from the cord, at 40 weeks gestational age and at 4 months corrected age (40 weeks + 4 months gestational age) from a group of preterm infants from both atopic and non-atopic backgrounds. Samples were analysed for both ovalbumin IgG and β-lactoglobulin IgG by in-house indirect enzyme-linked immunosorbent assays ELISA. A paediatrician assessed the infants at 8 months corrected age for signs of allergy by physical examination and parental questionnaire. Infants were subsequently assigned to allergic (n 10) and non-allergic (n 14) groups. The IgG results are shown in the Figure (mean and SE). Comparisons between groups were made using the non-parametric Mann-Whitney test, and within groups were made using the Wilcoxon signed ranks test.



In each group, mean values not sharing a common letter were significantly different, <sup>a,b,c</sup>P < 0.05; <sup>d,e,f</sup>P < 0.01.

Mean β-lactoglobulin IgG levels rose over time in both groups. The rise was statistically significant in the allergic group. All infants were fed on a combination of breast and formula milk, so the rise in β-lactoglobulin IgG levels may reflect the use of formula feeds which have been shown to be more allergenic than breast milk. Mean ovalbumin IgG levels decreased significantly over time in both groups; the high cord results reflect maternal IgG and the decrease seen indicates the degradation of these and normal weaning practices which do not encourage the introduction of egg into the diet until 6 months of age. There were no statistically significant differences between allergic and non-allergic groups for either mean β-lactoglobulin or ovalbumin IgG levels suggesting that at least until 4 months corrected age, these antibodies cannot predict subsequent allergic outcome in this sample of preterm infants. Further analysis of blood samples at 8 months corrected age and beyond is necessary to elucidate the role of food specific IgG antibodies in the development of allergic disease.

This research was funded by the MAFF Food Intolerance Programme.

**Use of red palm oil for promotion of maternal vitamin A status.** By G. Lietz<sup>1</sup>, G. Mulokozi<sup>2</sup>, C.J.K. Henry<sup>1</sup> and A. Tomkins<sup>3</sup>, <sup>1</sup>School of Biological and Molecular Sciences, Oxford Brookes University, Oxford OX3 0BP, <sup>2</sup>Tanzania Food and Nutrition Centre, PO Box 977, Dar es Salaam, Tanzania, <sup>3</sup>Centre for International Child Health, 30 Guildford Street, London WC1N 1EH

The vitamin A status of an infant is largely dependent on the retinol concentration of breast milk. High dose supplementation of vitamin A soon after delivery has been used as an intervention strategy (Stoltzfus *et al.* 1993). This may not be practical and sustainable in rural situations where home deliveries occur. Furthermore, this intervention concentrates on lactation rather than pregnancy. The teratology of retinol preclude its use during pregnancy. The present study aimed to use a food-based intervention to improve maternal vitamin A status. Ninety women 2 - 3 months before parturition were recruited and divided equally into three study groups. A red palm oil group was provided with 20 ml oil/woman and day, a sunflower oil group was provided with 20 ml oil/woman and day, and a control group was provided an incentive of 4 kg rice/family and month. The feeding trial was conducted in Singida rural district, Tanzania and lasted for 6 months. All groups were advised to consume pro-vitamin A - containing fruits and sun dried vegetables traditionally produced in the region. Concentrations of two carotenoids, retinol and α tocopherol in breastmilk 1 month after parturition, are presented in the Table.

	Red palm oil		Sunflower oil		Control	
	Mean	SE	Mean	SE	Mean	SE
α-carotene (µmol/l)	0.06 **	0.006	0.01	0.001	0.01	0.002
β-carotene (µmol/l)	0.11***	0.010	0.05 *	0.005	0.03	0.003
Retinol (µmol/l)	1.45	0.081	1.68	0.119	1.52	0.105
α-tocopherol (µmol/l)	7.00	0.427	9.71 **	0.588	7.40	0.413

Mean values were significantly different from control: \* p<0.05, \*\* p<0.01, \*\*\* p<0.001 (Student's unpaired t-test)

The preliminary results of this study suggest that supplementing pregnant women with pro-vitamin A - rich oil significantly increases the α- and β-carotene concentrations in breastmilk 1 month after delivery. However, it showed no impact on retinol concentration when expressed as µmol/l. Increasing the fat intake during pregnancy and lactation via dietary sunflower oil (containing no carotenoids) increased the concentration of β-carotene and α-tocopherol. Even if carotenoid absorption could be enhanced through dietary promotion of oil alone, this may not be sufficient to increase vitamin A status in pregnancy and lactation especially when other dietary sources of pro-vitamin A - containing foods are not readily available. This was the case for the present study groups as the intervention was carried out during the dry season. Since the pro-vitamin A concentration of breastmilk was increased in both the red palm oil and sunflower oil group, further research needs to be undertaken to examine possible effects on the immune response in lactating mothers and their babies, as well as its impact on the infants vitamin A status.

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**Assessment of dietary intake of vitamin A and iron by lactating Indian women.** By F.S.W. McCULLOUGH<sup>1</sup>, C.A. NORTHROP-CLEWES<sup>1</sup>, B.S. DAS<sup>2</sup> and D.I. THURNHAM<sup>1</sup>. <sup>1</sup>University of Ulster, Coleraine, BT 52 5SA. <sup>2</sup>Spat General Hospital, Rourkela, India

The National Nutrition Policy adopted by the Government in India in 1993 includes control of micronutrient deficiencies through intensified programmes. The present study was carried out in a slum housing area on the outskirts of Rourkela, presently not covered by any such programme. Sub-clinical vitamin A deficiency, anaemia and infection are major problems and low retinol concentrations have previously been reported from infants in this area (Das *et al.* 1996). The National Institute of India estimates that Indian diets should contain at least 20 mg Fe and 750 µg (2500 IU) Vitamin A/d to meet adult requirements. Natural food sources of pro-vitamin A from green, yellow and orange edible plants have been proposed as a sustainable public health solution to prevent vitamin A deficiency. The objective was to determine whether Indian women can meet dietary requirements of Fe and vitamin A from their normal food intake.

Forty housewives were recruited during March and April 1997 and baseline dietary information was recorded using a diet history, complemented with a food-frequency questionnaire. In addition anthropometrical, socioeconomic data and a 2 ml venous blood sample for measurement of haemoglobin using the portable Hemocue (Angelholm), ferritin by ELISA, and vitamin A and carotenoid concentrations by HPLC (Thurnham *et al.* 1988).

Table 1. Socio-economic, dietary and biochemical factors monitored in Indian women

Social & Dietary	Mean	Median	Range	Biochemistry	Mean	Median	Range
age (years)	20.3	20	16-38	retinol (µmol/l)	0.92	0.96	0.34-2.33
BMI (kg/m <sup>2</sup> )	19.5	20	17.3-21.9	β-carotene (µmol/l)	0.12	0.08	0.00-0.50
parity (number)	1.6	2	1-6	lutein (µmol/l)	0.52	0.45	0.19-0.99
Fe intake (mg/d)	6.21	6.2	2.7-8.7	Hb (g/l)	106.6	108.0	68.0-138.0
vitamin A (R.E/d)	776	699	127-2803	ferritin (mg/l)	3.14	3.46	2.13-4.48

The subject's daily dietary intake was calculated from the Nutritive Value of Indian Foods. Fat comprised 19.3% of the mean daily energy intake of 6.37 MJ. Haem iron is prohibitively expensive, therefore dietary Fe was provided almost exclusively from non-haem sources such as vegetables. The inadequate daily dietary Fe intake was reflected in the low Hb and ferritin results, suggesting that this population have inadequate iron status. 78% of the mothers being classified as anaemic (Hb < 120g/L). Hb correlated with ferritin (r = -0.71), and with BMI (r = 0.82), ferritin correlated with retinol (r = 0.41), all P < 0.05. The β-carotene intake shown in the Table suggests it is possible to meet daily dietary requirements for vitamin A as carotenoids from fruit and vegetable sources. However, the commencement of this study coincided with an abundance of dark green leafy vegetables being available in the market at very cheap prices e.g. 1 kg spinach cost 8p. Similarly, vitamin A intake was reported in poor Guatemalan homes to be 745 (SD 727) RE/d by Bulux *et al.* (1997). Using the serum retinol cut-off value of 0.70 µmol/L, 71% of these subjects had normal status at the time of investigation, the remainder being classified as sub-clinically deficient, with one retinol value of 0.34 µmol/L. During other months of the year few β-carotene-rich foods would be available, therefore dietary intake and serum retinol concentration measurements should ideally be compared during each season.

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**The effect of breast-feeding on vitamin A status of infants of human immunodeficiency virus positive (HIV+) women with or without vitamin A supplementation.** By: ANNE S.W. MBURU<sup>1</sup>, ANNA COUTSOUDES<sup>1</sup>, KUBEN PILLAY<sup>2</sup>, CHRISTINE A. NORTHROP-CLEWES<sup>1</sup> and DAVID I. THURNHAM<sup>1</sup>. <sup>1</sup> Northern Ireland Centre for Diet and Health (NICHE), University of Ulster at Coleraine, Cromore Road, Coleraine, BT52 5SA. <sup>2</sup> Department of Pediatrics and Child Health, University of Natal, Durban, South Africa.

In adequate maternal nutritional status, breast-milk provides essential nutrients and immunoprotective proteins for the infant in its first 6 months of life. However, in severe nutritional deficiency, breast-milk vitamin A levels remain low leaving the infant's nutritional requirements unmet (Stoltzfus & Underwood, 1995) and the infant at increased risk of vitamin A deficiency because of marginal vitamin A stores at birth. Vitamin A is recognized as an anti-infective agent with a role in reducing morbidity and mortality. However, plasma retinol decreases in the presence of infection. Low levels of circulatory vitamin A have been associated with a higher risk of HIV vertical transmission and higher breast-milk HIV viral load during pregnancy and lactation respectively (Nduati *et al.* 1995; Semba *et al.* 1995). As part of a vitamin A supplementation trial, carried out in Durban, South Africa, a sub-sample of study infants was followed-up to assess the association between reported breast-feeding and infant vitamin A status in HIV disease. The main trial was a randomized double-blinded placebo-controlled intervention looking into the effects of vitamin A supplementation on HIV vertical transmission. Ethical permission was obtained from the University of Natal and South African Medical Research Council's ethical committees. Consenting and counselled HIV + gravidae attending antenatal clinic at King Edward VIII Hospital, in Durban, South Africa were recruited onto the trial and provided with either a supplement capsule (5000 IU retinyl palmitate and 30 mg β-carotene) or placebo capsule, to be taken daily for a period of twelve weeks or up to delivery. Post-delivery, a one-off vitamin A megadose (200,000 IU retinyl palmitate) or placebo (40 IU vitamin E) was given. The code was broken using maternal post-supplementation β-carotene results; where a change in maternal plasma β-carotene concentration of greater than 0.5 µmol/l between baseline and delivery, was identified as having received supplement; group 1 denotes the identified placebo group and group 2 the supplement group. Preliminary findings for mean plasma retinol concentrations (µmol/l) with standard deviation and number per group are shown in the Table.

	Group 1 (Placebo)				Group 2 (Supplement)						
	Mean	SD	n	%	Mean	SD	n	%			
delivery	0.69	0.37	23	0.50	0.62	0.32	13	0.72	0.28	8	
1 week	0.87	0.57	20	0.77	0.28	1.03*	0.36	10	0.73	0.34	5
1 month	1.04	0.47	14	0.94	0.36	0.80	0.37	13	1.16	0.59	6
3 months	1.16	0.54	26	1.05	0.49	1.08	0.35	12	1.20	0.52	11
6 months	1.19	0.51	14	1.16	0.27	1.10	0.40	9	1.00	0.44	3

The mean plasma retinol concentration in the breast-feeding infants whose mothers received supplement was significantly different from corresponding placebo group at 1 week, \*P < 0.025. Vitamin A supplementation had no effect on plasma retinol at birth or at subsequent times of analysis up to 6 months. However, the greatest increase in plasma retinol was seen in the supplemented breast-fed group at 1 week (P < 0.025) indicating that breast-feeding as part of an antenatal maternal vitamin A supplementation intervention is an important dietary source of vitamin A for neonates within the first week of life. Additionally, there were fewer low retinol values (< 0.70 µmol/l) in the supplemented breast-fed group at all six time points than the comparable placebo group. However, this did not reach significance (P > 0.05). The possible benefits of vitamin A supplementation, shown by the somewhat lower proportion of low vitamin A results among the breast-fed infants whose mothers received supplements, may be confirmed once the study is completed.

Acknowledgment:- The second year of this research was supported by an International Training Fellowship, provided by the Nutricia Research Foundation (T3-1997).

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**Effects of vitamin A deficiency during pregnancy on reproductive outcome and plasma vitamin E and leptin concentrations in the rat.** By C. ANTIPATIS, G. GRANT, N. HOGGARD and C. J. ASHWORTH. *Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB*

Vitamin A is important for cell differentiation and fetal development. We have demonstrated dose- and duration-dependent reductions in fetal number and weight in vitamin A deficient mothers. In addition deficiency delays parturition and reduces neonatal survival. Dietary retinol supplementation in deficient mothers partially counteracted the effects on fetal weight (Antipatis *et al.* 1998). The effects of vitamin A deficiency during pregnancy on other fat-soluble vitamins and on factors mediating nutritional effects on reproduction, such as leptin, have not been determined. The aim of the present study was to investigate the effects of vitamin A deficiency during pregnancy on reproductive outcome and maternal plasma vitamin E and leptin concentrations and to determine whether vitamin A supplementation during pregnancy can reverse any changes in these variables.

Immediately after weaning, female rats received *ad libitum*, either a vitamin A free (VAF) diet, a vitamin A marginal (VAM) diet containing 0.45 mg retinol acetate/kg diet or a vitamin A sufficient (VAS) diet containing 7 mg retinol acetate/kg diet. All rats were mated after 8 weeks. Groups of rats were fed on the following dietary regimens:

- VAF diet for 8 weeks and during pregnancy.
- VAF diet for 8 weeks and up to day 7 of pregnancy and then VAS diet.
- VAM-F diet for 5 weeks and then VAF diet for 3 weeks and during pregnancy.
- VAM-F-S diet for 5 weeks, then VAF diet for 3 weeks and up to day 7 of pregnancy and then VAS diet.

(e) VAS diet for 8 weeks and during pregnancy.

Maternal weights were recorded twice weekly before mating and every day throughout pregnancy. Six animals from each group were killed on day 20 of pregnancy, whereas the rest were killed within 6 h of birth.

Fourteen out of twenty four rats fed on the VAF and VAF-S diet were pregnant (58.3%) compared with thirty five out of forty two (83.3%) from the VAM-F, VAM-F-S and VAS groups. Subsequent data relate to pregnant animals. At 7 weeks post-weaning there were no significant differences in rat weights amongst the groups, however, at mating dams fed on the VAF and VAM-F diets were lighter (22.9 (SE 2.5) v. 24.7.3 (SE 2.3) g;  $P < 0.001$ ) and gained less weight in the first week of pregnancy (22.2 (SE 1.1) v. 31.3 (SE 1.3) g;  $P < 0.001$ ) compared with dams fed on the VAS diet. Dams in the VAF and VAM-F groups gained less weight during the second (20.5 (SE 1.9) v. 29.1 (SE 1.6) g;  $P < 0.01$ ) and third (20.5 SE 2.9 v. 31.9 SE 2.4 g;  $P < 0.01$ ) weeks of pregnancy compared with the VAF-S, VAM-F-S and VAS groups. In VAF and VAM-F dams neonatal survival was lower (0.098 (SE 0.067) v. 0.788 (SE 0.092)) compared with dams fed on the VAF-S, VAM-F-S and VAS diets. Dams fed on the VAF-S and VAM-F-S diets had higher plasma leptin concentrations on day 20 of pregnancy (16.96 (SE 2.74) v. 4.13 (SE 1.39) ng/ml;  $P < 0.01$ ) and at birth (8.56 SE 2.00 v. 1.88 SE 0.33 ng/ml;  $P < 0.01$ ) compared with dams fed on the VAF, VAM-F and VAS diets. Day of pregnancy did not affect maternal plasma retinol,  $\alpha$ -tocopherol or  $\gamma$ -tocopherol concentrations. Therefore data from both time points were combined. In the VAF and VAM-F groups maternal plasma retinol,  $\alpha$ -tocopherol or  $\gamma$ -tocopherol concentrations were lower (0.030 (SE 0.014) v. 0.199 (SE 0.019)  $\mu$ g/ml;  $P < 0.001$ , 0.226 (SE 0.037) v. 0.661 (SE 0.088)  $\mu$ g/ml;  $P < 0.001$ , 5.481 (SE 0.471) v. 7.507 (SE 0.710)  $\mu$ g/ml;  $P < 0.05$  respectively) compared with the VAF-S, VAM-F-S and VAS groups.

These data suggest that pregnancy rate is dependent on the severity of vitamin A deficiency. Maternal weight and plasma tocopherol concentrations are reduced because of vitamin A deficiency but not maternal plasma leptin concentrations. Supplementation of vitamin A reverses the effects of deficiency on maternal weight, neonatal survival and plasma tocopherol concentrations. More interestingly, maternal plasma leptin concentrations are dramatically increased by supplementation. This result may be explained by the increased maternal weight and therefore body fat which in turn stimulates leptin secretion.

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**Assessment of goitre prevalence and nutritional status of schoolchildren in the mountainous region of Surakarta, Indonesia.** By PAM KAULDHAR<sup>1</sup>, JANE EARLAND<sup>1</sup>, RICAHRD TOVEE<sup>1</sup>, DR BAMBANG<sup>2</sup> & DR SENGENG<sup>2</sup>. <sup>1</sup>Centre For Human Nutrition, University of Sheffield, Herring Road, Sheffield S5 7AU, <sup>2</sup>Department of Nutrition, Faculty of Medicine, Sebelas Maret University, Jl. Ir 364 Sutarji, Indonesia

Iodine deficiency disorders (IDD) are an important worldwide nutritional problem and are a major impediment to human development (Administrative Committee on Co-ordination/ Sub-Committee on Nutrition, 1993). In Indonesia alone, approximately 9 million people suffer from goitre, despite a national iodization programme aimed at the eradication of IDD. In 1995, unpublished data revealed that more than 10% of the 6-12-year-old children had goitre and the study area was defined as endemic (Dr Tony, Personal communication, 1995). Although the 6-12-year-olds are not seen as a vulnerable group with respect to ill health, IDD nevertheless have specific effects including retardation of growth, leading to stunting and poor development of the child (ACC/SCN, 1993). Therefore the present study was carried out in order to assess both the current goitre prevalence and the nutritional status of the children.

In order to assess the scale of goitre prevalence in the area, all 6-12-year-old schoolchildren, who attended school in the village of Girimulyo, were examined by a trained midwife using the classification of goitre size adopted by WHO (ACC/SCN, 1993). One school was then randomly selected to participate in a case-control study. From a total of 196 children attending this school, sixty-two children were found to have goitre, of which every other child was selected from a register list. Subjects were matched with non-goitred children for sex, age and hamlet residence. Height and weight measurements were taken in accordance with the WHO guidelines (World Health Organization, 1983)

	Stunted ( $< 90$ H/A)	Wasted ( $< 80$ W/H)
Goitre	56%	6%
Non-goitre	39%	19%
Total	47% (n 34)	12% (n 8)

A, age; H, height; W, weight.

Results revealed a 29% goitre prevalence rate. According to the classification of goitre severity, this indicated that the 6-12-year-old children living in Girimulyo were suffering from moderate IDD. Results from the school under study showed a higher goitre prevalence rate of 32% indicating severe IDD which, at this level, have a profound impact on development. Assessment of nutritional status indicated that significantly more of the cases were stunted ( $P < 0.05$ ) and significantly more of the controls were wasted ( $P < 0.05$ ). These results appear to be consistent with other studies linking IDD to retarded linear growth. Further measures need to be implemented to eradicate IDD and improve the quality of life for millions of Indonesians.

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**Reproducibility of measurement of resting metabolic rate in boys and girls aged 6-11 years.** By JONATHAN C. VENTHAM and JOHN J. REILLY, *Department of Human Nutrition, University of Glasgow, Yorkhill Hospitals, Glasgow G12 8SJ*

A reduced resting metabolic rate (RMR) has been shown to be a predisposing risk factor for obesity (Ravussin *et al.* 1988). Various protocols are used for the measurement of RMR in children (Pencharz & Azcue, 1995). The aim of the present study was to determine the reproducibility of measurement of RMR in prepubertal boys and girls using a standard protocol employed by our department. We studied eighteen healthy children (nine boys, nine girls) aged 6.4-11.5 years. Subjects travelled to school by car and were measured in the school medical room. RMR was measured in a thermoneutral environment (22-24°C) with subjects supine at about 08.30 hours after an overnight fast (10-12 h) on three separate occasions over a 7-10 d period, using a Deltatrac metabolic monitor. On each occasion RMR was measured for 12-15 min once a steady state had been reached (about 5 min). Body composition was measured using bioimpedance (Reilly *et al.* 1996).

Physical characteristics of the eighteen children were: weight 33.1 (SD 9.0) kg, BMI 17.8 (SD 2.8) kg/m<sup>2</sup>, fat-free mass (FFM) 25.8 (SD 5.8) kg and percentage body fat 21.0 (SD 6.6) %.

Mean RMR for visits 1, 2 and 3 were 5359 (SD 732) kJ/d, 5267 (SD 736) kJ/d and 5334 (SD 727) kJ/d, respectively. There were no significant differences between RMR for visits 1, 2 and 3 (paired *t* tests *P*<0.05). The mean CV for the intraindividual RMR was 2.6 (SD 1.7) %. A repeated measures analysis of variance (ANOVA) showed no significant visit effect on the measurement of RMR for the group (*P*<0.01). The repeated measures ANOVA also showed that there was no significant visit effect on the measurement of RMR between different age groups and between boys and girls (*P*<0.01). From the repeated measures ANOVA, a reproducibility index was calculated as the variance between children divided by the variance between children plus the variance within a child expressed as a percentage, and showed good reproducibility for the measurement of RMR with a value of 95.0 % (Dunn, 1989).

The results indicate that reproducible measurement of RMR is possible using a short and simple protocol, with a measurement period of 12-15 min once a steady state has been achieved. The procedure is more acceptable to young children than classical protocols for measurements of RMR or BMR.

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**Whole-body plethysmography compared with hydrodensitometry for body composition analysis in children aged 8-12 years.** By ODILE DEWIT<sup>1</sup>, N.J. FULLER<sup>1</sup>, M. ELIA<sup>1</sup> and J.C.K. WELLS<sup>2</sup>, <sup>1</sup>MRC Dunn Clinical Nutrition Centre, Cambridge CB2 2DH, and <sup>2</sup>Childhood Nutrition Research Centre, Institute of Child Health, London WC1N 1EH

Although hydrodensitometry is a widely accepted reference method for measuring body volume, its use is limited because subjects may not tolerate full submersion under water. Recent developments in air-displacement whole-body plethysmography (McCrorry *et al.* 1995) offer an alternative way of measuring body volume, but the technique has not been validated in children. The aims of the present study were (1) to assess acceptability and feasibility of whole-body plethysmography in children 8-12 years old, and (2) to assess its agreement with hydrodensitometry. Eight girls and ten boys, 8.1-12.9 (median 9.6) years old, with a BMI ranging from 14.3 to 22.3 (mean 17.0) kg/m<sup>2</sup>, underwent two consecutive measurements of both plethysmography and hydrodensitometry on the same day. The same trained investigator performed all the hydrodensitometry procedures which included measurement of lung volume by He dilution. Whole-body plethysmography was performed using the BOD POD<sup>®</sup> Body Composition System (Life Measurement Instruments, USA). For the measurements, each child was sitting still in a fibreglass chamber, breathing normally. Each measurement lasted 50 s, but if the results of the two measurements differed by more than 0.15 litres, an additional one was performed, according to the software settings. Body volume was derived from the pressure changes in the chamber in the presence of the child, with corrections for predicted lung volume, body surface area, and for the isothermal conditions of lung air. Density of fat-free mass was 1.0864 kg/l and body fat (%) was calculated as (527/body density)-485 (JCK Wells, O Dewit, M Fawcett, M Elia and NJ Fuller, unpublished results; Brozek *et al.*, 1963), with body density in kg/l. Agreement between the two methods was assessed by bias (hydrodensitometry minus plethysmography) and 95% limits of agreement (bias ± 2 standard deviations of bias). The precision of the methods was derived from individual duplicate measurements and used to calculate the error due to methodology.

Plethysmography was well accepted by all children. The precision of body volume measurements by hydrodensitometry and plethysmography were 0.17 and 0.10 litre respectively (1 SD). Methodology accounted for a little more than half (SD 0.197 litres) of the difference between methods observed in these children (SD 0.37 litres, see table).

	Body Volume (litres)		Body density (kg/l)		Body fat (%)		Fat mass (kg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Hydrodensitometry	32.14	7.60	1.043	0.018	20.5	9.0	7.2	4.5
Plethysmography	32.19	7.58	1.041	0.016	21.3	7.7	7.5	4.1
Bias	-0.05	0.37	0.002	0.012	-0.8	5.9	-0.3	1.9
95% limits of agreement	-0.79 to 0.69		-0.022 to 0.026		-12.6 to 11.0		-4.1 to 3.5	

The study shows that (1) whole-body plethysmography using the BOD POD<sup>®</sup> Body Composition System is well accepted and easily performed in 8-12-year-old-children; (2) it may be used interchangeably with hydrodensitometry to compare mean results obtained in groups of normal children. It should not be used interchangeably to compare results obtained in individual children because of substantial individual differences between methods (95% limits of agreement), which are accounted for by methodological and biological factors.

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**The effects of rehydration beverage sodium concentration on restoration of fluid balance and plasma volume.** By C. VINCENT<sup>1</sup>, S. A. WOOTTON<sup>1</sup>, J. L. J. BILZON<sup>2</sup>, and A. J. ALLSOPP<sup>2</sup>, <sup>1</sup>Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD and <sup>2</sup>Institute of Naval Medicine, Gosport PO12 2DL

Whilst the addition of Na to rehydration beverages is believed to promote rehydration following strenuous exercise (Maughan & Leiper, 1995), the optimal Na concentration and effect on subsequent exercise performance remain unclear. The aim of the present study was to compare the effects of altering the Na concentration of rehydration beverages on changes in fluid balance and plasma volume during recovery following prolonged exercise in the heat.

Fifteen male subjects (mean age 33 years; weight 74.2 kg;  $\dot{V}O_2$  max 4.4 litres/min) participated in a within-subject double-blind trial of three randomised rehydration beverage conditions each containing 70g carbohydrate/l, Cl 15 mmol and K 5 mmol; high Na 60 mM (60Na), moderate Na 30 mM (30Na) or control with no Na (0Na). In each trial, the subject ran at 60%  $\dot{V}O_2$  max (room temperature of 35° and 40% relative humidity) for 90 min or until volitional fatigue or aural temperature reached 39°. During the 4 h recovery period the subjects drank a volume of the test beverage at a controlled rate of 1 litre/h, calculated to replace 125% of the loss in body mass during the run. Urine was collected throughout and the volume measured in order to calculate the percentage of ingested fluid which was retained. Changes in plasma volume (Apl Vol%) relative to a pre-exercise baseline were calculated from changes in the packed cell volume and haemoglobin concentration of venous blood sampled at the start (0 h) midpoint (2 h) and end (4 h) of the recovery period (Dill & Costill, 1974).

	0Na		30Na		60Na	
	Mean	SD	Mean	SD	Mean	SD
$\Delta$ Body Mass (kg)	2.20	0.12	2.08	0.15	2.21	0.11
Fluid intake (ml)	2740	577	2609	723	2751	527
Urine volume (ml)	945	390	920	292	617*	285
Fluid retained (%)	64	20	62	16	77*	12
Apl Vol% 0h	-7.7	6.7	-8.5	8.9	-8.9	8.6
Apl Vol% 2h	-0.8	5.1	1.7	3.7	4.5†	7.9
Apl Vol% 4h	-0.2	6.0	-0.5	5.0	5.9†§	8.4

Significantly different from 0Na: \*  $P < 0.01$ ; †  $P < 0.05$ . Significantly different from 30Na: ‡  $P < 0.01$ ; §  $P < 0.05$  (ANOVA)

No differences between the three conditions were observed in either  $\Delta$ body mass over the run or fluid intake during recovery. Urine output in the 60Na condition was attenuated compared with the other two conditions, leading to a greater fluid retention. Plasma volume was reduced at the end of the run and although re-established over the recovery period in all three conditions, the most rapid and largest changes were observed in the 60Na condition where the subjects were hypovolaemic at the end of the 4 h period. These results further demonstrate the importance of Na concentration of rehydration beverages on fluid balance and plasma volume following dehydrating exercise in the heat.

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**Influence of an electrolyte beverage on short-term rehydration following a prolonged field exercise.** By J.L.J. BILZON<sup>1</sup>, E.G. SCARPELLO<sup>1</sup>, R.J. PETHYBRIDGE<sup>1</sup>, A.J. ALLSOPP<sup>1</sup>, and S. A. WOOTTON<sup>2</sup>, <sup>1</sup>Institute of Naval Medicine, Gosport PO12 2DL, and <sup>2</sup>Institute of Human Nutrition, University of Southampton SO16 6YD

Following exercise-induced dehydration under controlled laboratory conditions, fluid is more readily retained and plasma volume restored when the beverage contains Na (Maughan & Leiper, 1995). The aim of the present study was to assess whether a carbohydrate-electrolyte beverage (CEB) was more effective for rehydrating dehydrated individuals than a flavoured placebo or water, under field conditions. Forty-five male volunteers were divided into three groups, which were balanced for previous endurance course performance. Subjects completed an 8 h (48 km) endurance exercise (EX), carrying about 18 kg additional weight, during which water consumption was assessed. On completion of the exercise subjects rested in a control room, where they consumed either: CEB (75g carbohydrate/l, 25 mmol Na/l); flavoured placebo (P); or water (W), *ad libitum* during a 4 h recovery period (REC). Volitional fluid intake and cumulative urinary volume were assessed during recovery and used in the determination of fluid retention. After voiding the bladder, body mass was recorded, pre- and post-exercise and again post-recovery, for the calculation of fluid deficit and overall fluid balance. Whole-blood samples were analysed for packed cell volume and haemoglobin concentration and used to determine changes in plasma volume ( $\Delta$ PV%) relative to pre-exercise. Fluid balance and  $\Delta$ PV% (post-exercise and post-recovery) are summarized in the Table.

	CEB		P		W	
	Mean	SD	Mean	SD	Mean	SD
Fluid deficit (ml)	-2564	1218	-2553	763	-2655	990
Fluid intake - recovery (ml)	1467	713§	1114	567	1108	522
Urine volume (ml)	377	147	456	229	450	189
Fluid retention (ml)	1099	604**	687	352	658	345
Fluid balance (ml)	-1465	870††	-1866	776	-1997	766
$\Delta$ PV% post-EX	-6.3	2.6	-6.6	2.0	-6.8	2.4
$\Delta$ PV% post-REC	2.4	4.2*†	-2.5	1.8	-2.1	1.4

Significantly different from P: \*  $P < 0.05$ ; †  $P < 0.1$ . Significantly different from W: ‡  $P < 0.05$ ; §  $P < 0.1$

There were no differences between groups in the mean fluid deficit incurred during the exercise period. Overall fluid retention during recovery was greater for the CEB than for the P and W groups. When expressed relative to fluid intake during exercise, the regression line for fluid retention during recovery was above and significantly greater for the CEB than for the P ( $P < 0.05$ ) and W ( $P < 0.05$ ) groups. CEB appeared to be more effective when fluid intake during exercise was lower and the fluid deficit higher. As a consequence of the higher fluid retention during recovery, the CEB group were rehydrated to 70 (SD 17) % compared with 58 (SD 19) % and 52 (SD 12) % for the P ( $P > 0.05$ ) and W ( $P < 0.05$ ) groups, respectively. Subsequently, post-recovery plasma volume was restored to a greater extent for the CEB compared with the P and W groups. It is concluded that, the addition of Na to a rehydration beverage improved fluid retention and the restoration of plasma volume during recovery from a field exercise. This effect is influenced by fluid intake during exercise, such that the inclusion of Na is more effective when fluid deficit following exercise is higher.

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Maughan R J & Leiper J B (1995) *European Journal of Applied Physiology* 71, 311-319.

**Measured calcium intakes of breast-fed rural Gambian infants aged 3 and 12 months.** By LANDING M. A. JARJOU<sup>1</sup>, ANN PRENTICE<sup>2</sup>, W. ANDY COWARD<sup>2</sup>, BAKARY DIBBA<sup>1</sup>, YANKUBA SAWO<sup>1</sup>, KEN DAY<sup>2</sup> and SUSAN FAIRWEATHER-TAIT<sup>3</sup>, <sup>1</sup>MRC Dunn Nutrition Unit, *Keneba, The Gambia*, <sup>2</sup>MRC Dunn Nutrition Unit, *Milton Road, Cambridge CB4 1XJ*, <sup>3</sup>Institute of Food Research, *Norwich NR4 7UA*

Ca insufficiency may contribute to the problem of infant growth retardation in developing countries, since the theoretical Ca accretion rate averages 100–200 mg/d in infancy and this may represent a substantial proportion of daily Ca intake during the first year of life (Prentice & Bates, 1993). However, quantitative data on the Ca intake of breast-fed babies are scarce. To assess dietary adequacy in a population where growth faltering is common, we have measured the total Ca intake of breast-fed rural Gambian infants aged 3 and 12 months.

Thirty Gambian infants (13M, 17F) were studied twice, at mean ages of 91(SD 7) and 366 (SD 6) days. All subjects breast-fed on demand; 27% and 100% consumed additional foods at 3 and 12 months. Breast-milk intake was measured over 14 d by the deuterium oxide dose-to-the-mother method (Coward *et al.* 1982). The Ca content of breast-milk was determined in whole-milk samples, after ashing and acid-digestion, using a validated spectrophotometric technique (Laskey *et al.* 1991). Ca intake from other sources was quantified by direct weighing over 5 d combined with the collection of recipes and the Ca analysis of representative samples of prepared foods, raw ingredients and water.

	3 months		12 months	
	Mean	SD	Mean	SD
Breast-milk intake (ml)	864	199	759	211
Breast-milk Ca concentration (mg/l)	203	27	152	24
Breast-milk Ca intake (mg/d)	177	52	116	38
Ca intake from other sources (mg/d)	12	38	73	105
Total Ca intake (mg/d)	188	58	190	95

The results are given in the Table. Breast-milk contributed an average of 94% and 62% to total Ca intake at 3 and 12 months. There was no relationship between breast-milk intake and breast-milk Ca concentration at either timepoint. Repeat measures ANOVA demonstrated that breast-milk Ca intake at 3 and 12 months was characteristic of the individual ( $P < 0.0001$ ), but that there was no correlation between total Ca intake at the two ages.

Total Ca intake averaged 189 mg/d and was similar at 3 and 12 months. The range was wide: 76–332 mg/d at 3 months and 55–566 mg/d at 12 months. These results demonstrate that the Ca intake of many rural Gambian infants is low and close to the theoretical Ca accretion rate. Further studies will determine whether such low Ca intakes are associated with poor growth performance.

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**Effect of calcium supplementation on plasma osteocalcin concentration of Gambian children.** By BAKARY DIBBA, ANN PRENTICE, DOROTHY STIRLING and ELIZABETH M. E. POSKITT, *MRC Dunn Nutrition Unit, Milton Road, Cambridge, CB4 1XJ and Keneba, The Gambia.*

Our recent Ca-supplementation study of rural Gambian children accustomed to a low Ca intake demonstrated a significant increase in bone mineral status with no concomitant effect on bone area or body height (Dibba *et al.* 1998). These results suggested that the Ca supplement may have acted by altering bone remodelling rather than by increasing bone growth (Prentice, 1995). To test this hypothesis we measured the concentration of osteocalcin, a marker of bone formation, in plasma samples collected before and after supplementation. The trial involved 160 children aged 10.3 (SD 1.0) years who were randomized, double-blind, to receive 714 mg Ca/d (Caco<sub>3</sub> Calcichew, Shire Pharmaceuticals) or placebo for 12 months (Dibba *et al.* 1998). Blood was collected after an overnight fast on the day before supplementation (baseline) and after 12 months (outcome). The sample was anticoagulated with lithium heparin, kept cool, separated and frozen within 45 min, and transported on solid CO<sub>2</sub> to Cambridge. Intact osteocalcin was measured on a subset of ninety-eight subjects (49M, 49F) using an immunoradiometric assay (N-TACT Osteocalcin, INCStar Corporation, Stillwater, USA). Multiple regression analysis, with continuous variables transformed to natural logarithms, was used to examine the supplement effect.

	Baseline		Outcome		Δ%†
	Mean	SE	Mean	SE	
Supplement	24.8	1.5	17.9***	1.0	-26.4***
Placebo	24.3	1.0	23.2	1.3	-3.9

†Significantly different from placebo group. \*\*\* $P \leq 0.001$ .  
 Δ%, percentage difference outcome-baseline  $(100[(\text{outcome}) - 100]/(\text{baseline}))$ , equivalent to 100 x difference/mean.

There was no significant difference between the groups at baseline. At outcome, the supplement group had a significantly lower plasma osteocalcin concentration and had exhibited a greater decrease over the 12-month period compared with the placebo group ( $P \leq 0.001$ ). After correcting for the initial value, the change in plasma osteocalcin was more positive in girls than boys (+17.8, (SE 7.1) %  $P = 0.015$ ) and in those who had entered puberty (+15.8, (SE 7.2) %  $P = 0.031$ ), but was not influenced by age. After adjusting for baseline value, sex and pubertal status, plasma osteocalcin in the Ca-supplemented group was significantly lower than in the placebo group by -22.2, (SE 6.8) %  $P = 0.002$ . There was no evidence of any interaction between the supplement effect and age, sex or pubertal status.

This study has confirmed that the increase in bone mineral status observed in Gambian children consuming a Ca supplement was associated with a decrease in bone formation, suggesting that the supplement had altered bone remodelling. This is in contrast to a recent supplementation study using milk in which an increase in bone mineral was not reflected in altered bone turnover (Cadogan *et al.* 1997). This raises the possibility that Ca salts and milk have different effects on the growing skeleton. More research is needed to determine the long-term benefit of calcium supplementation on the bone mineral accretion of children.

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**Nutrient intake in peri and early post-menopausal Scottish women: preliminary findings from a longitudinal study on bone health.** By H. M. MACDONALD<sup>1,3</sup>, S. A. NEW<sup>2</sup>, M. H. N. GOLDEN<sup>3</sup>, D. A. GRUBB<sup>4</sup> and D. M. REID<sup>1,3</sup> <sup>1</sup>Osteoporosis Research Unit, University of Aberdeen, Woolmanhill Hospital, AB25 1LD, <sup>2</sup>Centre for Nutrition & Food Safety, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH, <sup>3</sup>Department of Medicine & Therapeutics, University of Aberdeen, AB25 2ZD, <sup>4</sup>Computing Department, Rowett Research Institute, Aberdeen, AB21 9SB

In order to fully investigate the influence of nutrition on peri- and post-menopausal bone health, studies of longitudinal design are essential. In two recent cross-sectional studies, our group has identified intakes of K, Mg, Zn, fibre and vitamin C as being important to the bone health of pre-menopausal women (New *et al.* 1996, 1997). We now wish to address, for the first time, the relative strength of nutritional, genetic and physical activity factors on peri-menopausal and immediate post-menopausal bone loss.

A complete review of the 1065 women who took part in our original study between 1992 and 1994 is currently underway. They were recalled according to season (Summer, between April to September and Winter, October to March). Preliminary findings of changes in nutrient intake in the study population over the 5-year period are reported in this abstract. Current dietary intake has been assessed using our food-frequency questionnaire, which has been validated against 7 d weighed intakes and biochemical markers and its short-term (6 weeks) and long-term (12 months) reproducibility has been tested (New *et al.* 1993). Results for 553 women (281 Winter, 272 Summer) are shown in the Table.

Nutrients	Baseline			5 Years			P value
	Mean	SD	Range	Mean	SD	Range	
Energy (MJ)	8.2	2.3	3.4-16.8	7.8	2.1	3.4-16.9	0.001
Protein (g)	81.1	23.0	19.7-230.6	78.0	20.0	34.4-198.4	0.005
Fat (g)	73.5	28.7	18.1-182.8	68.2	24.9	22.4-224.4	0.001
Carbohydrate (g)	246	73	75-534	238	67	76-551	0.004
NSP (g)	15.6	5.7	4.7-47.3	16.0	5.4	4.3-43.4	NS
Calcium (mg)	1058	331	174-2394	1035	352	332-4461	NS
Potassium (mg)	3348	804	1475-6897	3212	779	1501-7941	NS
Zinc (mg)	10.1	2.9	2.4-23.7	9.4	2.4	3.6-21.0	0.001
Copper (mg)	1.31	0.43	0.45-4.42	1.21	0.35	0.39-2.89	0.001
Vitamin D ( $\mu$ g)	3.19	2.15	0.18-29.55	4.02	2.40	0.22-18.92	0.001
EI:BMR	1.43	0.41		1.33	0.35		0.001
Weight (kg)	63.7	10.8		67.5	11.4		0.001
BMI ( $\text{kg m}^{-2}$ )	24.4	3.8		26.1	4.2		0.001

These early findings indicate significant decreases in dietary intakes of energy, protein, fat, carbohydrate, Zn and Cu but a significant increase in vitamin D. The women have increased in weight suggesting a decrease in physical activity. Further analysis of quartile categorization is required to establish the full extent of dietary intake changes and whether these decreases are characteristic within the whole of the study population and peri-menopausal women in general.

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**Impact of a child-specific dual-energy X-ray absorptiometry analysis prototype on bone mineral content and body composition in children aged 8 to 12 years.** By N.J. FULLER<sup>1</sup>, J.C.K. WELLS<sup>2</sup>, O. DEWITT<sup>1</sup>, W. HIPGRAVE<sup>3</sup>, M. ELIA<sup>1</sup> and M.S. FEWTRILL<sup>2</sup>, <sup>1</sup>MRC Dunn Clinical Nutrition Centre, Cambridge CB2 2DH, <sup>2</sup>Childhood Nutrition Research Centre, Institute of Child Health, London WC1N 1EH and <sup>3</sup>Vertec Scientific Ltd, Aldermaston, Reading RG7 8JA

Dual-energy X-ray absorptiometry (DXA) software for analysis of bone mineral content (BMC) in adults may not be applicable to children because of inappropriate attenuation thresholds. Prototype child-specific software for the Hologic QDR-1000/W apparently uses more appropriate thresholds for children that assign correctly to bone those bone edges previously identified as fat-free soft tissue (FFST), especially in the hands and feet. Definitive validation of this prototype software is not possible at present due to the absence of a 'gold standard' or suitable reference for children's BMC. Therefore, the purpose of the present study was to evaluate the extent to which the new software might affect measurement of BMC and body composition in children.

Thirty healthy children aged 8-12 years volunteered for the study: sixteen boys; mean weight 31.6 (SD 7.6) kg; height 1.37 (SD 0.09) m; and BMI 16.7 (SD 2.0)  $\text{kg m}^{-2}$  and fourteen girls; mean weight 36.4 (SD 9.0) kg; height 1.43 (SD 0.13) m; and BMI 17.5 (SD 2.2)  $\text{kg m}^{-2}$  participated. Each underwent a whole body scan on the Hologic QDR-1000/W which was then analysed for BMC, body fat and FFST with both the Enhanced Whole Body V5.61 and the prototype Children's Whole Body (beta) V5.61 programmes, by the same operator. The respective BMC values were applied to a four-component model (4-CM), that also incorporated measurements of total body water and body density (Fuller *et al.* 1992), for comparative assessments of fat and fat-free mass (FFM). As the only differences between boys and girls were in group mean for BMC (1.10 (SD 0.3) v. 1.19 (SD 0.3) kg) and body fat (16.7 (SD 5.8) v. 24.3 (SD 7.5) %), bias and 95% limits of agreement were assessed between adult and children's mode for all subjects combined.

Group mean (using children's mode BMC)	DXA		4-CM	
	BMC (kg)	Fat (%)	FFST (kg)	Fat (%)
1.15 (SD 0.31)	20.2 (SD 7.6)	25.2 (SD 5.1)	20.8 (SD 7.3)	26.5 (SD 5.9)
Bias (adult minus children's mode)	-0.07***	-1.05***	0.44***	-0.30***
95% limits of agreement	-0.11 to -0.03	-2.44 to 0.34	-0.12 to 1.00	-0.44 to 0.16
***P < 0.001; paired t test for adult v. children's mode.				0.10***
				0.04 to 0.16

There was a slight trend for differences to increase between adult and children's modes for BMC and percentage fat by DXA due to greater size of measurement ( $P < 0.05$ ; 0.025  $\text{kg kg}^{-1}$  BMC), which itself was associated with body weight and height but not age; this was not observed for the 4-CM.

The significant increase in BMC determined by the new child-specific DXA software has a major impact on body composition as assessed by DXA suggesting that, if this new software proves acceptable, the adult version should no longer be used in children. In contrast, the near negligible effect that this increase in BMC has on body composition using the 4-CM implies that the adult and children's software may be interchangeable for this particular purpose.

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**Collagen crosslink ratios in bone and urine of guinea-pigs fed with graded intakes of vitamin C.** By CHRISTOPHER J. BATES and HARUMI TSUCHIYA, *MRC Dunn Nutrition Unit, Downhams Lane, Milton Road, Cambridge CB4 1XJ*

Of the biochemical lesions of vitamin C deficiency, that affecting collagen biosynthesis is best understood. The formation of hydroxyprolyl and hydroxylysyl residues is vitamin C-dependent (Kivirikko & Myllyla, 1982), and the two collagen crosslinks: pyridinoline (Pyr) and deoxypyridinoline (Dpyr) are formed from lysyl and hydroxylysyl residues, in different proportions. Vitamin C-deficient guinea-pigs showed crosslink ratio changes in bone and urine, consistent with an effect on lysyl hydroxylation (Tsuchiya & Bates, 1997). The present study defines the range of vitamin C intakes over which crosslink ratios respond. Six groups (A-F) of male weanling Dunkin-Hartley guinea-pigs, mean initial body weight 314 (SD 27) g were fed on a vitamin C-free purified diet (Tsuchiya & Bates, 1997) with vitamin C, 0.5 to 1128 mg/d, for 46 days. Urine samples were collected on days 43–45; animals were killed on day 46, and plasma and organs were extracted for vitamin C analysis. Femurs and urines were acid-hydrolysed for collagen crosslink analyses (ELISA: Metra Biosystems (UK) Ltd), and for femur hydroxyproline (hypro) (Table).

Index	Diet group												P for trend (1)	P for trend (1) <sup>†</sup>		
	A	B	C	D	E	F	Mean	Mean	Mean	Mean	Mean	Mean				
Vitamin C intakes (mg/d)	0.5	1.0	5.0	49	205	1128										
Plasma vitamin C (µmol/l)	2.1	6.0	26.1	109	144	160										<0.0001
Adrenal vitamin C (µmol/g)	0.6	1.3	4.7	10.9	11.4	11.8										<0.0001
Body-weight increase (g)	115	120	153	166	177	258										<0.0001
Bone collagen crosslinks																
Pyr/hypro (nmol/mol)	0.81	0.77	0.82	0.91	0.91	0.87										0.019
Dpyr/hypro (nmol/mol)	0.093	0.092	0.075	0.071	0.064	0.070										0.003
Pyr/Dpyr (mol/mol)	8.9	8.9	11.2	14.4	14.8	13.4										0.003
Urine collagen crosslinks																
Pyr/creatinine (µmol/mol)	216	231	184	206	251	270										0.15
Dpyr/creatinine (µmol/mol)	31.2	31.2	20.8	22.7	23.1	24.9										0.13
Pyr/Dpyr (mol/mol)	7.0	7.8	8.9	10.0	11.1	11.0										0.0002
<sup>†</sup> Linear regression of index v. log (vitamin C intake).																
<sup>‡</sup> Linear regression of index v. log (vitamin C intake) after adjustment for body-weight increase (multiple regression).																

Results show that vitamin C in plasma and adrenals increased sharply between 0.5 and 49 mg/d vitamin C intake, but approached a plateau at higher intakes. Body-weight increases were higher at greater vitamin C intakes. Of the collagen crosslink indices, the ratio Pyr:Dpyr in bone and urine was more consistently correlated with vitamin C intake than the other ratios were. The linear correlation between the crosslink ratio and log (vitamin C intake) was highly significant, and remained significant after adjustment for differences in body-weight increase.

Thus, Pyr:Dpyr ratios increase as tissue vitamin C increases and this is not secondary to the effects of vitamin C on growth. Thus, crosslink ratios may prove useful in defining vitamin C adequacy, in animals and man.

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**Validity of tissue composition in leg sections obtained by dual-energy X-ray absorptiometry against magnetic resonance imaging as reference.** By N.J. FULLER<sup>1</sup>, C.R. HARDINGHAM<sup>2</sup>, N. SCREATON<sup>2</sup>, M. GRAVES<sup>2</sup>, A.K. DIXON<sup>2</sup>, W. HIPGRAVE<sup>3</sup> and M. ELIA<sup>1</sup>, <sup>1</sup>MRC Dunn Clinical Nutrition Centre, Cambridge CB2 2DH, <sup>2</sup>Department of Radiology, Addenbrooke's Hospital, Cambridge CB2 2QQ and <sup>3</sup>Vertec Scientific Ltd, Aldermaston, Reading RG7 8JA

Assessment of leg muscle and adipose tissue (AT) composition may be obtained using a theoretical model based on dual-energy X-ray absorptiometry (DXA) measurements of body composition (Fuller *et al.* 1992). However, not only has this model never been validated against an established reference method, such as magnetic resonance imaging (MRI), but also it has never been applied to sections or sub-regions of the leg. Therefore, the aim of the present study was to assess the validity of the DXA-based model against MRI for estimating tissue composition of predetermined sections of thigh and lower leg.

Sixteen volunteers (eight males and eight females) participated in the study: age range, 41–62 years; BMI, 18.1–38.4 kg/m<sup>2</sup>. Each was subjected to a whole body scan on the Hologic QDR-1000/W and an MRI scan of the legs (Hardingham *et al.* 1998). Muscle and AT were analysed by both techniques over a 200 mm section of thigh, centred two-fifths of the distance from knee joint space to anterior superior iliac spine, and a 100 mm section of lower leg, centred two-fifths of the distance from knee joint to ankle joint space. Skin covering these sections was accounted for equally in both techniques by the use of anthropometric estimates. Where appropriate it was assumed that density of skeletal muscle was 1.0414 kg/l and that of AT was 0.916 kg/l. As results from both legs were similar, only those from the right leg are reported. Bias and 95% limits of agreement ( $\pm 2$  SD) were assessed between MRI and DXA (MRI minus DXA).

	Group value by MRI		Bias	95% Limits of agreement
	Mean	SD		
Thigh muscle volume (litres)	2.29	0.64	0.05**	-0.30 to 0.40
Thigh AT volume (litres)	1.48	0.80	0.24	-0.12 to 0.60
Calf muscle volume (litres)	0.60	0.19	0.09**	-0.02 to 0.20
Calf AT volume (litres)	0.18	0.12	-0.05	-0.19 to 0.09

Difference between methods became more negative with increasing size of measurement; \*\**p* < 0.01.

These results indicate that the DXA model may be used in these sections with reasonable confidence for estimating muscle tissue, especially in the thigh, but that, apparently, it is not applicable to AT. The bias between methods for muscle in calf and AT in thigh and calf sections, coupled with substantial discrepancies for individual differences, may be attributable in part to the relatively small size of the sections and constituent tissues.

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**Nutrient intake and bone health in ballet dancers and healthy age-matched controls: preliminary findings from a longitudinal study on peak bone mass development in adolescent females.** By SUSAN A. NEW<sup>1</sup>, ADELEYE SAMUEL<sup>1</sup>, SALLY-ANN T. LOWE<sup>1</sup> and NICOLA J. KEAY<sup>2</sup>. <sup>1</sup>Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH. <sup>2</sup>Department of Endocrinology, St Thomas's Hospital, London SE1 7EH

Non-modifiable factors such as hereditary influences, sex and race account for much of the variation in peak bone mass (PBM) development. However, it is considered that at least 20% of the variation may be explained by modifiable factors, the most important being those of hormonal status, physical activity and nutrition. It is well established that females engaged in physical activities which require extremely low body weights and which are of high intensity may experience reduced PBM attainment due to the later age of menarche and high incidence of amenorrhoea.

The present abstract reports the baseline nutritional data from a longitudinal study on the effect of dance training on PBM attainment (Keay 1997). The study population includes a total of twenty-eight elite female ballet dancers (group 1), twenty-eight healthy age-matched controls (group 2) and twenty-nine musical theatre pupils involved in less intense dance training (group 3). Nutrient intake was assessed using two 7 d estimated food diaries completed during term and holiday time. Food diaries were coded using the Diet 5 computer program which is based on McCance and Widdowson's food composition tables and supplements. Whole-body, lumbar spine and femoral neck bone mineral densities (BMD) were measured using dual X-ray absorptiometry (DXA) (Hologic QDR4000).

No differences were found in nutrient intakes between term and holiday diaries except for energy intake (EI) in group 3. Results are therefore presented for average year intakes. Preliminary results for key nutrients are shown in the Table for group 1 (n 18), group 2 (n 10) and group 3 (n 8).

	Group 1 (n 18)		Group 2 (n 10)		Group 3 (n 8)	
	Mean	sd	Mean	sd	Mean	sd
Age (years)	13.6 <sup>a</sup>	1.6	13.3 <sup>a</sup>	1.7	15.8 <sup>a</sup>	1.5
Weight (kg)	41.4 <sup>a</sup>	5.2	47.1 <sup>a</sup>	6.6	52.0 <sup>b</sup>	8.4
Height (m)	1.53	8.6	1.56	9.4	1.63	5.8
Energy (MJ/d)	6.5 <sup>a</sup>	1.3	7.1 <sup>b</sup>	1.2	7.2 <sup>b</sup>	1.2
EI:BMR	1.2	0.2	1.31	0.2	1.24	0.2
Calcium (mg/d)	706	214	716	205	751	221
NSP (g/d)	10.2	3.64	10.7	2.6	11.6	3.5
Iron (mg/d)	9.6	2.54	10.1	3.4	9.9	2.5

a, b Mean values with unlike superscripts were significantly different, P<0.05 (ANOVA).

EI:BMR ratios demonstrated that on average, the food diaries had been completed to a satisfactory standard. No differences were found in weight, height or other nutrients except energy between the dancers (group 1) and controls (group 2). Group 3 subjects were older and heavier than the dancers and had higher intakes of energy and protein. Preliminary dietary intake and BMD correlations indicate little association between nutritional factors and bone health. Although further cross-sectional analysis of the study population will be useful, longitudinal data on changes in bone mass will enable the full extent of the influence of modifiable factors on PBM development to be determined.

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Keay N (1997) *British Journal of Sports Medicine* 31, 143-147.

**Dietary intake and bone health in young adolescent females: a comparison between elite gymnasts and healthy age-matched controls.** By LUCY-ANN PRIDEAUX<sup>1</sup>, JACKI A. BISHOP<sup>2</sup> and SUSAN A. NEW<sup>2</sup>. <sup>1</sup>Department of Medicine & Therapeutics, University of Aberdeen, AB25 1GR. <sup>2</sup>Centre for Nutrition & Food Safety, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH

Females involved in physical activities requiring low body weights such as endurance athletes and gymnasts, often report a high incidence of amenorrhoea or late onset of menarche. Both are likely to affect achievement of peak bone mass (PBM) and result in poor skeletal health. Although, few data exist on gymnasts, these are potentially a very useful model to examine the effect of nutrition and physical activity on bone mass. Gymnastic training involves high bone strain rates and the physical requirements of repeated bone loading activities such as jumping, leaping and vaulting should have beneficial influences on bone mass. However, low circulating levels of oestrogen may reverse any training effects.

The present abstract reports preliminary cross-sectional data on elite female gymnasts. Twenty elite gymnasts selected from three top gymnastics clubs in South-east England were compared with twelve healthy age-matched controls from a Sussex secondary school. Bone mass of the os calcis was measured by a recently developed method for assessment of bone health, namely, broadband ultrasound attenuation (BUA). BUA and velocity of sound (VOS) are believed to relate to calcaneus bone mass and possibly structure, although this has yet to be fully defined. Weight, height and Tanner stage were also measured and dietary intake was assessed from 7 d estimated food diaries.

	Gymnasts		Controls	
	Mean	SD	Mean	SD
Age (years)	12.4	2.0	12.7	1.1
Weight (kg)	38.6 <sup>a</sup>	8.7	48.6 <sup>b</sup>	9.5
Standing height (m)	1.42 <sup>a</sup>	0.1	1.20-1.67	0.1
Sitting height (m)	0.72 <sup>a</sup>	0.4	0.64-0.80	0.5
Tanner stage	1.9 <sup>a</sup>	0.7	1-3	0.7
Birth weight (kg)	2.6	0.6	1.2-3.9	3.0
BUA (dB MHz)	52.8	15.8	30.0-83.6	61.3
VOS (m/sec)	1679 <sup>a</sup>	36.2	1623-1750	1669 <sup>b</sup>
Energy intake (MJ/d)	6.8 <sup>a</sup>	1.4	3.9-10.1	8.6 <sup>a</sup>
Calcium (mg/d)	606.9	151.4	227-849	677.7
Iron (mg/d)	11.4	5.9	6.7-25.2	11.9
EI:BMR	1.34	0.34	1.53	0.34

a, b Values with unlike superscripts were significantly different, P<0.05 (ANOVA).

As shown in the Table, the groups were well matched for age. Gymnasts were lighter, shorter and were at a significantly earlier stage of pubertal development than the controls. VOS was not related to body weight and was significantly higher in the gymnasts. These data point to a possible association between low energy intake in gymnasts and delayed puberty which has been found in other groups (Bale *et al.* 1996). Eight of the gymnasts were known to be dieting for competition. Higher intakes of Se and Cu were associated with higher BUA in the controls. As yet no data exist on micronutrient intake and bone health in children and further longitudinal investigations will undoubtedly be helpful.

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Bale P, Dost J & Dawson D (1996) *Journal of Sports Medicine and Physical Fitness* 36, 49-53.

**Food intake and bone mineral status of Beijing adolescent girls.** By XUEQIN DU,<sup>1</sup> HEATHER GREENFIELD,<sup>1</sup> DAVID R. FRASER<sup>2</sup> and KEYOU GE,<sup>3</sup> *University of New South Wales, Australia, <sup>1</sup>University of Sydney, Australia, <sup>2</sup>Chinese Academy of Preventive Medicine, China*

Environmental factors such as nutrition and physical activity have been reported to be determinants of bone mineral status, although with a less striking role than genetic factors (Matkovic, 1992). A study of a random sample of 1200 girls aged 12–14 years in Beijing habitually consuming a plant-based diet (Du *et al.* 1997a) provided an opportunity to examine the relationship between food intake and bone mineral status. Food and nutrient intakes over the past year were estimated by use of a specially designed semi-quantitative food frequency questionnaire validated against 24-hr recalls (Du *et al.* 1997b). FFQ nutrient intakes were adjusted down by about 20%, the levels indicated by the 24-hr recall. Bone mineral content (BMC, g/cm) and bone width (BW, mm) at distal 1/3 and 1/10 radius and ulna were measured by single-photon absorptiometry (BMD-4, BBTI, China). Stepwise multiple regression analyses of BMC on foods and nutrients as well as confounding factors were performed for 517 girls with complete data from the subsample of 650 for whom bone mineral measurements were made. Nine variables which were significantly correlated with BMC were included as independent variables in the analysis, i.e. BW, body weight, height, bone age (Greulich and Pyle Atlas method), Tanner stage of breast development, School Physical Activity Score (SPAS, scale 1–4 where 1 = most active), milk, Ca, and vitamin D intakes. Among the thirteen food groups, namely cereals, milk, vegetables, fruits, legumes, fish, iced confections, nuts, eggs, meat and sugar, only the milk group had significant partial correlation with BMC and could therefore be included in the analysis.

Variable	BMC distal 1/10 radius	BMC distal 1/3 radius	BMC distal 1/10 ulna	BMC distal 1/3 ulna
BW (mm)	0.710***	0.726***	0.738***	0.654***
Milk (g/d)	0.173***	0.175***	0.172***	0.110***
Bone age (years)	0.124***	0.075*	NS	NS
SPAS	-0.058*	-0.105***	NS	-0.095**
Body weight (kg)	NS	0.082**	NS	0.147***
Tanner stage	NS	NS	0.073*	NS

Values are  $\beta$  values (standardised regression coefficient)

SPAS: scale 1–4 where 1 = most active

As the Table shows, milk intake was included in all four models for BMC at all bone sites measured. The models explained 54–64% of the variation in BMC, and milk alone accounted for up to 3.2% of the variation, meaning that milk intake was a determinant of bone mineral status of these girls. The milk group included fresh milk, powdered milk, vitamin D fortified milk and yoghurt. The average intake for the whole group of the girls was 64 (range 0.3–501) g/d, however only two-thirds of the girls consumed milk. BW, bone age, body weight, SPAS and Tanner stage were also predictors or determinants of BMC. BW accounted for most of the  $R^2$ , whereas physical activity measured as SPAS was associated with less than 1% change in  $R^2$ . Other factors also contributed to a small change in  $R^2$  whereas height, and Ca and vitamin D intakes did not meet the criteria for inclusion in the models. This study suggests that milk intake is the major dietary determinant of bone mineral status of Beijing adolescent girls aged 12–14 years. Since only small amounts of milk were consumed and about one-third of these girls did not consume milk at all, milk consumption should be considered for promotion in this age group.

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