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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at the University of Ulster at Coleraine on Monday–Friday, 24–28 June 1996, when the following papers were presented.

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

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Influence of maternal nutrition during early pregnancy on placental development in sheep. By LINDSAY HEASMAN, LYNNE CLARKE, MONIQUE HUDSON and MICHAEL E. SYMONDS, *School of Animal and Microbial Sciences, The University of Reading, Whiteknights, PO Box 228, Reading RG6 6AJ*

Maternal nutrition during the first half of pregnancy has been proposed to have a major influence on placental growth, which peaks during mid gestation in sheep (Kelly,1992). Placental size is known to have a pivotal role in the regulation of fetal growth, and this has been highlighted in recent studies in which reduced placental size was associated with an increased incidence of hypothermia in near-term lambs delivered by Caesarean section (Symonds & Clarke, 1996). The present study aimed to determine the effect of high and low levels of nutrition, between 30 and 80 d of gestation on the development of fetal cotyledons and maternal caruncles within the ovine placenta.

Ten Welsh Mountain ewes which had similar body weights (47.3 (SEM 1.8) kg) and fat distribution as assessed from measurement of body condition score (BCS; 2.0 (SEM 0.3)) (Russel, 1984) at mating were entered into the study. At 30 d of gestation (term=147) ewes were individually housed and fed daily on a diet of chopped hay and a barley-based concentrate to supply either 50% of the energy requirements for maintenance and pregnancy (0.5M; 85-145 g concentrate, plus 370-610 g hay, *n* 5) or 200% of energy requirements (2M; 205-260 g concentrate, plus 890-1125g hay, *n* 5). All diets contained adequate minerals and vitamins. Ewes were scanned by ultrasound 40 d after mating in order to confirm that they were monotocous, and humanely slaughtered at 80 d of gestation to enable placental and fetal sampling.

	Maternal (80 d)				Placental weight (g)					
	Weight (kg)		BCS		Maternal		Fetal		Fetal weight (g)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0.5M	41.2	1.4	1.5*	0.0	238	37	226*	21	320	23
2M	43.0	2.8	2.4	0.3	186	34	357	37	362	10

* Significantly different from 2M (Student's *t* test): $P<0.05$.

The level of maternal nutrition had no influence on maternal body weight, but ewes fed on 0.5M had a lower BCS at 80 d. The weight of the maternal caruncular component of the placenta was not significantly affected by maternal nutrition, although total fetal cotyledon weight was significantly lower in ewes fed on 0.5M. This difference was not reflected by any change in fetal weight.

In conclusion, a reduced level of maternal nutrition between 30 and 80 d gestation can compromise growth of the fetal, but not maternal component of the placenta. This has no immediate effect on fetal weight but may have the potential to compromise growth and development during late gestation when fetal nutrient demands are greatly increased.

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Lean body mass and human pregnancy. By CHOY H. LEE and PAUL G. WHITTAKER, *University Department of Obstetrics and Gynaecology, RVI, Newcastle, Tyne and Wear NE1 4LP*

Metabolic studies may require results to be standardized for lean body mass, the proportions of which may change during pregnancy. A simple non-invasive method for measuring body composition is bioelectric impedance analysis (BIA) giving an indirect measure of total body water (TBW) which can be converted to lean body mass (LBM_W). It has been validated in pregnancy (Van Loan *et al.* 1995) but LBM_W post-delivery was not studied. We have studied twenty healthy non-obese women at 35 weeks gestation and again at 15 weeks post-delivery when breast-feeding ceased. We used a Holtain body composition monitor, giving a within-subject CV of 2.6% for LBM_W during pregnancy and a linear relationship over the weight range. LBM_W and TBW were calculated using the equations of Villar et al (1992) and van Raaij et al (1988) respectively. Total body K counting (TBK) was used as an alternative measure of lean body mass (LBM_K) and calculated according to Pipe et al (1979).

	Late pregnancy		Post-delivery		Post-delivery change	
	Mean	SD	Mean	SD	Mean	SD
Wt (kg)	77.8	10.2	68.9	9.5	-8.9	3.7
LBM_K (kg)	44.2	5.4	41.8	4.5	-2.3	3.0
LBM_W (kg)	52.6	5.2	46.9	4.6	-5.7	3.0
TBW (kg)	39.2	3.8	34.0	3.4	-5.2	2.2
$\%LBM_K$	57.0	4.7	61.2	5.6	+4.2	3.6
$\%LBM_W$	67.9	3.4	68.5	3.5	+0.7	2.2
%TBW	50.6	2.5	49.6	2.5	-1.0	1.6

Our values of $\%LBM_K$ during pregnancy by TBK were similar to Pipe et al (1979) but may underestimate post-delivery LBM change since loss of 2.3 kg LBM was much less than would be accounted for by loss of infant, placenta and maternal tissues, estimated at 5 kg or more (Hytten 1995). However if TBK at 35 weeks was corrected, as suggested for late pregnancy, by 5% (Godfrey & Wadsworth, 1970) the mean change in LBM_K (-4.5 kg) became more realistic and similar to the change in LBM_W.

Our mean values for %TBW and $\%LBM_W$ by impedance were similar to Van Loan et al (1995) and others obtained using isotope dilution. BIA gave higher values of LBM than TBK, not only in pregnancy but also in non-pregnant subjects, as shown by Pipe et al (1979) and others using TBW to estimate LBM_W.

We have shown that BIA can be used as a reliable non-invasive method to estimate TBW and LBM in pregnancy. When proportional to body weight, %TBW and $\%LBM_W$ did not change significantly ($P>0.1$) in non-obese women between late pregnancy and 15 weeks post-delivery.

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Whole body protein turnover during pregnancy in healthy English women. By S. DUGGLEBY and A.A. JACKSON, *Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

Previous studies measuring protein turnover during pregnancy have used different methodologies and results are conflicting. de Benoist *et al.* (1985) found marked differences in protein turnover between trimesters in a group of Jamaican women. In contrast, there was no change in rates of protein turnover during pregnancy in a sample of English women (Thompson & Halliday, 1992). We measured protein turnover in English women following a similar method to that of de Benoist *et al.* (1985) to establish whether these differences were real or methodological.

Healthy pregnant women were recruited from antenatal clinics when they presented at 12-16 weeks. Seven women were studied in early pregnancy (17-20 weeks) and six in late pregnancy (30-32 weeks). Five of these women were studied at both time points. Whole-body protein turnover was measured by the end-product method using [^{15}N]glycine (Fern *et al.* 1984). A single dose of 125 mg [^{15}N]glycine was taken orally and urine was collected for the following 24 h. Enrichment in urinary urea and NH_3 was measured by mass spectrometry. Rates of N flux were calculated from the arithmetic average of the two end-products (Fern *et al.* 1985).

	Nitrogen flux (mgN/kg per h)		Protein synthesis (mgN/kg per h)		Protein degradation (mgN/kg per h)	
	Mean	SD	Mean	SD	Mean	SD
Early pregnancy	30.0	2.1	23.8	1.4	21.9	2.0
Late pregnancy	28.2	2.7	24.1	3.2	21.1	2.9

There were no differences in values for N flux, protein synthesis or protein degradation between early and late pregnancy. Our results are in contrast with the Jamaican findings, despite using similar methodology. Whilst methodological issues may account for part of the observed differences between studies, these results suggest that there are genuine differences in protein turnover between pregnant Jamaican and English women. These findings are also in agreement with measurements of urea kinetics carried out in these two populations of women (McClelland *et al.* 1993).

Therefore, we conclude that there is a marked variation in the way that Jamaican and English women adapt to pregnancy in terms of protein turnover.

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The relationship between calcium intakes in early pregnancy and iron status. By S. ROBINSON, K. GODFREY, C. OSMOND and V. COX, *MRC Environmental Epidemiology Unit, University of Southampton, Southampton SO16 6YD*

In theory the Fe requirements of pregnancy are met through the combined actions of increased absorption of Fe from the diet, mobilization of Fe stores and from the savings made during the period of amenorrhoea. In women with adequate Fe reserves there should be no need to increase Fe intake during pregnancy (Department of Health, 1991). However, not all women in the UK have adequate Fe stores and, whilst large increases in Fe absorption have been demonstrated in pregnancy in women consuming test diets (Barrett *et al.* 1994), it is not clear how these increases would be influenced by diets of poorer Fe bioavailability.

In a recent general population survey of 569 pregnant women, nearly half the group (274) reported that their consumption of milk in early pregnancy was increased relative to non-pregnant levels. Since Ca has been shown to be a potent inhibitor of Fe absorption, we investigated whether Ca intakes in the first trimester were related to maternal Fe status at 14 weeks gestation, as assessed by circulating haemoglobin and ferritin levels. Blood samples were available for 547 women. Other maternal and dietary factors known to influence Fe status and Fe absorption were also considered. Dietary intakes were assessed retrospectively at 15 weeks gestation using a 100 item food-frequency questionnaire (Robinson *et al.* 1996). Meal-pattern information from 4 d food diaries kept by the women in the 16th week of pregnancy was used in order to examine the separate effects of nutrient intakes from meals or snacks. Nutrient intakes and iron status variables were transformed before analysis.

No relationship was found between circulating haemoglobin level and intake of any dietary factor examined. There were no associations between serum ferritin level and intake of Fe, vitamin C, phytate or meat protein, but serum ferritin level was inversely related to Ca intake; P=0.0002. Mean serum ferritin level fell from 30 μg/l (95% CI 26 to 34) in women in the lowest quarter of calcium intake (≤ 947 mg/d) to 20 μg/l (95% CI 18 to 23) in women in the highest quarter (> 1512 mg/d). After allowing for Ca intake, weak positive relationships emerged between intakes of Fe, vitamin C and meat protein with serum ferritin level, but these were not statistically significant. When food diary nutrient intakes were used, after allowing for Ca intake, serum ferritin level was found to be positively related to between-meal vitamin C intakes; P=0.004, rising from a mean of 21 μg/l (95% CI 18 to 24) in women in the lowest quarter of intake (≤ 5 mg/d) to 27 μg/l (95% CI 24 to 31) in women in the highest quarter (> 37 mg/d). Serum ferritin level was not related to vitamin C intake during meals.

These findings suggest that high intakes of Ca in early pregnancy impair Fe absorption and result in reduced Fe stores. However, the effect whilst statistically strong, is relatively modest in size and should not be used as the basis of dietary advice to pregnant women to reduce milk intakes. Furthermore it appears that the effects of Ca may be offset by increasing vitamin C intakes between meals, and it is this feature which deserves further exploration and which could prove to be a useful part of dietary advice in the future.

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Does maternal calcium intake influence the calcium nutrition of the breast-fed baby? By M. ANN LASKEY, LANDING JARJOU, BAKARY DIBBA and ANN PRENTICE, *MRC Dunn Nutritional Unit, Milton Road, Cambridge CB4 1XJ*

Breast-feeding mothers have a high Ca requirement for breast-milk production. Breast-milk Ca secretion is known to vary widely between women. The present study aimed to determine the influence of current maternal Ca intake, and Ca intake during the previous pregnancy, on breast-milk Ca output in forty mothers at 2 months lactation (60 (SD 11) d). These mothers were taking part in a detailed longitudinal study to investigate the effect of lactation on bone and Ca metabolism.

The mothers were aged 31 (SD 4) years, parity 1-2, height 1.65 (SD 0.11) m, weight 67 (SD 12) kg. Maternal Ca intake was determined using a prospective 7 d food diary using photographs to aid portion size estimations (Braddon *et al.* 1988). A food-frequency questionnaire (FFQ) was also used to assess current Ca intake and intake during the previous pregnancy (Nelson *et al.* 1988). Breast-milk volume was determined, according to the mother's preference, by deuterium-oxide dilution (Coward *et al.* 1982) or test weighing. Breast-milk samples were collected using a standard protocol from both breasts, and analysed for Ca using a semi-automated micromethod (Laskey *et al.* 1991). The mothers were in full lactation; six were also giving their infants occasional bottles of formula milk.

Mean Ca intake, as measured by food diary, was 1235 (SD 456) mg/d and ranged from 637 - 2280 mg/d. Estimates of current Ca intake and intake during pregnancy, as estimated by FFQ, were similar. There was reasonable agreement between current Ca intake determined by diary and FFQ (r 0.62, slope 0.53, P <0.0001), and between current FFQ and pregnancy FFQ (r 0.72, slope 0.81, P <0.0001), but less so between the diary and pregnancy FFQ (r 0.30, slope 0.31, P = 0.03).

Breast-milk Ca output (mg/d) ranged widely from 83 - 450, with a mean of 252(SD 87). Breast-milk Ca concentration was not correlated with breast-milk volume. No associations were evident between Ca intake and breast-milk Ca secretion using regression analysis. When mothers were divided into four equal groups according to their diary Ca intake, no significant differences were observed in breast-milk Ca concentration, volume or Ca output (Table). All mothers in groups 1 and 2 had a Ca intake below the reference nutrient intake for lactating women (1250 mg/d), and those in group 1 were all below the lower reference nutrient intake (LRNI; 950 mg/d). Restricting the analyses to exclusively breast-feeding mothers gave similar results.

In summary, breast-milk Ca output was determined at 2 months of lactation and no evidence was found that this was related to Ca intake, even in women consuming less than the LRNI.

Quarter of Ca distribution	1		2		3		4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Maternal Ca intake (mg/d)	756	25	1011	30	1272	26	1899	88
Breast-milk volume (ml/d)	848	106	869	48	896	103	802	43
Breast-milk Ca concentration (mg/l)	280	12	302	22	319	18	278	12
Breast-milk Ca output (mg/d)	240	33	267	28	283	32	220	9

There were no statistical differences between the groups (ANOVA, P>0.05).

This study was supported by the Sainsbury Charitable Fund.

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Bile-salt-stimulated lipase in preterm and term human milk. By A.M. MC KILLOP, M.M.T. O'HARE and J.A. DODGE, *Department of Child Health, The Queen's University of Belfast, Institute of Clinical Science, Grosvenor Road, Belfast BT12 6BJ*

Human milk contains a lipase stimulated by bile salts (bile-salt-stimulated lipase, BSSL, *EC* 3.1.1.3) which is important for fat digestion in the newborn period. This enzyme is not present in cows' milk. The aim of the present study was to investigate the discrepancies in reported molecular mass of this enzyme which have ranged between 90 and 125 kDa (O'Connor *et al.* 1994) and to determine the incidence of multiple molecular forms in both term and preterm milk.

BSSL was purified from preterm (*n* 7, 3-40 postnatal days) and term human milk (*n* 25, 4-175 postnatal days) by heparin-Sepharose chromatography and molecular forms were characterized by SDS-PAGE and gel permeation chromatography.

Approximately 40% of the milk samples contained two molecular forms of BSSL of variable molecular mass (one form was 120 kDa and the other ranged from 98-105 kDa). The remainder of the milk samples contained one molecular species of 115 or 120 kDa. The number of molecular forms present was not related to maternal age or length of lactation, however, a higher incidence of two molecular forms was found in preterm milk (more than half) whereas only about one third of the term milk samples contained two forms. The specific activity of BSSL purified from term milk (32.4 $\pm$ 4.0 U/mg protein; Mean $\pm$ SE) tended to be higher than that from preterm milk (26.6 $\pm$ 3.7 U/mg protein), but there was no difference in activity whether one (28.5 $\pm$ 3.9 U/mg protein) or two (34.4 $\pm$ 4.8 U/mg protein) molecular forms were present.

This study demonstrates heterogeneity of both molecular mass and molecular forms, and explains why there has been no consensus regarding the molecular mass of this enzyme.

This work was supported by the Martha Moffett and John Alexander Moore research funds in Child Health.

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A comparison of two infant soyabean formulas containing different protein preparations. By FIONA CLARKE[1], VALDA BUNKER[2] and ANGELA HOUNSLOW[3], *[1]Dietetic Department, Musgrove Park Hospital, Taunton TA1 5DA, [2]School of Pharmacy and Biomedical Science, University of Portsmouth, PO1 2DZ, [3]Institute of Human Nutrition, Southampton SO16 7PX*

Soyabean infant formula is used as the sole source of nutrition in infants who are intolerant to lactose, cows' milk formula, or both, and an increasing number of parents choose to feed their infants with soyabean formula. The protein in Wysoy (SMA Nutrition, Maidenhead, Berks), which is the most commonly used soyabean formula in the UK, has conventionally been a soyabean protein isolate. Recently a method of preparation has been developed which incorporates the Ca and P with the soyabean protein isolate rather than adding them separately as salts. This improves the stability of the formula and may increase the bioavailability of Ca and P.

A double-blind, 72 h metabolic balance study was employed to compare the intake, absorption and retention from (1) the current and (2) the new soyabean formula. Sixteen full-term healthy infants, aged 8-13 weeks, previously fed the current Wysoy formula, were randomized into the two feeding groups (*n* 8) and fed *ad libitum* for 8 d. The intake of formula was measured, and all stools and urine were collected separately according to methods previously described (Widdowson,1965; Alexander & Delves,1972). There was no statistically significant difference in birth weight, gestational age, weight gain or volume of formula taken between the two groups. The mean study weight of infants was 5.29 kg (SD 0.35) and 5.11 kg (SD 0.36) for groups 1 and 2, respectively. Portions of the pooled 3 d samples of the formula, urine and faeces were analysed for fat, N, P, Ca and Mg. The results are expressed as means, the difference (Diff) between the means and 95% confidence intervals (CI) for the difference between the means:

Nutrient		Intake	Diff	95%CI	Absorption	Diff	95%CI	Retention	Diff	95%CI
Fat	1	5.26	0.00	(-0.82,	4.74	-0.11	(-0.89,			
(g/kg per d)	2	5.26		0.82)	4.85		0.67)			
Nitrogen	1	481.88	26.63	(-35.33,	383.0	15.0	(-28.92,	153.25	14.0	(-16.49,
(mg/kg per d)	2	455.25		88.58)	368.0		58.92)	139.25		44.02)
Calcium	1	96.18	-15.85	(-32.48,	29.98	-2.28	(-22.03,	26.46	-3.18	(-22.84,
(mg/kg per d)	2	112.02		0.78)	32.35		7.48)	29.64		16.49)
Phosphorus	1	71.96	-4.96	(-16.22,	42.36	2.65	(-7.19,	20.61	-2.13	(-10.27,
(mg/kg per d)	2	76.93		6.3)	39.71		12.49)	22.74		6.02)
Magnesium	1	11.89	-0.07	(-1.46,	3.96	-0.13	(-1.98,	1.78	0.04	(-1.58,
(mg/kg per d)	2	11.95		1.33)	4.09		1.73)	1.74		1.65)

There was no statistically significant difference (unpaired *t* test at the 5% level) in the intake, absorption and retention of nutrients between the two formula groups, which are similar to those of infants of the same age fed on cows' milk formula. Although the difference in the intake of Ca approached significance this was not reflected in its absorption and retention. However, the CI for Ca absorption and retention are wide and include values that would be of clinical significance; the need for a larger sample size is indicated to improve the precision.

This work was supported by Wyeth Laboratories (UK) Ltd.

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Relation of infant diet to childhood health and growth: The Dundee Infant Feeding Study. By A.C. WILSON[1], J.S. FORSYTH[1], S.A. GREENE[1], C. HAU[2], P.W. HOWIE[3], *[1]Departments of Child Health, [2]Epidemiology and Public Health and [3]Obstetrics and Gynaecology, Ninewells Hospital and Medical School, Dundee DD1 9SY.*

The aim of the present study was to determine the relationship between infant diet and subsequent respiratory illness, growth and blood pressure (BP) in childhood. The infant feeding data had previously been collected prospectively during the first 2 years of life as part of the Dundee Infant Feeding Study (Howie *et al.* 1990). The 674 children recruited to the original study (1983-1986) were contacted again aged 7-8 years. A history of respiratory illness was obtained by standardized questionnaire. Growth was assessed by measuring height, weight, skinfold thickness (four sites) and BP by random zero sphygmomanometer. The relationships between infant feeding and childhood respiratory illness, growth and blood pressure were investigated by linear modelling and adjusted for confounding variables.

The final number of completed questionnaires available for analysis was 544, mean age 7.2 years. BP measurements were obtained in 301 children, and growth measurements in 405 children, mean age 7.7 years. The percentage expected probability of respiratory symptoms occurring in the last 7 years and the mean fitted values for systolic BP are shown in the table.

	Wheeze			Cough		Breathless		Respiratory		Blood Pressure		
Feeding Groups	n	%	SD	%	SD	%	SD	%	SD	n	mmHg	SD
Exclusive Breast > 15 Weeks	141	12.8	9.1	11.3	3.3	8.5	6.9	17.0	6.6	74	90.3	3.6
Partial Breast	203	21.2	11.5	22.2	6.2	17.7	10.8	31.0	9.6	122	90.9	3.6
Bottle	199	18.6	11.5	24.6	7.1	12.1	8.9	32.2	10.5	105	94.2	3.8

After adjusting for confounding variables of sex, parental allergy and social class, there was a considerably lower fitted probability of ever having respiratory illness ($P<0.01$), breathlessness ($P<0.025$) and cough ($P<0.01$) in children who received breast milk exclusively for more than 15 weeks. In addition early solid feeding (<15 weeks) was associated with an increased probability of wheeze ($P<0.01$). Exclusive bottle feeding was associated with a higher systolic BP with a mean increase of 3..5 mmHg in children who were exclusively bottle fed compared with those who had received breast milk ($P<0.01$)(adjusted for maternal BP, sex, age and BMI). After adjustment for the confounding variables of maternal height and BP, sex, birth weight, weight at first solid feed, social class and BMI, the early introduction of solids was associated with increased body fat (BF) and weight in childhood (16.5% BF solids <15 weeks *v.* 15.5 % BF solids >15 weeks;$P<0.05$; Weight: 0.04 standard deviation score(SDS) solids <15 weeks *v.* -0.11 SDS solids >15 weeks;$P<0.05$).

In conclusion, the results from this present study indicate that in a developed society, exclusive breast feeding and delaying the introduction of solids until at least 15 weeks confers significant independent health benefits to the child. The significant differences in BP and body composition may have a beneficial effect on the development of subsequent adult disease, particularly in relation to cardiovascular illness. This provides clinical evidence to support the current national recommendations on duration of breast feeding and the timing of introduction of solids (Department of Health, 1994).

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Do early changes in dietary fatty acid composition alter lipoprotein metabolism in adulthood in the rat? By C. CHAPMAN, L.M. MORGAN and M.C. MURPHY, *Centre of Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

The development of coronary artery disease has been linked to maternal diet during pregnancy, early diet of the offspring and to the amount and type of dietary fat consumed in later life. Lipoprotein lipase (LPL; *EC* 3.1.1.34) is the key regulatory enzyme involved in the removal of triacylglycerol (TAG) from plasma and its deposition in adipose tissue. We have shown previously that a high-fish-oil diet increases LPL mRNA levels in adult rats (Murphy *et al.* 1993) and postheparin LPL activity in humans (Zampelas *et al.* 1994). The present study investigated whether a fish-oil diet in pregnancy and early life can set a level of LPL gene expression and activity in offspring. Ten pregnant female Wistar Albino rats were given a diet containing 50g fish oil or mixed oil/kg for the last 14 days of gestation and 21 days post-partum. The litter sizes were standardized at birth and offspring fed on the maternal diet for 2 weeks post-weaning. Half the offspring were killed at 2 weeks post-weaning (young); the remaining rats were placed on normal chow and culled 5 weeks later (adults). Rats were starved on the day before culling, fed *ad libitum* on a standard high fat diet (250g mixed oil/kg) overnight and killed at 09.00 hours the following day after blood collection by cardiac puncture. Heparin-releasable LPL activity was measured in omental adipose tissue and LPL mRNA quantitation by Northern blot was measured in epididymal adipose tissue in the males. Statistical differences were identified following ANOVA using a Tukey-Kramer multiple comparison test.

	TAG (mmol/l)		Insulin (pmol/l)		Glucose (mmol/l)		Glucose: insulin ratio		GIP (pmol/l)		LPL Activity (mU)		% LPL/ACTIN mRNA	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fish 1	4.46[a]	0.47	249.85	31.26	7.83[a]	0.53	0.04	0.005	608[a]	391	15.05[a]	6.53	16.90	4.7
Fish 2	1.55[b]	0.13	418.64	50.18	9.73[b]	0.36	0.03	0.004	236[b]	72	2.50[b]	0.94	64.97[a]	11.0
Mixed 1	2.77[b]	0.19	272.39	72.70	7.75	0.45	0.04	0.007	630[a]	242	6.38	1.15	17.62[b]	4.8
Mixed 2	1.55	0.13	257.62	31.96	8.89	0.50	0.04	0.004	170[b]	65	1.13	0.62	280.4[b]	27.3

1 and 2 represent young and adults respectively.

[a,b] Mean values within a column not sharing a common superscript letter were significantly different ($P < 0.05$).

There were no significant differences in food intake or weight gain between the groups throughout the study. The fish-oil-fed young had significantly higher TAG, glucose-dependent insulinotropic polypeptide (GIP) and LPL activity levels than the fish-oil-fed adults. These responses were mirrored in the mixed-oil-fed group but only the change in GIP reached significance. LPL mRNA was significantly lower in the fish-oil-group adults and mixed-oil-fed young when compared with the high level seen in the mixed oil fed adults. There were no significant changes in the glucose:insulin ratios. GIP and TAG levels were significantly correlated ($P < 0.001$). The majority of the differences shown were related to the age of the animals. Significant differences between the dietary groups occurred only in TAG levels in young animals and LPL mRNA in adults. LPL activity and expression measured in different tissues showed no correlation.

In conclusion, most of the differences shown were between young and adults and suggest that changes in the postprandial handling of a standard test meal occur with age. Early diet affects LPL expression but not activity later in life; however, future studies are required to elucidate the mechanisms of these effects.

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The response of blood pressure to feeding composite diets of differing macronutrient composition in rats with hypertension of fetal origin. By C.L. PICKARD[1] and H.D.McCARTHY[2], [1]*Department of Human Nutrition, University of Southampton, Southampton SO16 7PX;* [2]*School of Life Sciences, University of North London, London N7 8DB*

Exposure *in utero* to a maternal diet marginal in protein content induces hypertension in rats when maintained on standard laboratory chow post-weaning (Langley & Jackson, 1994). We have previously shown that this hypertension can be reversed by feeding a macronutrient self-selection diet and postulated that this might be due to a greater fat:carbohydrate ratio in the diet (Pickard *et al.* 1996). The aim of the present study was to clarify this by measuring systolic blood pressure (SBP) while feeding diets differing in macronutrient composition post-weaning, to offspring with hypertension induced *in utero*.

Thirty-two female offspring were used from dams which had either been maintained on 90 g casein/kg or 180 g casein/kg diet 2 weeks before and throughout pregnancy. At age 4 weeks, offspring were weaned and SBP measured using the tail-cuff method. Eight rats from each prenatal dietary group were fed on one of four postnatal diets, standard laboratory chow SDS CRMX (SLC), high-carbohydrate (540g maize starch and 100g sucrose/kg), high-fat (295g lard and 100 maize oil/kg) and high-protein (490g casein/kg). Food intake, weight gain and SBP were measured at 2 week intervals.

		Systolic blood pressure (mm Hg)							
Age (weeks)		4		6		8		10	
Offspring diet	Maternal diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE
SLC:	90 g/kg	140.5*	2.1	145.2*	3.7	145.8*	1.6	147.1*†	1.9
	180 g/kg	112.7	0.7	123.3†	2.6	129.5†	1.5	130.8†	1.5
High-Carbohydrate [a]	90 g/kg	136.5*	3.3	144.4*	4.3	145.1*	1.4	143.0*	1.6
	180 g/kg	111.9	2.6	126.4†	4.5	125.0†	2.4	127.0†	2.1
High-fat [b]	90 g/kg	151.9*	4.3	132.0†	7.0	126.8†	7.5	118.3*†	3.3
	180 g/kg	128.4	1.7	136.4	7.9	141.1†	6.7	144.3†	5.4
High-protein [c]	90 g/kg	151.9*	4.3	143.8	7.4	130.9†	6.2	127.5†	3.9
	180 g/kg	128.4	1.7	132.8	6.3	133.5	5.6	137.1†	5.0

*Mean values were significantly different from those for the 180 g/kg group P<0.05
†Mean values were significantly different from weaning value, P< 0.05
[a]20%protein (p), 61.5%carbohydrate (c), 10%fat (f); [b]20%p, 32%c, 39.5%f, [c]49%p, 32%c, 10%f

The SLC results confirm those shown previously. The high-carbohydrate diet also maintains high blood pressure in the 90 g casein/kg offspring indicating that it is not a specific component in SLC causing the increase in blood pressure. The high-fat feeding quickly abolished and high-protein feeding gradually reversed the hypertension in the 90 g casein/kg group without affecting the age-related increase in SBP in the 180 g casein/kg group.

These results support our previous observations that postnatal diet can influence the expression of the hypertension in this model. However the inhibitory effect is not unique to a high-fat diet but occurs also with a high-protein diet. This suggests that a low dietary carbohydrate intake may underlie this hypotensive effect.

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Haemoglobin as a predictor of birth weight in women of North West Frontier Province, Pakistan.

By B. AKRAM[1], P.I. PARACHA[2], J. KHALIL[2], L. RUKH[3] AND B. AFRIDI[3]. *[1]Family Health Project, Government of NWFP, Peshawar, [2]Department of Human Nutrition, NWFP Agricultural University, Peshawar, [3]Department of Gynaecology and Obstetrics, Hayat Shaheed Teaching Hospital, Peshawar, Pakistan*

A hospital-based study was conducted on pregnant women to assess the prevalence of anaemia and its effect on anthropometric measurements of newborn babies. One hundred pregnant women in the last week of third trimester who had completed 37 weeks of gestation were enrolled for the study. Information regarding demographic and socioeconomic characteristics was collected by interviewing the women. A food-frequency questionnaire was designed to estimate the food intake and the consumption of vitamins and minerals. Venous blood was collected from the women before delivery to assess haemoglobin (Hb). Women with Hb < 110 g/l were characterized as anaemic while those with Hb $\geq$ 110 g/l were categorized as non-anaemic. Anthropometric measurements including weight, length and head circumference of the newly born babies were taken. Weight and length of the babies were then compared with the National Center for Health Statistics (NCHS) reference standards for US babies (Hamill et al. 1979) to obtain the Z-scores.

Demographic and socioeconomic results revealed that mean number of people was about five per family and that anaemic women had a greater number of people in the family than the non-anaemic women. Mean family income of the anaemic women was also found to be significantly lower than that of the non-anaemic women. The dietary practices of the women indicated that the majority of the women did not consume any additional food during pregnancy and that frequency of dairy, meat, vegetables and fruit consumption of the anaemic group was significantly lower than that of the non-anaemic group. Biochemical analysis of blood samples revealed that 56% of the women were anaemic with mean Hb of 97.5 g/l while 44% were non-anaemic with mean Hb of 126.9 g/l. Results of the anthropometric measurements showed that 20% of the babies were of low weight (<2.5 kg). Among the fifty-six anaemic women, ninteen gave birth to underweight babies compared to only two out of forty-four of the non-anaemic women. Similarily, mean weight-for-age, height-for-age and weight-for-height Z-scores of the babies from the anaemic women which were recorded to be -0.98 (SD 1.08), -0.04 (SD 0.77),-1.52 (SD 0.95), respectively, were significantly lower than those of the non-anaemic women. Regression analysis revealed that Hb level of the pregnant women was the strongest predictor of weight-for-age Z-score of the babies accounting for 45.3% of the total 56.1% of the variance explained by the model.

The results of the study suggest that anaemia is highly prevalent among pregnant women increasing the chance of an unfavourable pregnancy outcome i.e., low birth weight babies.

Hamill, P.V., Drizd, T.A., Johnston, C.L., Reed, R., Roche, A.F. & Moore, W.M. (1979). *American Journal of Clinical Nutrition* **32**,607-629.

A comparison of urine-bags and super-absorbent nappies for collection of urine to measure intestinal permeability. By F.S.W. McCULLOUGH, C.A. NORTHROP-CLEWES and D.I. THURNHAM, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

The dual sugar permeability test (DSPT), using lactulose (L) and mannitol (M) provides a non-invasive technique for assessing pathogenic alterations in the structure and integrity of the small intestinal mucosa and the method is ideally suited for field conditions. Traditionally, urine bags have been used for the 5 h collection in infants. However, difficulties have been experienced with bags leaking, and the objective of the present study was to test an alternative method of urine collection using nappies. Disposable nappy manufacturers claim that the Aridall polymer in super-absorbent nappies combines with the urine, holding the wetness away from the skin and helping to prevent it mixing with solid waste. Nappies have been successfully used for urinary steroid analysis (Taylor & Curran, 1994). However, the DSPT relies on the collection of sugars in the urine and the presence of contaminating faecal matter, which contains many bacteria, may result in loss of sugars.

Thirty healthy Irish babies (aged 3-15 months) were given L (400 mg) and M (100 mg) per kg body weight in 2 ml water. Urine was collected for 5 h on two occasions, first using a paediatric urine bag (Hollister) and secondly a super-absorbent nappy (Pampers), used with a disposable nappy liner to help prevent any faecal matter contaminating the nappy. Urine from the nappies was extracted according to the method of Taylor & Curran (1994). Faecal contamination was mostly retained in a separate part of the nappy from the urine. Urinary creatinine was measured using a modification of the Jaffe reaction (Jaffe, 1986), while L and M were measured enzymatically (Lunn *et al.*, 1989, Northrop *et al.*,1990) on a Cobas-Bio autoanalyser (Roche product). The urine-bag and nappy results were compared statistically using the Wilcoxon matched-pair signed-rank test.

	n	Urine Bag			Nappy			*P* Value
		Median	CI 5%	CI 95%	Median	CI 5%	CI 95%	
Lactulose:mannitol	30	0..185	0.187	0.500	0.100	0.120	0.357	0.399
Lactulose:mannitol	30	0.375	0.241	1.086	0.235	0.224	0.804	0.621
Mannitol:creatinine	30	2.090	1.740	3.819	2.255	1.870	3.335	0.658

The Table shows no significant differences between the two methods of urine collection. Therefore it may be possible to use nappies with DSPT. This could have many advantages such as cost savings, being more acceptable to mothers, no leakage problems and a potentially complete urine collection. During the study 31% nappies were soiled, possibly due to the mild laxative effect of the sugar solution but these results indicate that bacterial contamination did not occur to any significant extent or alternatively, equal amounts of both sugars may have been lost. In developing countries diarrhoea and nappy soiling are more likely, therefore we intend to investigate further the feasibility of using nappies for the DSPT in the Third World.

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Growth rates of infants in the Upper Nuimi District of The Gambia, West Africa. By A.E. CATHCART[1], C.A. NORTHROP-CLEWES[1], E.M.E. POSKITT[2], and D.I. THURNHAM[1], *[1]Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA, and [2]Medical Research Council, Dunn Nutrition Centre, Keneba, The Gambia, West Africa.*

Most previous studies on growth in The Gambia have been reported from the West Kiang district where the predominant tribal group is Mandinka and the diet is based mainly on rice (*Orzya sativa*). In this report, we describe growth rates of infants in the Upper Nuimi district where the main tribal group is Wollof and the diet is mainly millet (*Pennisetum gambiense*). While working in Upper Nuimi for 18 months I was suprised at my lack of sickness and this, together with the apparently healthy population stimulated us to collect growth statistics on infants in the area.

A cross-sectional study was carried out in five Mother and Child Health Clinics in the Upper Nuimi district of The Gambia during October and November 1995. The predominant tribal group in three clinics was Mandinka with the remaining two being Wollof. All infants who attended the clinics and were under the age of 24 months were included. Weight and length were measured and sex, date of birth, and tribal group were recorded from the infant clinic cards. Anthropometric measurements were compared with National Center For Health Statistics (NCHS) standards (Hamill *et al.* 1979) and the weight-for-age (WAZ) and length-for-age (LAZ) Z-scores were calculated.

In the present report we discuss these data in relation to growth rates for infants from the West Kiang district of The Gambia (Lunn *et al.* 1991). Our results are expressed as age group means with both sexes and all tribes taken together. The West Kiang results are available for 3, 14 and 15 months of age.

		Upper Nuimi district					West Kiang district		
Age (months)		0-3	3-6	9-12	12-15	18-24	3	14	15
LAZ	Mean	0.00	-0.20	-0.70	-1.00	-0.90	-0.781	-2.13	-2.094
	SD	1.74	1.37	1.31	0.89	0.98			
	n	82	90	87	96	119	119	119	119
WAZ	Mean	-0.31	-0.24	-1.50	-1.66	-1.54	-0.252	-2.03	-2.33
	SD	1.80	1.35	1.21	1.01	0.88			
	n	82	90	87	97	127	119	119	119

The LAZ and WAZ for both groups are shown. Z-scores of -2.00 and 0.00 are comparable with the NCHS 5th and 50th centiles for growth. There were no significant differences within the data for sex or tribal grouping (results not shown). For the first 3 months of life, the mean LAZ was 0.00 showing that length growth was on the 50th centile, while weight gain was slightly below the 50th centile with a WAZ of -0.31. From 3-6 months onwards mean length and weight growth started to fall, stabilizing at 12-15 months with a LAZ of -1.00, and WAZ of -1.66. At this age infants were on the 25th centile for length growth and on the 5th centile for weight gain. West Kiang data showed a similar growth pattern to that described here but the overall Z-scores were lower than those of Upper Nuimi infants except at 3 months when the WAZ was -0.252 for West Kiang. At no point did mean growth rates in the Upper Nuimi district fall to -2 Z-scores suggesting that growth faltering was less severe than in the West Kiang district.

The results discussed here highlight a difference in infant growth rates between two areas of The Gambia. These two areas differ in their tribal representation, cropping pattern and in the adequacy of medical services available. An investigation into the possible reasons for these differences may be warranted in the light of the extent of growth faltering.

Hamill, P.V.V., Drizd, T.A., Johnston, C.L., Reed, R., Roche, A.F. & Moore, W.M. (1979). *American Journal of Clinical Nutrition* **32**, 607-629.

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The accuracy of data describing the onset of weaning in South Africa's Birth to Ten study. By GEORGE T.H. ELLISON[1], LUCY WAGSTAFF[2] and THEA DE WET[3], [1]*Institute for Behavioural Sciences, University of South Africa, Pretoria;* [2]*University of the Witwatersrand Medical School, Johannesburg; and* [3]*Birth to Ten, Medical Research Council, Johannesburg, South Africa*

Recall accuracy for infant feeding transitions, such as sevrage and the introduction of formula or solid food, depends upon the time elapsed since the transition took place and how memorable or salient the transition was (Quandt, 1987). To assess whether these factors influenced the reliability of infant feeding data collected in the Birth to Ten study (Richter *et al.* 1995) we calculated the percentage agreement between data collected at different times during the first year of the study. Information was available at both 6 and 12 months for 1354 of the children enrolled in the study, and duplicate questionnaires had also been completed for ninety-seven of these children whose carers were interviewed twice at either 6 months (*n* 40) and/or 1 year (*n* 61). During these interviews carers were asked when the child had stopped breast-feeding (sevrage), and when he or she had first received bottle and/or cup feeds (containing formula or cows milk) and other (solid) foods. If children were reported to have stopped breast-feeding, or to have started other feeds before both interviews, it was possible to calculate the percentage agreement between the two reports, to the nearest week, 2 weeks or 1 month.

Percentage agreement between reports of infant feeding transitions collected at the 6 and 12 month interviews

Interviewees:	Mothers and carers		Mothers only		Mothers only	
Interview:	Repeat interviews at 6 and 12 months		Repeat interviews at 6 and 12 months		Duplicate interviews at 6 and/or 12 months	
	%	*n*	%	*n*	%	*n*
Duration of breastfeeding:						
to the nearest week	35.8	260	37.4	198	58.3	12
to the nearest 2 weeks	49.6	260	52.0	198	66.7	12
to the nearest 4 weeks	76.2	260	78.3	198	75.0	12
Age at introduction of bottle and/or cup feeds:						
to the nearest week	27.3	788	27.5	612	41.1	34
to the nearest 2 weeks	34.6	788	35.3	612	50.0	34
to the nearest 4 weeks	55.2	788	56.0	612	67.6	34
Age at introduction of other foods:						
to the nearest week	33.4	1254	35.3	1041	38.1	63
to the nearest 2 weeks	36.5	1254	37.9	1041	39.7	63
to the nearest 4 weeks	65.5	1254	67.1	1041	68.3	63

The percentage agreement between repeat reports of feeding transitions was low, ranging from 30-70%. To some extent this poor reliability may have been the result of using data from different interviewees (carers and/or mothers) at 6 and 12 months. When the analyses were restricted to maternal reports at both interviews there was a consistent, albeit modest, improvement in reliability. An additional improvement in reliability occurred when we compared maternal reports of infant feeding transitions obtained from duplicate interviews conducted at either 6 months or 1 year. The potential effect of memory deficit was lower in this group because there was a shorter time delay between the two interviews. However, because the events reported in both interviews could have occurred up to 6-12 months earlier, memory deficits might still have caused much of the remaining inaccuracy. Reliability was highest for reports of sevrage, which suggests that this was the most memorable feeding transition. Yet even the reliability of sevrage was modest, with only 75% agreement to within 1 month between repeat reports. Perhaps these are levels of inaccuracy inherently associated with maternal reports of infant feeding patterns, because they closely resemble the poor validity described by Quandt (1987).

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The nutritional status and weaning foods of infants and young children in Central Uganda. By JOYCE K. KIKAFUNDA,[1,2] ANN F. WALKER,[1] RICHARD B. KAJURA,[2] and RICHARD BASALIRWA,[2] [1]*Department of Food Science and Technology, The University of Reading, Whiteknights, Reading RG6 6AP and* [2]*Department of Food Science and Technology, Makerere University, P. O. Box 7062, Kampala, Uganda*

Recent health surveys in Uganda have revealed that the nutritional status of children is very poor (Ministry of Health, 1988/89). Early work done in Uganda indicated that the traditional banana (matoki)-based starchy foods fed to young children do not provide the growing child with adequate energy and nutrients (Rutishauser, 1973). Therefore the present study was undertaken to assess the nutritional status of young children in Uganda and to determine the nutritional quality of the traditional starch-based weaning foods.

The investigation was a cross-sectional study-survey that included an administered questionnaire in a predominantly matoki and maize zone in Central Uganda. A total of 261 children aged 3-28 months (15.7 (SD 6.4) months) participated in the study. Their nutritional status was assessed by anthropometry, interpreted against the US National Center for Health Statistics (NCHS) international reference population.

Of the children studied, 24.1% and 23.8% were found to be underweight and stunted respectively (2 or more SD below the mean of the reference population for weight or height-for-age and gender) and 17.2% had low mid-upper-arm circumference (<135 mm). Children from low economic status households were significantly ($P \leq 0.034$) more stunted than those from higher economic status households.

During the study-survey, "ready-to-feed" traditional weaning food samples were requested from the mothers, oven-dried (100 °C) and air-freighted to Reading for analysis. The solid foods were made up of a starchy staple mixed with a sauce. The staples were dominated by matoki and the sauces by beans (*Phaseolus spp.*). The porridges were prepared mostly from maize flour. The samples were analysed for the macronutrients and Zn using standard analytical procedures. The results are shown in the Table (values per kg food as fed).

	DM (g/kg)		Energy (kJ/kg)		Protein (g/kg)		Fat (g/kg)		Zn (mg/kg)	
Food type	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Solid food	240	40	3590	830	29	13	12	13	4	2
Porridge	110	30	1530	390	11	4	1	1	2	1

The energy density of the foods was found to be very low. The mean energy density of the solid foods was only about half the recommended energy density for young children. The mean energy density of the porridges was about half the energy density of human milk. The protein content of the foods was found to be adequate but the fat content was very low, contributing to the low energy density of the foods. The Zn content of the foods was also found to be low.

In view of these findings, strategies to improve the quality of starch-based traditional weaning foods need to be put in place to alleviate the general poor nutritional status of children in Uganda.

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Effect of zinc supplementation on growth of school children in Uganda. By JOYCE K. KIKAFUNDA,[1,2] ANN F. WALKER,[1] RICHARD B. KAJURA[2] and JAMES K. TUMWINE,[3] [1]*Department of Food Science and Technology, The University of Reading, Whiteknights, Reading RG6 6AP,* [2]*Department of Food Science and Technology, Makerere University, P. O. Box 7062, Kampala, Uganda and* [3]*Department of Paediatrics, Makerere University, P. O. Box 7062, Kampala, Uganda*

It has been postulated that a mild Zn deficiency may limit growth in otherwise healthy children. High rates of poor growth have been reported in Ugandan children under 5 years of age. The present study was undertaken to investigate the effect of Zn supplementation on growth of Ugandan school children.

A total of 153 children aged 33-89 months (55.8 (SD 11.2) months), from three nursery schools in the suburbs of Kampala city, Uganda, participated in the present study. Over half of the children (50.3%) were below the 20th height-for-age centiles. The children were randomly assigned to one of two supplementation groups; Zn or placebo. The Zn group received a daily supplement of 10 mg Zn (as $ZnSO_4$) in 200 ml fruit juice for a total treatment period of 6 months. The placebo group received the same juice with placebo tablets instead of the Zn tablets. Anthropometric measurements of weight, height and mid-upper-arm circumference (MUAC) were taken at baseline and after 2, 3, 6, 7 and 8 months. Sickness records were kept throughout the trial. The trial was two-phased, each supplementation phase lasting 3 months with a 2-month period in between with no supplementation.

	LSM changes in weight (kg)				LSM changes in height (cm)				LSM changes in MUAC (mm)			
	Zinc		Placebo		Zinc		Placebo		Zinc		Placebo	
Time†	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2‡	0.22	0.03	0.18	0.03	0.64	0.05	0.57	0.05	-1.22	0.38	-1.23	0.39
3‡	0.39	0.04	0.31	0.04	1.20	0.06	1.13	0.06	-0.08	0.42	-1.25	0.42
6§	1.02	0.05	0.88	0.06	2.98	0.10	3.20	0.10	0.30	0.56	-0.24	0.59
7§	1.17	0.06	1.04	0.07	3.88	0.10	3.92	0.10	0.03*	0.55	-1.72	0.46
8§	1.33	0.07	1.21	0.07	4.38	0.10	4.25	0.10	0.86*	0.57	-0.82	0.60

LSM, Least square means.

* Mean values were significantly different from placebo, $P<0.05$.

† Months.

‡ *n* 153; of which 78 were given Zn and 75 placebo.

§ *n* 113; of which 59 were given Zn and 54 placebo.

MUAC showed a significant response to Zn supplementation compared to placebo after 7 months ($P\leq0.033$) and 8 months ($P\leq0.029$) from the start of the trial. Although the Zn-supplemented group had higher weight increments throughout the trial than the controls, the effect did not reach statistical significance. Mean increments in height were variable between the two groups. Sickness rates were lower in the Zn-supplemented group than the controls with children in the Zn group falling sick at an average rate of 1.36 times per group per week compared with 1.82 times per group per week in the controls.

A mild but significant effect of Zn supplementation on MUAC of children has been established, in agreement with the work of Bates *et al.* (1993). However, more research is needed involving children from a wider cross-section of the country in order to ascertain the role of Zn in the nutrition of children in Uganda.

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Effect of zinc supplementation on growth and morbidity of undernourished Jamaican children. By J. MEEKS GARDNER[1], M. WITTER[2] and D. RAMDATH[1], *Tropical Metabolism Research Unit, University of the West Indies, Mona, Jamaica, West Indies,*[2] *Stanford University School of Medicine, Stanford, California, USA,* [3]*Department of Biochemistry, Faculty of Medical Sciences, University of the West Indies, St. Augustine, Trinidad, West Indies.*

Low Zn status is associated with poor growth in children. However, the results of Zn-supplementation studies are inconsistent (Allen, 1994). We investigated whether there was a response to Zn supplementation of undernourished children in Kingston, which would indicate Zn deficiency.

Sixty-one undernourished children were recruited from nutrition clinics in Kingston. Children selected were singletons aged 6-24 months, below -2.0 SD length for age (National Center for Health Statistics references, Hamill et al., 1979), with no physical or mental handicap, who were not being breast-fed. The children were stratified by sex and age (6-11, 12-17,18-24 months), and randomly assigned to receive Zn supplementation (n 31) which comprised 5 mg elemental Zn daily as $ZnSO_4.7H_2O$ in 5 ml flavoured syrup, or 5 ml placebo daily (n 30), which comprised the syrup alone. Parents were interviewed to obtain social background data. Anthropometric measurements were carried out on enrolment and after 6 and 12 weeks. Each week, seven individual doses of the supplement or the placebo were delivered to the homes, and the empty bottles of the previous week's doses retrieved. At these weekly visits, a morbidity questionnaire was given to the caretakers to determine the children's health. Any days with apathy, anorexia, cough, colds, ear infections, diarrhoea, rash, vomiting or other illnesses which occurred during the previous week were recorded. Clinic visits and hospitalizations during the study period were recorded. The reliabilities of the interviewers and anthropometrists were checked before the study began. Appropriate transformations were used to normalize the data. Five children, all from the control group, were hospitalized during the study period. Four children were in hospital for extended periods. They were excluded before final measurements were taken, and their data were not included in the growth and morbidity analyses.

The Zn-supplemented and control groups were very similar on enrolment. There were no significant differences in mean age, length (Zn-supplemented 7.1 kg SD 0.9 kg, placebo 6.9 kg SD 1.1 kg), weight (Zn-supplemented 68.7 cm SD 4.8 cm, placebo 68.6 cm SD 4.9 cm), head circumference, caretaker's age, number of siblings, sanitation (water and toilet scores), or number of possessions (proxy for socio-economic status).

Regression analyses showed that there were no significant effects of supplementation on length (+12 weeks:Zn-supplemented mean 71.3 cm SD 4.6 cm, placebo mean 71.8 cm SD 80.8 cm), weight (+12 weeks: Zn- supplemented mean 7.5 kg SD 0.9 kg, placebo mean 7.5 kg SD 1.1 kg) or head circumference during the first 6 weeks, the second 6 weeks, or over the whole 12 weeks. Analyses of covariance of the number of episodes of each illness, and their mean duration, were calculated with group (Zn-supplemented or control) as the between-subjects factor. Covariates were child's sex and age on enrolment, toilet and water ratings, and crowding. Zn supplementation had no significant effect on incidence of any symptom. Mean duration of the episodes was significantly shorter for skin rashes in the Zn supplemented group (median 5.9 days, upper and lower quartiles, 1.8, 7.2) compared with the control group (median 9.0 d, quartiles, 5.2, 12.8) (P=0.02), and longer for vomiting (median 2.0 d, quartiles 1.2,5.0 v. median 1.0 d, quartiles, 0.5, 1.8, P=0.02). The reported number of clinic visits was not significantly different between the groups. The incidence of hospitalization was significantly greater in the control group (Fisher's exact test, two-tailed, P=0.02), which suggests that the children's Zn status may have been marginal.

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Zinc intake from non-breastmilk sources in the first year of life in a poor urban slum in Manaus, Amazonas, Brazil. By *Roger Shrimpton, UNICEF, PO Box 1202, Jakarta 10012, Indonesia.*

Despite the fundamental importance of Zn for cell division, little is known about Zn intakes in infancy, a phase when growth is predominantly by cell multiplication. Previous studies suggest that Zn may be limiting in Amazonian household diets (Shrimpton, 1984). In the present study the Zn intake was assessed for forty-two boys and forty girls in the first year of life by 24 h recall dietary interview with their mothers, in a poor slum area of urban Manaus. The Zn concentration of food mixtures used to feed infants was calculated using values for the Zn content of infant foods obtained by analysis. Energy and protein values were obtained from the manufacture's labels and food composition tables. Mothers giving food mixtures in addition to putting their infants to the breast more than three times daily were classified as "complementing" breastmilk. Those breast feeding 1-3 times daily and giving other foods were classified as "supplementing" breastmilk. Those giving no breastmilk had "substituted" other foods for breastmilk. The adequacy of these three types of food mixtures was assessed by comparing nutrient energy densities with recommended values.

The majority (62.2%) of the eighty-two mothers interviewed were breast-feeding their infants. Few infants were exclusively breast-fed (4.9%) and many (36.6%) were not breast-fed at all. The most common feeding method was a combination of breastmilk and various food mixtures. Four foods were common to 40.0% of all food mixtures: cassava flour; sugar; infant formula and whole-milk powder. The use of whole-milk powder was as frequent as the use of infant formulas. Half of the mothers who used infant formulas mixed them with cassava flour, sugar or other such foods.

	Infants 0-5 months				Infants 6-11 months			
	Mean	SD	*n*	%RDA	Mean	SD	*n*	%RDA
Zinc:energy ratios (mg/MJ)								
Breastmilk substitutes	0.54	0.22	11	44.6	0.62	0.25	19	51.2
Breastmilk supplements	0.45	0.19	11	37.2	0.67	0.41	6	55.3
Breastmilk complements	0.62	0.18	19	51.2	0.63	0.24	6	52.0
Zinc intakes (mg/d)								
Breastmilk substitutes	1.31	1.2	11	43.7	2.44	2.26	19	48.8

The mean Zn:energy ratio (mg Zn/MJ) of these food mixtures ranged from 37-55% of the recommended ratios. None of the sixty-two food mixtures met the recommended Zn:energy ratio of 1.22 mg/MJ, and 87.5% were below 70% of the recommended level. The total Zn intake of non-breast-fed infants was 45-50% of the recommended daily amount.

In thirty non-breast-fed infants the average adequacy of protein intake was double the recommended level, and the mean energy intake was the same as the recommended level. The mean protein energy percentage (PE%) of the food mixtures was 10.2 (SD 3.4) % and the Zn:energy ratio 0.59 (SD 0.24) mg/MJ Only 23% of the diets had a PE% of less than 8, the recommended value for infants assuming a net protein utilization rate of 80. The PE% values and the Zn:energy ratios of the diets of thirty non-breast-fed infants were significantly correlated (r 0.83, $p < 0.001$). A typical food mixture would need to have a PE% of 21.1 in order to meet the recommended Zn:energy ratio. These results suggest that food mixtures used to feed infants in Manaus, whilst largely adequate for protein, are not adequate for Zn.

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The role of oxidative stress and DNA damage in the aetiology of malnutrition-related diabetes mellitus. By MARGARET P. MCDONAGH[1], LIAQUAT ALI[2], AZAD KAHN[2], PETER R. FLATT[1], YVONNE A. BARNETT[1] and CHRISTOPHER R. BARNETT[1], [1]*School of Biomedical Sciences, University of Ulster, Coleraine, BT52 1SA. and* [2]*Research Division , BIRDEM, Dhaka, Bangladesh*

Chronic undernutrition over a lifetime may be an important determinant of diabetes in an individual either by increasing the susceptibility of the individual to other genetic and environmental diabetogenic influences or by progressively impairing beta cell function (Rao, 1984). Undernutrition is a pre-eminent feature of the two major clinical syndromes, protein-dependent diabetes mellitus (PDDM) and fibrocalculous pancreatic diabetes (FCPD). These forms of diabetes are common in tropical countries and have been grouped together under the term malnutrition-related diabetes mellitus (MRDM).

Antioxidant status and DNA damage determined by an ELISA measuring single strand DNA (ssDNA) were examined in malnourished patients in Dhaka, Bangladesh displaying PDDM (*n* 24), FCPD (*n* 14) and in non-insulin-dependent diabetes mellitus (NIDDM) patients (*n* 46). Comparisons were made with age and sex-matched controls (*n* 67).

	Control		Malnourished Control		NIDDM		PDDM		FCPD	
	(*n* 26)		(*n* 41)		(*n* 46)		(*n* 24)		(*n* 14)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Vitamin A (μmol / l)	1.67	0.10	1.47	0.06	1.45	0.06	1.13**	0.09	1.04**	0.09
Vitamin C (μmol / l	6.71	0.96	4.76	0.77	4.49	1.20	3.10	1.34	3.48	1.95
Vitamin E (μmol/l)	13.56	0.78	11.88	0.50	20.09†††	1.02	15.10	1.13	10.54	1.30
Catalase (EC 1.11.1.6) (U/gHb)	45.1	2.3	43.3	2.0	37.6†	1.8	33.1***	2.1	36.1**	1.8
Superoxide dismutase(U/gHb) (EC 1.15.1.1)	998	27.8	1149	25.4	1137†††	33.6	1111	47.5	994***	26.9
Glutathione peroxidase (U/gHb) (EC 1.11.1.9)	32.0	1.8	27.7	1.7	27.2	1.8	31.8	2.5	37.7***	2.2
Caerulo-plasmin (U/l) (EC 1.16.3.1)	625	28.5	656	26.9	942†††	46.4	858***	44.3	838*	55.2
%ss DNA	34.28	8.4	42.39	12.9	31.46	7.24	43.19	12.9	36.35	9.94

Mean values were significantly different from those for malnourished control *p<0.05,**p<0.01 and *** p<0.001.

Mean values were significantly different from those for normal control †p<0.05, ††p<0.01 and †††p<0.001.

The Table shows that levels of plasma antioxidant vitamin A were reduced in all malnourished diabetics (PDDM and FCPD), as was catalase activity. In contrast caeruloplasmin levels and glutathione peroxidase activity were increased. Plasma vitamin E levels were significantly increased in the NIDDM patients when compared with the normal contrtols. Superoxide dismutase activity was decreased only in the FCPD subgroup. Percentage single strand(%ss) DNA breaks were elevated in malnourished diabetics when compared with NIDDM patients. These results indicate that MRDM is associated with increased DNA damage and oxidative stress.

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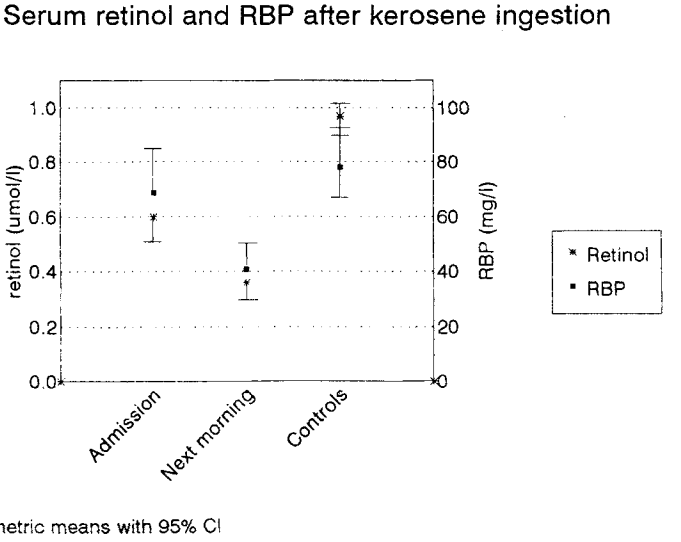
Effect of acute toxic epithelial damage on vitamin A metabolism in South African children. By JUANA F. WILLUMSEN[1], KARIN SIMMANK[2], SUZANNE M. FILTEAU[1], LUCY WAGSTAFF[2] and ANDREW M. TOMKINS[1], *[1]Centre for International Child Health, Institute of Child Health, London WC1N 1EH and [2]University of Witwatersrand, Johannesburg, South Africa*

Infections acutely alter vitamin A distribution and may reduce liver stores through increased demand for, or excretion of, vitamin A. Animal studies suggest that epithelial infections place a particular burden on retinol stores, as retinol may be required for epithelial repair. In the present study we have investigated acute, toxic lung epithelial damage caused by accidental kerosene ingestion and aspiration. Kerosene poisoning is still a major cause of childhood hospitalization in many developing countries. Children aged 1-3 years admitted to Baragwanath Hospital in Soweto after kerosene ingestion were eligible for this study.

Blood samples were collected on admission and the following morning. Both samples were analysed for serum retinol, retinol binding protein (RBP) and two positive acute-phase proteins (α_1-acid glycoprotein and C-reactive protein). Neighbourhood controls of similar age were also recruited. There was no significant difference between cases and controls with respect to weight or height.

Final laboratory results show serum retinol levels (geometric means) of cases (*n* 23) were 0.60 (95% CI 0.52-0.70) μmol/l on admission and 0.36 (95% CI 0.30-0.43) μmol/l the next morning, compared with control levels of 0.97 (95% CI 0.88-1.07) μmol/l. Serum RBP also showed a marked decrease the day after admission, possibly as a result of high urinary losses on the first day (34.33, 95% CI 21.57-54.63 μg RBP/mmol creatinine) compared with controls 9.82, 95% CI 6.80-14.18 μg RBP/mmol creatinine)



geometric means with 95% CI

These results suggest that serum retinol decreases earlier and to a greater extent than does serum RBP. Therefore acute toxic epithelial damage, with a systemic acute-phase response indicated by a sharp rise in C-reactive protein levels,affects retinol metabolism by mechanisms separate from thos of decreased RBP secretion or increased urinary RBP losses.

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Crop production and its influence on economic status and the nutritional status of children in Malawi.
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Food production and availability, economic development and health interact substantially to determine the present world nutrition situation Administrative Committee on Coordination/Subcommitmittee on Nutrition (ACC/SCN 1987). The economy of Malawi is based on agriculture, in particular crop production. Changes in crop production are therefore expected to result in changes in quality of life which would be reflected in child nutritional status. In the present study the effect of agricultural production in Malawi on child nutritional status was explored, by examining the relationship between child growth and economic status of the population. The hypothesis assumes either (1) crop production is responsible for food supply and directly affects levels of consumption and nutritional status or (2) crop production affects economic status of the population and thereby nutritional status, or a combination of (1) and (2).

The analysis used raw data from the nationally representative Malawi Demographic Health Survey conducted in 1992. Data on sources of water, presence of toilet facilities, housing condition, ownership of radio and lamps were obtained from a total of 4849 women aged 15-49 years. Anthropometric data were obtained from their 3248 children. The National Centers for Health Statistic (NCHS) reference population (Epi Info. Version 5) was used to calculate the height -for -age -Z- scores. Prevalence of stunting defined - Z-scores or less was the measure of nutritional status and dependent variable. A regression analysis was used to derive a statistical predictive model. In the regression model per cent diarrhoea, cough, fever, use of toilet facilities, sources of water, roof and floor materials, ownership of radio, paraffin lamp and food supply were independent factors. The equation for predicting stunting was chosen based on the values of the adjusted R^2, T values of the independent factors and the spread of the residual values.

Table. Regression analysis for association with Stunting

*Var.	%Toilet	%Water	%Lamp	%Cough	%Floor	MTFood	Constant
B	-0.85	-0.30.	-0.68	0.73	-1.10	0.22	191
P-value	0.006	0.009	0.04	0.01	0.01	.30	0.00
				Adjusted R^2 0.98		Sig. F =0.016	f-value=61.2

* Var. = Variable, MTFood = Total food produced in metric tonnes, %Toilet = %families with a toilet, %Water = % families with acess to protected water, %Lamp = % families with a lamp, % cough = % children with a cough, %Floor = % families with a cement floor.

The best fit for predicting stunting was: %Stunting = 191 - (0.30water) - (0.85toilet) - (0.68lLamp) + (0.73cough) - (1.1floor). The results suggest that 98% of the variability in stunting could be explained by type of toilet facilities, sources of water, the state of the floor of the house, ownership of a lamp, and the prevalence of a cough. Crop production as indicated by total food (MTfood) produced at district level did not appear to strongly affect levels of stunting. Since the model only considered linear associations, care should be taken in concluding that other factors have no significance in affecting child nutrition. Other associations may also be important.

We conclude that in Malawi crop production affects economic statuses which in turn affects housing, sources of water, toilet facilities and ownership of property, which relate directly to the nutritional status of the children.

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Seasonal differences in the intake of imported milled rice in the village of Chilla in The Gambia, West Africa. By A.E. CATHCART[1], E.M.E POSKITT[2], M.B.E. LIVINGSTONE[1] and D.I. THURNHAM[1], [1]*Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA, and* [2]*Medical Research Council, Dunn Nutrition Centre, Keneba, The Gambia, West Africa.*

In the rainy season of 1988, an outbreak of beriberi occurred in and around the village of Chilla in the Upper Nuimi District of the Gambia, West Africa. At least 140 people were affected and twenty-two deaths occurred (Tang *et al.* 1989). Beriberi has occurred before in The Gambia (Walters & Smith, 1952; Marsden & Harling, 1967) but never before has a free-living community been affected. Beriberi is typically associated with communities eating milled rice in the Far East and Asia. In this area of The Gambia, pearl millet (*Pennisetum gambiense*) is the staple crop but imported milled rice (*Orzya sativa*) is popular and there is evidence to suggest that its consumption may be increasing as more highly-milled rice is imported each year (IFPRI, 1987).

In the present report, we describe data showing the total cereal energy intake, the proportion supplied by imported polished rice (Platt, 1962) and differences between the seasons. Throughout 1995, 4d, observed-weighed-meal intakes (WMI) were measured five times within two family units (FU). WMI 1 and 2 were pre-rainy season (March-May), WMI 3 and 4 were during the rainy season (July-September) and WMI 5 was post rainy season (October-November). Results are expressed as mean energy intakes (EI) per d for the two FU.

		WMI 1 (*n* 8)	WMI 2 (*n* 8)	WMI 3 (*n* 8)	WMI 4 (*n* 7)	WMI 5 (*n* 7)	ANOVA (P<)
EI (MJ/day)	Mean	179.2^{a}	186.9^{a}	$166.4^{a,b}$	135.0^{b}	180.5^{a}	0.05
	SD	17.9	27.6	23.0	55.4	35.3	
Rice EI (MJ/day)	Mean	58.0^{a}	68.4^{a}	59.1^{a}	58.9^{a}	19.0^{b}	0.02
	SD	7.7	19.1	17.2	44.0	33.7	
% EI as rice EI	Mean	$32.5^{a,b}$	$36.0^{b,c}$	$37.5^{b,c}$	60.6^{c}	8.7^{a}	0.01
	SD	4.3	5.2	18.5	49.8	14.9	
FU number	Mean	37.7^{a}	37.6^{a}	40.1^{b}	36.4^{a}	37.7^{a}	0.03
	SD	1.5	3.2	1.2	2.6	1.5	

^{a,b,c} Mean values within a row not sharing a common superscript letter were significantly different, P<0.05, LSD test

Throughout the study period imported rice supplied 35.1% of cereal energy. During the rainy season (WMI 4) this was significantly increased to 60.6%, however the consumption of rice in the two FU differed considerably at that time, being 100% in one and only 8% in the other. A higher percentage of energy from rice occurs at this time due to low reserves of millet while use of imported rice hardly changes. Following the rainy season, the consumption of rice energy fell to 8.7% but then increased fairly quickly to about 30% during WMI 1 - 3.

The rainy season is the farming season, a time of year typically known as the hungry season, when the food reserves from the previous year's harvest are almost depleted. This was reflected in the lowered energy intake during WMI 4. However, as the Table shows, the percentage of rice energy remained high throughout the year with most rice energy being consumed just before the rainy season. The high proportions of milled rice in the diet of Gambian villagers, like those in Chilla, indicates a dependency on an imported food which may lead to health problems of major importance within an area of limited resources like The Gambia. It may not be a coincidence that the outbreak of beriberi occurred at the start of the rainy season.

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Nutritional intakes of a rural and an urban population sample in South Central Cameroon, West Africa. By S. SHARMA[1], S. CHUNGONG[1], J.C. MBANYA[2], J. CADE[1] and J.K. CRUICKSHANK[1]. [1]*Clinical Epidemiology Unit, Manchester University Medical School, Manchester M13 9PT,* [2]*Department of Internal Medicine, University of Yaounde I, Cameroon*

Assessing nutritional intake of population samples in Cameroon presents a major challenge as very little work has been done previously (Sharma *et al.* 1994). This work is part of an international study examining nutritional influences on the emergence of diabetes and hypertension in populations of African origin in Cameroon, Jamaica and Britain.

We collected 2 d food diaries in a rural site (Evodoula) and a suburb (Cite Verte) of the capital Yaounde to determine the foods that should be included in a food- frequency questionnaire (FFQ) that would be used on a larger population sample (Sharma *et al.* 1996). The present report compares the nutrient intakes obtained in the rural site with those in the urban site using self completed 2 d diaries spread throughout the week and market days (response rate; rural 97% (62/64), urban 79% (60/76)).

Nutrient intake	Urban men		Rural men		Urban women		Rural women	
	(*n* 25)	(CI)	(*n* 29)	(CI)	(*n* 36)	(CI)	(*n* 33)	(CI)
Mean age (years)	25		45		37		45	
Energy (MJ)	11.3	(9.7-12.9)	16.3	(13.7-19.0)	9.7	(8.6-10.8)	16.6	(14.1-19.0)
Carbohydrate (g)	306.2	(260-353)	341.8	(275-409)	295.5	(261-330)	434	(364-504)
Fat (g)	120.8	(97-145)	198.0	(160-236)	96.9	(78-116)	182.9	(147-219)
Protein (g)	87.8	(73-103)	94.4	(76-113)	74.1	(65-83)	104.7	(88-122)
Alcohol (g)	9.2	(2.8-15.6)	86.0	(57-115)	6.5	(2.8-10.1)	36.6	(21-52)

Four of the diaries were incomplete and could not be used and the age of four rural women was unknown. The rural men sampled were on average 20 years older and rural women 8 years older than those in the city. Energy intake was much greater in the rural site reflecting the energy-demanding occupation of farming. Percentage of energy provided by carbohydrate was similar between the men (urban 45.7%, rural 46.5%), however the women consumed a greater percentage of energy as carbohydrate; urban women having 2% more than rural women (urban 49.7%, rural 47.7%). Alcohol intake was much greater in the village (palm wine being freely available) contributing 14.8% of total energy in rural men *v.* 2.4% in the urban men and 6.5% in rural women *v.* 2% in the urban women. Fat consumption was higher in the village (mainly from palm nuts) contributing 3% more energy in men and 4% more in women (men: urban 41%, rural 44%; women: urban 37.6%, rural 41.7%). These estimates, while higher in rural women than might be expected, probably reflect expenditure although diaries collected on days during and following market day may have raised values. From these food diaries a FFQ was developed and is being used to assess the diets of 1500 rural and urban Cameroonians in more detail.

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Nutritional status of tribal women of Orissa. By BASANTI RATH, Department of Anthropology, *Utkal University, Bhubaneswar 751 004, India*

Notwithstanding the fact that food production has gone up substantially over the decades, malnutrition is a major health problem and a majority of Indian women belonging to the underprivileged communities are undernourished to a large extent. The present paper attempts to highlight the nutritional status of women including female children of tribal communities of Orissa and to make an assessment of the determinants of protein-energy malnutrition for evolving strategies to combat such disabilities.

Women of the studied tribal communities consume foods which are lower in energy, protein and essential vitamins than those of their male counterparts. The average values for consumption of energy protein among the Saora, Khond, Koya, Kolha, Juang are 4.6 MJ, 18.56 g, 7.8 MJh. 42.96 g, 4.4MJ, 34.88 g, 7.4 MJ 40.00g and 3.9MJ 26.32 g respectively (energy-protein scale from H. Gopalan et al. 1993)

The present mothers were found hardly meeting the extra loads of energy and protein which are needed during pregnancy months and the average haemoglobin level was 92.3g/l. This led the mothers to remain in moderate severe anaemic condition (Shiva, H. 1992)

The incidence of malnutrition is greater in pre-school girls than in boys with a rise in the severe grade malnutrition. However, the difference is not statistically significant (UNICEF, 1991).

Poverty coupled with low purchasing power, illiteracy, repeated pregnancies and discriminating attitude and practices in family were observed to have been responsible for high incidence of malnutrition among the tribal women of Orissa (UNICEF, 1991)

The paper submits remedial measures for alleviating the nutritional status of the tribal women of Orissa.

Remedial Measures.

1. Female health care facilities need to be augmented in tribal areas.
2. Female education, nutritional and health care education are to be vigorously persued in tribal areas.
3. Age at marriage for female has to be raised.
4. Increasing employment opportunity for females would ensure better income and better living conditions.
5. A change in social attitude towards girl child through proper extension education is necessary.
6. The public distribution should be made effective so that the essential food items are provided to poor tirbal women.
7. The tribals should be allowed to obtain sufficient amount of freely collected food items from forest showing the period of food scarcity.
8. To open "Food Grain Bank" in each and every village for the lean months.

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Assessing malnutrition through anthropometry among free-living elderly in a rural site of Malaysia. By SUZANA SHAHAR [1], JANE EARLAND [1], ANTHONY M. WARNES [2] and SURIAH A. RAHMAN [3], [1] *Centre For Human Nutrition, University of Sheffield, Herries Road, Sheffield S5 7AU;* [2] *Department of Health Care for Elderly People, University of Sheffield, Herries Road, Sheffield S5 7AU;* [3] *Department of Food Science and Nutrition, Universiti Kebangsaan Malaysia, Malaysia*

As other countries in the world, Malaysia is experiencing a rapid demographic transition, with an increase in the number and the proportion of elderly people. Between 1980 and the year 2000, the proportion of those aged 60 and above will have increased from 5.3% to 6.8% of the total population (Malaysian Department of Statistics, 1983). Although the elderly are a particularly vulnerable group with respect to ill health and malnutrition, there is a lack of scientific information on the nutritional status of this group, particularly in developing countries. Therefore, the present study was designed to assess the current prevalence and magnitude of malnutrition among elderly Malays through anthropometry. Anthropometry is a non-invasive and reliable way of assessing malnutrition and provides information on body stores of fat and muscle (Chumlea *et al.* 1989).

This cross-sectional study was carried out in Mersing district which is on the east coast of Malaysia. A total of eleven traditional villages were randomly selected. The sample included all major economic activities of the rural population. A total of 350 rural elderly Malays, who were aged 60 years and above, and with no known terminal or mental illnesses were studied. The following anthropometric measurements were taken by a trained female nurse and a male college graduate: standing height, demispan (DS), weight, mid-arm circumference (MAC), triceps skinfold thickness (TSF), biceps skinfold thickness (BSF), subscapular skinfold thickness (SSF), and suprailiac skinfold thickness (SISF). Mid-arm-muscle circumference (MAMC) and BMI were calculated.

Results revealed that weight, height, DS, MAC and MAMC were greater in men than women ($P<0.005$). Measurements for TSF, BSF, SSF, and SISF were greater in women than men ($P<0.005$). Cross-sectionally, weight, MAMC, MAC, and BSF were greater in the younger than the older age group ($P<0.05$). Approximately half the subjects were within their normal range for BMI as shown in the Table. Nearly 40% were underweight using the chronic energy deficiency (CED) and obesity classifications (James *et al.* 1988), with more women being severely underweight (CED1). There was also a trend for more women to be overweight.

CED indexes	Men		Women		Total	
	n	%	*n*	%	*n*	%
CED I (<16.0)	21	12.1	34	19.2	55	15.7
CED II (16.0-16.9)	11	6.4	9	5.1	20	5.7
CED III (17.0-18.4)	34	19.7	23	13.0	57	16.3
Normal (18.5-24.9)	93	53.8	82	46.3	175	50.0
Obese I (25.0-29.9)	12	6.9	24	13.6	36	10.3
Obese II (30.0-39.9)	2	1.2	5	2.8	7	2.0
Obese III (40.0+)	0	0	0	0	0	0
Total	173	49.4	177	50.6	350	

The differences found between sexes were similar to the findings of other studies in developed countries (Fidanza *et al.* 1991). Although, in this study, the majority of rural elderly Malays were in the normal range for BMI, obesity is likely to increase as it has in many urban areas in developing countries. In spite of the rapid economic growth in Malaysia, CED is still a problem which needs urgent attention.

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Macronutrient sources in the South Asian diet. By N.A. KARIM[1] and B.M. MARGETTS[1,2], [1] *Institute of Human Nutrition and* [2] *Wessex Institute of Public Health Medicine, University of Southampton SO16 7PX*

Dietary intake studies in the South Asian community have been carried out in the UK since the late 1970s. Various dietary assessment methods such as household inventory, weighed records and dietary diary have been used in these studies (McKeigue *et al.*1985; Sevak *et al.*1994). The main objective of the present study was to develop a food-frequency questionnaire (FFQ) to be used for dietary evaluation in the South Asian community in UK. As part of this study, a preliminary survey using the 24 h recall was carried out to determine the macronutrient sources in the South Asian diet. A total of sixty-two men and women of South Asian origin between the ages of 18 and 72 participated in this survey.

Foods	Sources of energy in the South Asian diet (% of total energy intake)	Cumulative %
1.Chapatti	17.6	17.6
2.Meat curry	16.4	34.0
3.Milk and dairy products	14.6	48.6
4.Breads	6.9	55.5
5.Vegetable curry	4.1	59.6
6.Rice	3.8	63.4
7.Paratha and naan	3.5	66.9
8.Buns and pastries	3.3	70.2
9.Fruits	2.8	73.0
10.Sugar	2.7	75.7
11.Breakfast cereals	2.3	78.0
12.Eggs	2.2	80.2
13.Chips	2.2	82.4
14.Crisps	1.9	84.3
15.Savouries	1.6	85.9
16.Lentils	1.5	87.4
17.Soft drink	1.2	88.6
18.Meat, not curry	1.0	89.6
19.Puddings	0.4	90.0

The Table shows the sources of energy in the Asian diet in order of the size of their contribution to the total energy intake. A cumulative percentage distribution of energy was calculated for each food list until at least 90% of the total energy intake had been included. The main sources of energy in the South Asian diet were chapatti and meat curry. Important sources of fat, protein and carbohydrate in the diet were also concentrated in the meat curry, chapatti and milk products. The present study thus demonstrates that in the Asian diet, chapatti and meat curry contribute between 30% and 50% of the energy and macronutrient intake. The food sources of these nutrients are different from those reported in the English diet (Cade & Margetts,1988).Thus in developing a FFQ for the South Asian community it is essential that a different food list, which will cover at least 90% of the total energy intake of the adult South Asians, is included.

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Foods consumed by a Bengali population in a British hospital. By P.C. McGLONE[1], G.J. DAVIES[1], A. MURCOTT[2], J. POWELL-TUCK[3] and J.W.T. DICKERSON[1], [1]*Nutrition Research Centre, South Bank University, 103 Borough Road, London SE1 OAA,* [2] *School of Health and Social Care, South Bank University, Erlang House, Blackfriars Road, St. Georges Circus, London SE1 8QE* [3]*Rank Department of Human Nutrition, Royal London Hospital, Whitechapel, E1 1BB*

Patients may be more at risk of malnutrition in hospital if unfamiliar foods are being provided (McGlone *et al.* 1996). Butler (1967) also reported that "immigrants from India and Pakistan will frequently do without customary food items if they are not available", rather than consume alternatives. Of the elderly Asians in the UK 80% cannot read their own language (Aslam & Healy, 1985). The 1990 Food Safety Act has led to a loss of ward kitchens for use by patients, because of concern over liability for negligence in the event of food poisoning. Patients have now become more dependent on the hospital for food since prepared meals from home are difficult to sanction.

As part of a larger study addressing the problem of hospital malnutrition, the researcher spent one month on each of two general medical and two general surgical wards in a London teaching hospital, observing the eating habits of patients during mealtimes and at other times during the day. All Asian patients who were eating and were going to be in the ward for either 3 or 4 d were asked to participate. An interpreter was available for those unable to speak English.

The bulk system of food distribution was used and food was provided from the kitchen for twenty-seven patients in the ward. Food appropriate for Asian patients was sent up daily, however extra food was provided only if ordered by nurses. Menus were in the English language only and were placed in bedside notes.

At different mealtimes 100 observations were made over either 3 or 4 d on fifteen different Bengali patients. Of the meals provided 38% of meals were entirely from home. Four (27%) out of the fifteen patients did not eat any hospital food; six (40%) ate hospital food at some mealtimes whilst at other meals they ate food from home. A further two patients ate only hospital food and two patients ate food from home between meals. One patient ate both hospital food and meals from home.

Half (seven) of the patients spoke little English and three patients could not speak any English. The remaining five spoke English quite well. The two patients who received only hospital food were amongst those who spoke little English.

These findings suggest that despite legislation and hospital policy, many patients are dependent on their relatives if they are to receive appropriate food and thus have an opportunity of being adequately nourished. Staff available at mealtimes had to make food choices for Asian patients who were unable to speak English.

The Act may be strictly applied in some hospitals because of concern for food poisoning. If however, current hygiene regulations are strictly applied according to the Food Safety Act (1990), patients become totally dependent on the hospital for food. The fact that many Asian patients were dependent on receiving food from home gives cause for concern and raises debate as to what policy the hospital should adopt towards this, bearing in mind concerns about undernutrition of patients in hospital. Alternative foods will have to be provided which mirror the diet of those patients who reside in the area, taking into account the wide cultural diversity of the hospital population.

The results of this study suggest the need for investigation into the foods consumed by Asian patients in hospital and barriers to the uptake of Asian foods in hospital. The inability to communicate in the English language and therefore ask for certain foods also warrants further investigation.

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Trends in birth outcomes of Asian babies in Southampton. By S. MOHD-YUSOF[1] , B.M. MARGETTS[1,2], N. KARIM[1], Z.S.M. AL-DALLAL[1] and A.A. JACKSON[1]. [1]*Institute of Human Nutrition and* [2]*Institute of Public Health Medicine, University of Southampton, Southampton SO16 7PX*

Long-term follow-up studies of European men and women in Britain have shown that birth weight, birth length, head circumference and the birth weight : placental size ratio are associated with to chronic diseases in later life (Barker, 1995).The first generation South Asian immigrant s in this country have a higher rate of coronary heart disease than the general population (Balarajan, 1995). To date there is little information linking birth outcome to risk of chronic diseases in South Asians. The current study presents trends in birth outcome in South Asians born in Southampton.

Birth record for all babies born in Southampton between 1960 and 1979 were checked for Asian names, and the data abstracted. The birth records included information on the mother (age at booking or date of birth, religion, gravida, weeks of gestation or the last menstrual period, height and husband's occupation) and the infant (date of birth, sex, birth weight, placental weight, head circumference and birth length). The total number of South Asians births recorded for the years 1960-9 and 1970-9 were 376 and 474 respectively. Birth weights were available for almost all infants but records on placental weights, head circumference and length were not present in all the records. Only babies born at full term (≥37 weeks gestation) were selected for this analysis. Data was analysed by one-way ANOVA using SPSS for Windows (Release 6.1).

	Male			Female			
	n	Mean	(95% CI)	*n*	Mean	(95% CI)	F Ratio ; F Prob
1960-1969							
Birth wt (g)	190	3206	(3137 - 3276)	158	3062	(2989 - 3135)	8.0 ; 0.005
Placental wt (g)	173	602	(584 - 619)	142	586	(565 - 608)	1.2 ; 0.27
Head circumference (mm)	120	343	(340 - 345)	92	338	(335 - 341)	4.9 ; 0.03
Length (cm)	32	507	(495 - 520)	20	507	(493 - 522)	0.0 ; 1.00
1970-1979							
Birth wt (g)	207	3126	(3064-3189)	203	3046	(2989-3103)	3.5 ; 0.06
Placental wt (g)	192	592	(575-608)	191	590	(575-606)	0.0 ; 0.89
Head circumference (mm)	172	345	(343-347)	161	337	(335-340)	25.8 ; 0.0000
Length (mm)	119	508	(502-514)	112	501	(496-506)	3.5 ; 0.06

The Table shows that female infants had lower birth weights than male infants. There was no difference in the mean birth weight, placental weight, head circumference and length of Asian infants born in the 1960s compared with those born in the 1970s. When compared with white infants the Asians had lower birth weight, placental weight and head circumference (Godfrey et al. 1996). For both sexes the prevalences of low birth weight (<2500g) among all full-term South Asian babies during 1960-9 and 1970-9 were 11.5% and 10.6% respectively. This compares with about 7% in the whole UK population (Office of Population Censuses and Surveys, 1986). We plan to follow these babies up as adults to test the hypothesis that those babies who were born small-for-dates have higher chronic disease risk.

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Comparing the dietary iron intakes during weekdays and weekends among South Asian adolescent girls in Southampton by using a 24 h dietary recall. By ZUHAIR SALMAN MAJED AL-DALLAL[1] and BARRIE M. MARGETTS[1-2], *[1]Institute of Human Nutrition, Bassett Crescent East, University of Southampton, SO16 7PX, [2]Wessex Institute of Public Health Medicine, Level B, South Academic Block, Southampton General Hospital, SO16 6YD*

The main cause for the high incidence of low Fe stores (Fe depletion) in adolescent girls is a discrepancy between a high need of Fe for haemoglobin formation and a low intake due to the comparatively low Fe content of foods most commonly used. Few studies have assessed the prevalence of low Fe stores (status) and the influence of the dietary intake among the South Asian adolescent girls in the UK. We have conducted a pilot study to assess the range of food eaten by sixteen South Asian adolescent girls aged 11-16 years during weekdays and at weekends. We used a 24 h dietary recall to assess the dietary Fe intakes; all the girls were school-students and they had their weekday lunch meal at school.

	Weekdays		Weekends		
Nutrients	Mean	SD	Mean	SD	2-tail significance
Vitamin C (mg)	67.68	53.3	58.4	63.9	0.543
Iron (mg)	10.07	2.55	10.46	3.12	0.677
Protein (g)	58.70	19.4	65.4	28.60	0.366
Energy (MJ)	7.0	1.5	6.7	1.7	0.539

Intakes of energy, protein, vitamin C and Fe were measured. The Table shows that the estimated mean vitamin C intakes were higher on weekdays compared with weekend days although the difference was not statistically significant. Estimated total energy was higher on weekdays, while in both days the total energy intakes were lower than the Reference Nutrient Intakes (RNI) for adolescents (10.5 MJ). There was no statistically significant difference in the Fe intakes on weekdays and weekend days, while the sources of Fe were slightly different. The main sources of Fe on weekdays were peas, pizza and fish consumed as the school meal and chicken and other meat consumed as the evening meal at home. On the other hand, the main sources of Fe at the weekend were meat, chicken, curry, fish and chapatti. The Fe intakes reported here are similar to those seen in previous studies (Department of Health, 1989; Southon *et al.* 1992; Nelson *et al.* 1993,1994). We plan to use the food pattern data obtained from this pilot study to develop a more reliable estimate of Fe intakes, which takes account of variation in food choices adopted in this population.

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Composition of the diet of a population sample of African Caribbeans living in Manchester; a comparison with a white population. By S. SHARMA, J. CADE and J.K. CRUICKSHANK, *Clinical Epidemiology Unit, Manchester University Medical School, Manchester M13 9PT*

Very little is known regarding the diets of British African Caribbeans despite a population of almost 500 000. We have designed a food frequency questionnaire (FFQ) specifically for this group to assess food and nutrient intake.

The FFQ contained foods and drinks contributing to at least 90% of the intake of energy, fat, carbohydrate and protein; this totalled 108 food items including both traditional West Indian and European foods (Sharma *et al.* 1993,1996).

The proportion of energy of the diets of the first 210 randomly selected African Caribbean (AfC) subjects (mean age; men 57 years, women 51 years, mean BMI; men 27.0, women 28.7) completing a FFQ (response rate 84%) are given below compared with results from a mainly white population (Gregory *et al.* 1990) obtained by 7 d weighed intake (men and women aged 50-64 years).

	AfC men (*n* 85)		White men (*n* 273)	AfC women (*n* 125)		White women (*n* 283)
% Energy from nutrients	Mean	SD	Mean	Mean	SD	Mean
Energy (MJ)	9.8	3.73	10.0	8.1	2.94	6.7
Carbohydrate (incl. alcohol)	52.4	5.9	47.8	52.3	5.8	44.5
Fat	32.0	5.1	37.6	32.6	5.1	39.5
Protein	14.9	2.0	14.7	14.7	2.1	16.1
Alcohol	3.6	4.7	6.4	1.3	1.6	2.2

There wss little difference in macronutrient contribution to energy between AfC men and women (60% born in Jamaica); men consumed rather more of their energy as alcohol, although the total energy from carbohydrate was the same for both groups. However energy composition was different from that of the white population. The white men had 4.6% less of their total energy provided by carbohydrate and 5.6% more provided by fat compared with the AfC men. The white men are consuming almost twice as much energy as alcohol. The white women were consuming 7.8% less of their energy as carbohydrate and 6.9% more as fat compared to AfC women. The white women were also consuming more of their energy as alcohol.

The AfC diet appears to meet and fall below the *Health of the Nation* (1991) target for fat to provide no more than 35% of food energy.

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Variation in food beliefs and dietary practices by ethnic group in pregnant women in Birmingham. By FATEMEH RABIEE, *School of Health and Policy Studies, University of Central England, Birmingham B42 2US*

Women (*n* 400), taken equally from five ethnic groups (Pakistani, Bangladeshi, Indian, Afro-Caribbean and white British) using quota sampling, were studied to investigate dietary practices and changes in food intake during pregnancy. Two maternity hospitals and a large health centre were chosen to ensure broad socio-economic representation. Data were collected on socio-economic status, health behaviour, food beliefs and food frequency using a structured questionnaire with some open-ended questions.

The mean age was 25.6 (range 16-43) years. Bangladeshis had a lower mean age (22.4 years), a higher proportion of first-time pregnancy (39%), and more were recent immigrants. There were marked socio-economic differences within the population; Bangladeshis had the highest unemployment rate (44%) and the lowest non-manual employment rate (11%). Analysis of food-frequency data suggested that dietary practices differed by ethnic group. Bangladeshis consumed less dairy produce, bread (wholemeal and/or brown), raw vegetables, high-fat snacks (somasa, pakora, peanuts, Bombay mix, crisps) chocolate and sweets, but more fish and rice. White British consumed least fruit, more semi-skimmed and/or skimmed milk, sweets and chocolate and high-fat snacks (Table).

Significantly fewer Bangladeshis than whites or Afro-Caribbeans ($P<0.01$) changed their food intake during pregnancy. The foods avoided were: red meat, offal (45%), yoghurt and cheese (45%), fried and fatty food (19%), raw eggs (16.5%), cooked meat products (16.5%). The main reasons given were: fear of contamination, advice by health professionals about harm to the baby, nausea and sickness. All Asians, and 64% of Afro-Caribbeans and whites stopped alcohol intake and more than 40% gave up smoking during pregnancy. During pregnancy, 55% of women ate more fruit, dairy produce, vegetables, cereals and bread. The most frequent reasons given were: more healthy, high in vitamins and/or protein, Ca, Fe and fibre (24%), cravings and/or liked the taste (19%), better for the baby (11%). A higher proportion could offer no reason (46%).

Type of food	Total (%)	Pakistani (%)	Bangladeshi (%)	Indian (%)	Afro-Carib. (%)	White (%)
Wholemeal/brown bread	64b	64b	23a	71b	49b	60b
Pulses>3 times weekly	29a	31a	16a	57b	22a	20a
Fruit>2 pieces/d	57	62	51	65	57	49
Vegetables, cooked >2/d	51	39	50	60	54	60
Vegetables, raw, every day	25ab	38b	11a	50c	18a	16a
Fish>3 times weekly	20b	13b	75c	0a	14b	9b
Semi-skimmed/skimmed milk	31a	16a	11a	24a	47b	58c
Unsaturated fat used in cooking	88	82	96	74	97	90
Butter spread on bread	26	19	49	19	20	21
High-fat snacks > 5 weekly	30b	29b	15a	35b	35b	36b
Sweets/chocolate every day	31b	29b	19a	30b	35b	44b

abc Mean values within a row not sharing a common letter were significantly different : $P<0.05$ (ANOVA).

In conclusion, dietary practices of pregnant women differ between ethnic groups, but are moving towards nutritional targets particularly in relation to fruit and vegetable consumption (Department of Health, 1994). The changes in health behaviour of pregnant women echo findings of recent studies (Gray, 1991; Anderson *et al.* 1993). Foods avoided or eaten in greater quantity are consistent with current dietetic advice for pregnancy (Health Education Authority, 1995). Dietary patterns and health behaviour are much closer between groups of Afro-Caribbeans and whites, Pakistanis and Indians compared with Bangladeshis, possibly due to the marked socio-demographic variations and shorter length of stay in the UK amongst the Bangladeshis.

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Health Education Authority (1995). *The New Pregnancy Book*, pp. 8-9. London: HEA.

Do the intergenerational differences in the diets of first and second generation Pakistani Muslims affect the diets of their children? By J.H.GODSON, S.PARSONS and S.A.WILLIAMS, *Oral Health and Ethnicity Unit, Leeds Dental Institute, The University of Leeds, Leeds LS2 9LU*

Previous reports of intergenerational differences in the diets of immigrants have shown greater signs of acculturation in the second generation immigrants than in the first generation (Kalka 1988). The present study examined the diets of 3-year-old children born to first generation (*n* 117) and second generation (*n* 109) Pakistani Muslim mothers in Bradford, West Yorkshire. The mothers were interviewed by one of three multi-lingual interviewers who spoke English, Punjabi and Urdu, using a semi-structured questionnaire, which examined their social characteristics including language ability and employment status, and their infant feeding practices. It also contained a 24h diet record.

The table below shows that no differences existed between the breakfast meals eaten by first and second generation's children, as both groups ate eggs, toast, cereals and biscuits, with the second generation's children being more likely to eat cereal for breakfast then the first generation's children.

Rank	First Generation (n 117)	Number (%) of individuals consuming food	Rank	Second Generation (n 109)	Number (%) of individuals consuming food
1	Eggs	31 (26)	3	Eggs	28 (26)
2	Toast	30 (25)	2	Toast	36 (33)
3	Cereals*	29 (24)	1	Cereals*	43 (39)
4	Biscuits	13 (11)	4	Biscuits	10 (9)

*Cereals = Rice Krispies, Corn Flakes. Ready Brek and Coco Pops

At main meals, both of the first and second generation's children were equally likely to eat the traditional Muslim staples of chappattis and curry (64% of first generation children and 69% of second generation children). The second generation's children were more likely to eat less traditional meals, for example fish and chips, compared with the first generation children (14% and 3% respectively).

The table below reveals that no differences existed in the snacks eaten by both of the first and second generation's children, with crisps, biscuits, fruit, sweets and cereal being eaten by both groups. Snacking, appeared to be the most acculturated part of the children's diets, as traditional Asian foods were eaten as snacks only in very small quantities.

Rank	First Generation (n 117)	Number (%) of individuals consuming item	Rank	Second Generation (n 109)	Number (%) of individuals consuming item
1	Crisps	44 (38)	1	Crisps	49 (45)
2	Biscuits	34 (29)	2	Biscuits	39 (36)
3	Fruit	30 (26)	3	Fruit	32 (29)
4	Sweets	15 (13)	4	Sweets	13 (12)
5	Cereal*	12 (10)	5	Cereal*	3 (3)
6	Toast	4 (3)	5	Toast	3 (3)
6	Chocolate	4 (3)	5	Chocolate	3 (3)
6	Sandwiches	4 (3)	5	Sandwiches	3 (3)

These findings indicate that whether a parent is a first generation or a second generation immigrant does have some effect on their child's diet, as the diets of the second generation's children appear to be closer to the western diet than the diets of the first generation's children. This is perhaps due to social factors such as the mothers' language ability, education and employment status.

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Improving the acceptability of potassium chloride as a salt substitute, using coconut milk powder. By M.IMHOF[1], C.J.K. HENRY[1], and D.J. MELA[2], *[1]School of Biological and Molecular Sciences, Oxford Brookes University, Oxford OX3 0BP, [2]Consumer Sciences Department, Institute of Food Research, Earley Gate, Reading RG6 6BZ*

Partial replacement of NaCl with KCl could help to achieve the current dietary recommendations (Department of Health, 1994), for reducing Na and increasing K intakes. However, widespread use of KCl as a salt substitute is limited, largely because of its unpalatable bitter aftertaste. Pilot studies in our laboratory had suggested that coconut milk powder could potentially increase the acceptability of KCl in foods. The aim of the present study was to evaluate the effect of coconut milk powder on the perceived saltiness, bitterness and pleasantness of KCl in a model, rice-based food system.

Cooked rice samples were prepared with 40 g rice and 80 g water, with or without coconut milk powder or sucrose, and in combination with NaCl or KCl, as specified in the Table. Thirty untrained panellists (mean age 37.5 [SE 2.09] years) attended two taste sessions on each of three separate days. They were presented with 5 g of each sample, in random order, and assigned ratings twice for bitterness, saltiness, and pleasantness. Line scales, with the anchor points designated as no taste (0 mm) and extremely strong (100 mm), were used to assess the taste intensity judgements.

Category	Test samples		Taste intensity judgements (mm)					
	Coconut (g/120 g)	Sucrose (g/120 g)	Salt		Bitter		Pleasant	
			Mean	SE	Mean	SE	Mean	SE
No Salt	0	0	8.2	2.1	20.2	3.5	36.7	3.7
	8	0	12.9	2.5	26.2	4.6	37.1	4.5
	0	0.9	11.2	2.4	21.5	3.8	37.4	4.1
NaCl	0	0	59.8^{a}	3.4	30.2	4.0	41.5	4.1
(1 g)	8	0	48.1^{b}	3.9	27.3	2.8	49.1	3.8
	0	0.9	44.8^{c}	4.6	31.0	4.4	48.6	4.1
NaCl + KCl	0	0	35.7^{a}	4.1	34.8^{a}	3.8	44.9	3.7
(0.5 g each)	8	0	$30.2^{a,b}$	3.3	25.2^{b}	2.9	49.1	3.5
	0	0.9	22.2^{b}	2.5	19.2^{c}	2.4	44.8	3.2
KCl	0	0	24.4	3.0	43.6^{a}	5.5	31.5^{a}	4.1
(1 g)	8	0	23.3	3.3	31.9^{b}	4.5	42.0^{b}	3.9
	0	0.9	30.2	3.4	43.1^{a}	4.6	31.0^{a}	3.3

^{a,b,c} Values with non-identical superscripts, within a salt and taste category were significantly different (repeated measures ANOVA, $P<0.05$).

These results indicate that the addition of coconut powder markedly reduced the perceived bitterness and enhanced the pleasantness of KCl samples without reducing their saltiness. Although the level of bitterness relative to saltiness was still elevated in the KCl samples, their absolute bitterness and pleasantness were similar to NaCl alone. Further studies are being directed at identifying components of the coconut powder, and other materials, which may specifically improve the acceptability of KCl as a salt substitute.

This work was supported by a research grant from MAFF.

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The effect of seasonal variation and light cycle changes on selected factors associated with heart disease. By JENNIFER C. FREEBURN and WILLIAM S. GILMORE, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

Deaths from ischaemic heart disease (IHD) are considerably higher in winter than summer (Elwood *et al.* 1993). Keatinge *et al.* (1984) showed that 6 h of mild surface cooling in vivo increased platelet count, erythrocyte count and blood viscosity of healthy subjects, thereby demonstrating that temperature may explain the seasonal variation in IHD. However, it has been suggested that light may also be involved (Scragg *et al.* 1990). We modified the experiment of Keatinge *et al.* (1984) by varying the length of the light cycle and holding temperature (20°) constant.

Blood variables were measured in ten healthy subjects (five males and five females) aged 23-29 years. Subjects were placed in either artificial natural light or complete darkness for 6 h. Blood samples were taken following a low-fat meal, before and after the light condition. In addition a winter sample (February) was taken from nine of the subjects to allow comparison between summer (June) and winter samples. Results were analysed by one way ANOVA. Significant differences between light cycle groups were tested by least significant differences (LSD) test. Differences between winter and summer groups were tested by paired t tests.

	Light cycle variation								Seasonal variation			
	Before light (*n* 10)		After light (*n* 10)		Before dark (*n* 10)		After dark (*n* 10)		Summer (*n* 9)		Winter (*n* 9)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Haemoglobin (Hb) (g/dl)	134	1.8	133	5.8	132	5.4	131	5.5	131	5.8	136**	6.2
Packed cell volume	0.40	0.02	0.43	0.04	0.39	0.02	0.39	0.02	0.38	0.02	0.41**	0.02
Erythrocyte count ($\times 10^{12}$/l)	4.44	0.18	4.24	0.34	4.43	0.18	4.39	0.19	4.40	0.19	4.61***	0.17
Platelet count (10^9/l)	219	11.9	231	20.5	194	16.4	205	12.8	206	14.4	230*	9.9
Plasma viscosity (cp)	1.47	0.003	1.47	0.003	1.46	0.002	1.31	0.016	1.4	0.016	1.58***	0.014

Mean values were significantly different from those for summer: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

ANOVA results from variations in the light cycle showed no change in any of the selected factors associated with heart disease. However, there were significant differences in some of these factors when summer and winter samples were compared. These seasonal changes are consistent with those observed elsewhere and with the effects on whole body surface cooling observed by Keatinge *et al.* (1984). Therefore it, seems probable from these results that temperature, rather than light exposure, may be responsible for the increased incidence of IHD in winter.

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Chronic effect of varying the proportions of sucrose and starch in the diet on insulin sensitivity in healthy adults. By C. VALE[1], M. DALY[1], A. LITTLEFIELD[1], M. WALKER[2], K.G.M.M. ALBERTI[2] and J.C. MATHERS[1], [1]*Human Nutrition Research Centre, Department of Biological and Nutritional Sciences and* [2]*Department of Medicine, University of Newcastle, Newcastle upon Tyne NE1 4LP*

Insulin insensitivity appears to be central to a cluster of metabolic abnormalities including impaired glucose tolerance, hypertension, dyslipidaemia and obesity, which are major risk factors for cardiovascular disease. Studies in laboratory animals suggest that high-fructose or high-sucrose diets impair insulin sensitivity (Thorburn *et al.* 1989) but results from human studies have been inconclusive.

Sixteen healthy, non-obese subjects were recruited to a randomized partial crossover trial in which four diets, of a week's duration each, were provided such that each diet was completed by eight subjects. Diet 1 was similar to the current average UK intake (Gregory *et al*, 1990). Diets 2-4 were of lower fat content (35% of energy, saturated: monounsaturated: polyunsaturated fatty acid ratio of 1:1:1) with sucrose providing 23, 14 or 4% and starch 20, 30 or 40% of energy respectively. The total diet was provided as conventional foods for the volunteers in amounts estimated to supply 1.5 x BMR.

A modified insulin tolerance test (Akinmokun *et al.* 1992) was used to assess insulin sensitivity at the beginning (day 0) and end (day 8) of each dietary period. Insulin sensitivity was quantified as the rate constant (Kitt) for fall in glucose (from 3 to 15 minutes) and non-esterified fatty acid (NEFA) (from 6 to 15 minutes) concentrations after the intravenous administration of insulin at a dose of 0.05U/kg body weight.

	Mean % Δ Kitt				
	Diet 1	Diet 2	Diet 3	Diet 4	SEM
Glucose	-0.9	6.7	37.4	10.6	12.3
NEFA	-26.2	-2.4	-15.6	16.0	13.4

Compared with the subjects' habitual intake, no significant difference in insulin sensitivity was found after imposition of either of diets 2-4. However, a significant fall in insulin sensitivity for NEFA (Δ = -26.2% day 8 *v.* day 0, $p<0.004$) was observed with the average UK diet (diet 1). It may be relevant that fat provided 34% of the non-ethanol energy in the habitual diet of these subjects. The apparent improvement in $Kitt_{glucose}$ with diet 3 was associated with the lowest day 0 value for any treatment period.

Therefore, in this population group, dietary fat may have more effect on insulin sensitivity than altering carbohydrate type. This would be in line with epidemiological studies which suggest that increased dietary fat, in particular saturated fat, leads to insulin resistance. NEFA metabolism appears to be more sensitive to change in dietary fat than glucose metabolism.

This research was supported by MAFF (Project ANO 0309).

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Do people with impaired glucose tolerance on two occasions have higher levels of cardiovascular risk factors than those with impaired glucose tolerance once only?

By J. OLDROYD[1], N. UNWIN[2], M. WHITE[2], and J.C. MATHERS[3], [1] *Newcastle Nutrition, Royal Victoria Infirmary, Newcastle-Upon-Tyne NE1 4LP,* [2] *Department of Epidemiology and Public Health, University of Newcastle-Upon-Tyne, NE2 4HH,* [3] *Department of Biological and Nutritional Sciences, University of Newcastle-Upon-Tyne, NE1 7RU, on behalf of the Newcastle Heart Project and Impaired Glucose Tolerance Study*

The usefulness of impaired glucose tolerance (IGT) as a independent marker of risk for developing diabetes and coronary heart disease has been questioned (Yudkin *et al.* 1990). People with IGT may revert to normal, or go on to develop overt diabetes, or merely remain impaired. People who are persistently impaired would be expected to be at greater risk than those who improve. We tested the hypothesis that people who have IGT on two occasions 2-6 weeks apart have more metabolic abnormalities and risk factors for coronary heart disease than people identified with IGT only once.

Caucasian subjects aged 25-74 years were randomly chosen from the Newcastle Family Health Services Authority register and invited for a World Health Organization standard oral glucose tolerance test; IGT was defined using WHO guidelines (WHO, 1985). Participants identified as having IGT had a repeat oral glucose tolerance test 2-6 weeks later. Subjects were then grouped according to whether they had reverted to normal glucose tolerance or had persistent abnormal glucose tolerance (IGT or diabetes). We then compared cardiovascular factors measured at the time of the first oral glucose tolerance test between these two groups. Comparisons were made using the independent-sample *t* test. Non-normally distributed variables such as insulin and triacylglycerols were log transformed before analysis.

Eighty-one subjects initially identified with IGT had a repeat oral glucose tolerance test. Forty-one subjects (51%) had IGT or diabetes on two occasions (six subjects (7%) developed diabetes). Forty (49%) had IGT on the first occasion only.

IGT	*n*	Age (years)	BMI (kg/m^2)	2 h plasma glucose (mM)
Once only	40	57	26.7	9.2
Twice	41	61	29.2	9.6
P		0.057	0.029	0.059

People with persistent abnormal glucose tolerance tended to be older, and more obese than those found to have IGT on only one occasion, but there was little evidence of differences in other metabolic variables (high blood pressure, plasma lipids, insulin resistance).

Those with IGT will be invited to participate in the Newcastle IGT study, a pragmatic intervention study testing the efficacy of diet and exercise in a randomized controlled trial.

This study is supported by the British Heart Foundation (Study Number: PG/94155)

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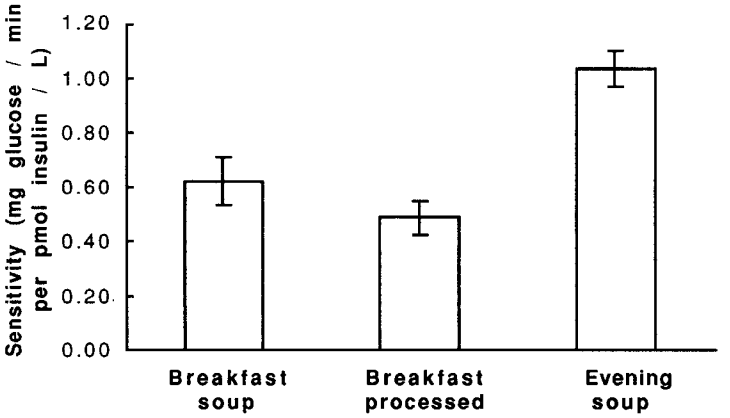
Post prandial insulin sensitivity of glucose disposal measured with [6,6^{2}H]-D-glucose is markedly dependent on prior meal consumption. By G. LIVESEY[1], R. FAULKS[1], P. WILSON[1], J. BROWN[1], M. ROE[1], T. NEWMAN[1], K. TAYLOR[1,2], S. HAMPTON[2] and R. GREENWOOD[3], *[1]Department of Nutrition, Diet and Health, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, [2]School of Biological Sciences, University of Surrey, Guildford GU2 5XH, [3]Department of Medicine, Norfolk and Norwich Hospital, Norwich NR1 3SR.*

Poor insulin sensitivity (I_S) of glucose disposal (G_d) from the circulation is an early event in the progress towards diabetes, cardiovascular disease and hypertension, and is associated with poor blood-lipid risk factors. Diet is probably an important contributor to the development of this metabolic condition and so its improvement is an important goal. Therefore, we have developed a dynamic model to quantify the I_S of G_d during the post prandial period when gut-derived hormones may be important modifiers of the sensitivity of G_d to insulin. We have used this model to investigate whether post prandial I_S is dependent on prior meal consumption.

Our volunteers were six 'healthy' adults (3M, 3F, mean age 42 years, mean BMI 25 kg/m^2) who had no immediate family history of diabetes. They entered the IFR Human Nutrition Unit on three occasions, each time after eating foods for 2 d that were prescribed in their individual 'habitual' food intake diaries. At 2 h after the start of a primed, constant i.v. deuterated glucose infusion, the volunteers ate 100 g peas (*Pisum sativum*); at breakfast as soup, at breakfast after processing in $CaCl_2$ to make them more difficult to digest, and as soup after prior consumption of mixed meals at breakfast (–8 h) and lunch (–4 h). G_d was computed using Steel's non-steady state kinetics on a single compartmental model.



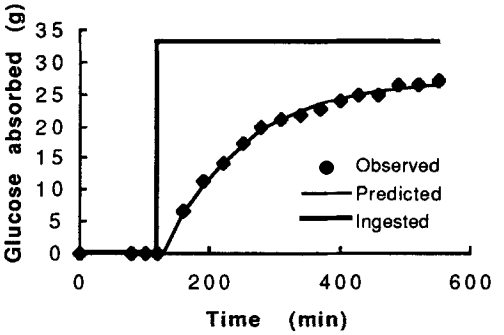
Sensitivity was derived using cumulative G_d as the dependent variable and time and integrated insulin concentration as independent variables. The results (mean, SEM) show that prior meal consumption nearly doubled the sensitivity. This derivation also gave a non-insulin dependent glucose disposal rate, a value which was similar for all the treatments. An implication is that carbohydrate consumption is tolerated most easily after prior mixed meals. Either mixed meals markedly enhance or fasting overnight impairs the post prandial sensitivity of G_d to insulin.

Supported by the Ministry of Agriculture, Fisheries and Food.

Development of a dual stable-isotope method for determining glucose absorption from ^{13}C-enriched starchy foods. By G. LIVESEY[1], R. FAULKS[1], P. WILSON[1], M. ROE[1], J. BROWN[1], T. NEWMAN[1], F. MELLON[1], J. EAGLES[1], J. DENNIS[2], I. PARKER[2], R. GREENWOOD[3] and D. HALLIDAY[4], *[1]Department of Nutrition, Diet and Health, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, [2]Central Science Laboratory, Norwich Research Park, Norwich, NR4 7UQ, [3]Department of Medicine, Norfolk and Norwich Hospital, Norwich NR1 3SR, [4]Unit of Metabolic Medicine, St. Mary's Hospital Medical School, London W2 1PG.*

Post prandial glycaemia is often used to assess the digestion and absorption of orally administered starchy food. However, glycaemia is regulated by changing both the rate of hepatic glucose entry into the circulation and the rate of clearance from the circulation into insulin-sensitive tissues. Therefore, it is impossible to characterize absorption from starchy foods based on glycaemic response alone. Since hepatic glucose recycling was minimal during glucose absorption, we hypothesized that the entry of glucose derived from ^{13}C-enriched starchy foods into the circulation could be assessed if the rate of glucose disposal from the circulation could be determined simultaneously using [6,6^{2}H]-D-glucose.

We grew peas (*Pisum Sativum*) in an atmosphere periodically enriched with $^{13}CO_2$. We fed 100 g peas containing 30.3 g starch (30% above natural enrichment) to six 'healthy' adults (3M,3F, mean age 42 years, BMI 25 kg/m^{2}) 120 min after initiation of a primed, continuous i.v. infusion of [6,6^{2}H]-D-glucose. Using Steel's equations, a non-steady state one compartment model of the kinetics in arterialized plasma showed that glucose absorption from the peas fitted a lagged rising exponential curve characterized in each volunteer by three parameters. First, a lag period between ingestion of the peas and first appearance of absorbed ^{13}C-glucose; second, a fractional rate constant for absorption, and third an absorption plateau. Unabsorbed starch was considered to be resistant to small-intestinal digestion.



A representative absorption curve from one volunteer is shown. The standard error of fit was equal to 2% of the available starch. In the six healthy adults given pea soup at breakfast the lag period was 17 (SEM) 3 min, the fractional rate of absorption was 0.0076 g glucose / g available starch per min and the plateau indicated that 85 (SEM 5) % of starch was available. All the starch absorption curves behaved as if only two forms of starch occurred, available and unavailable.

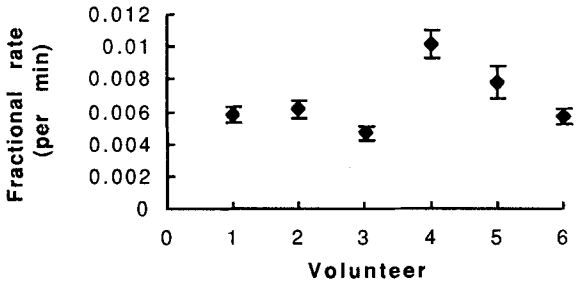
Supported by the Ministry of Agriculture, Fisheries and Food.

Individual rates of glucose absorption from ^{13}C-enriched starch in peas eaten by healthy adults can be very different: Is there a role in the aetiology of disease? By G. LIVESEY[1], R. FAULKS[1], P. WILSON[1], M. ROE[1], J. BROWN[1], T. NEWMAN[1], F. MELLON[1], J. DENNIS[2], I. PARKER[2], R. GREENWOOD[3], and D. HALLIDAY[4], *[1]Department of Nutrition, Diet and Health, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, [2]Central Science Laboratory, Norwich Research Park, Norwich NR4 7UQ, [3]Department of Medicine, Norfolk and Norwich Hospital, Norwich NR1 3SR, [4]Unit of Metabolic Medicine, St. Mary's Hospital Medical School, London W2 1PG.*

Individual variation in post prandial hyperglycaemia is usually attributed to variation in the sensitivity of glucose disposal to insulin, variation in pancreatic ß-cell function and variation in the susceptibility to suppression of hepatic glucose production in the post prandial period. Because of an absence of suitable methodology for assessing the rate of glucose entry into the circulation from oral starch it has not been possible to ascertain whether variation in glucose delivery from the gut after consumption of starchy foods might contribute to variation in stress on the gluco-regulatory mechanisms and postprandial glycaemia.

In an earlier communication at this meeting we have described the development of a dual isotope method that characterizes glucose appearance in the circulation from the gut in three terms, the major one in the present context is the rate of absorption, expressed as the fraction of available starch digested and absorbed per min. We investigated whether this rate differed between individuals.

Volunteers were six 'healthy' adults (3M, 3F, mean age 42 years, mean BMI 25 kg/m^2) who had no immediate family history of diabetes. The volunteers received a primed continuous i.v. infusion of [6,6^{2}H]-D-glucose and ate 100 g ^{13}C-enriched peas (*Pisum sativum*); at breakfast as soup, at breakfast after processing in $CaCl_2$ to make them more difficult to digest, and as soup after prior consumption of mixed meals at breakfast (–8 h) and lunch (–4 h). Mean (±SEM) fractional rates were obtained in each subject and blocking factors were used to minimize variation due to the different meal treatments.



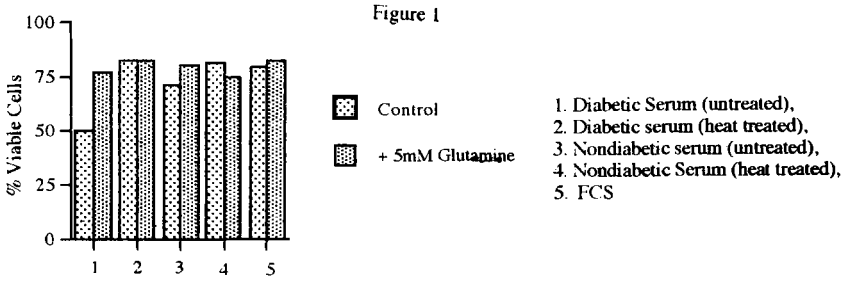
Some individuals had significantly different fractional rates ($P<0.05$, Bonferroni). Should similar differences occur in diabetic and glucose intolerant patients this may contribute towards their dietary carbohydrate intolerance. Should variation in the susceptibility of carbohydrate to digestion be an important factor, it would go undetected by the oral glucose tolerance test.

Supported by the Ministry of Agriculture, Fisheries and Food.

Evidence that complement is involved in the mechanism of IDDM serum induced ß-cell cytotoxicity and that provision of glutamine can be cytoprotective. By E. M. CARAHER and P. NEWSHOLME, *Dept. of Biochemistry, University College Dublin, Dublin 4, Ireland*

Insulin-dependent diabetes mellitus (IDDM) is an auto immune condition which involves a self-directed attack on the insulin producing ß-cells of the pancreas. Juntti-Berggren *et al.* (1993) reported that serum from newly diagnosed IDDM patients caused apoptosis in ß-cells in culture as measured by DNA fragmentation, via an unknown mechanism. We have determined that complement components in newly diagnosed IDDM serum can cause ß-cell death via a mechanism which includes DNA strand breakage in primary rat islet cells as well as the ß-cell line CRI-G1. We found that depletion of C1q and C3 components in IDDM serum increased cell viability (determined by Trypan blue exclusion) from 56% (no complement depletion) to 74% (C1q and C3 depletion) when primary rat islet cells were incubated in 10% IDDM serum for 48 hours. DNA strand breaks were measured in rat islet cells exposed to 10% IDDM serum using the method of *in situ* nick translation as described by Fehsel *et al* (1994). After 24 hours 29% of cells incubated in IDDM serum had detectable DNA strand breaks compared with 7% of cells incubated in non-diabetic serum or 9% of cells incubated in heat treated (so destroying complement activity) IDDM serum. The mechanism of DNA damage may have involved the pathway typically utilised by cytokines whereby NFκB is activated, causing genes to be transcribed which specifically cause production of free radicals which subsequently damage DNA. We have used specific inhibitors of NFκB action (PDTC, Deferoxamine Mesylate or Curcumin) and have found that the latter drugs have no effect on the loss in cell viability induced by IDDM serum. However Zn^{2+} did increase cell viability measured after 24 hours exposure to 10% IDDM serum (56% in the absence of 2mM Zn^{2+} to 73% in the presence of 2mM Zn^{2+}). As Zn^{2+} is a known inhibitor of Ca^{2+}/Mg^{2+} dependent endonucleases the latter result suggests that complement, possibly via a rise in intracellular Ca^{2+}, activates an endonuclease which subsequently damages DNA. The strand breaks which occur in ß-cell DNA can cause activation of the nuclear DNA repair enzyme Poly (ADP-ribose) polymerase. Overactivation of latter enzyme depletes cellular pools of NAD^+ and ATP, thus strategies based on increasing ATP production may help the cell to recover from IDDM induced damage. Consequently we determined that 5mM glutamine can significantly ($P<0.01$) enhance cell viability measured 48hrs after rat islet cell exposure to IDDM serum (Figure 1).

Figure 1



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Effects of glycated and non-glycated gastric inhibitory polypeptide on glucose transport and metabolism in isolated abdominal muscle from mice. By F.P.M. O'HARTE, A.M. GRAY, Y.H.A. ABDEL-WAHAB and PETER R. FLATT, *School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

Diabetic hyperglycaemia can lead to glycation of functional proteins and alterations in their biological activity. The present study investigated the effect of glycation of the gut hormone gastric inhibitory polypeptide (GIP) on glucose uptake and metabolism in isolated abdominal muscle. Glycated GIP was prepared by incubation with 220 mM-D-glucose in 10 mM-sodium phosphate buffer (pH 7.4) with a 1000-fold molar excess of $NaBH_3CN$ and purified by reversed-phase HPLC (O'Harte *et al.* 1994).

The ability of non-glycated and glycated GIP to stimulate 2-deoxy-D-[1-^{3}H]glucose uptake was assessed using abdominal muscle strips from 3-5-week-old lean mice. Non-glycated GIP (10^{-10} to 10^{-8} M) stimulated (1.4-1.5-fold) 2-deoxy-D-[1-^{3}H]glucose uptake (626-664 (SE 28-40) dpm/mg tissue per h, $P<0.001$, n 10) during 30 min incubations at 30°, compared with control incubations without GIP (441 (SE 32) dpm/mg tissue per h, n 19). The stimulatory effect of non-glycated GIP on glucose uptake was decreased by 14-20% following glycation of the peptide ($P<0.05$, n 10) between 10^{-10} and 10^{-9} M. Non-glycated GIP (10^{-9} and 10^{-8} M) induced a stepwise 1.4 and 1.7-fold increase ($P<0.01$, $P<0.001$, respectively) in [^{14}C]glucose oxidation in muscle (0.57 and 0.70 (SE 0.04 and 0.06) nmol CO_2 /mg tissue per h), as assessed by $^{14}CO_2$ production at 37°, compared with control incubations without GIP (0.41 (SE 0.04) nmol CO_2 /mg tissue per h, n 15). The stimulation of glucose oxidation by non-glycated GIP was diminished by 32% with 10^{-8} M glycated GIP ($P<0.05$, n 15). Non-glycated GIP (10^{-9} and 10^{-8} M) induced a 1.4-1.8-fold increase in [^{14}C]glucose incorporation into glycogen (glycogenesis) in isolated muscle (0.32 and 0.42 (SE 0.03 and 0.05) nmol glucose/mg per h), compared with control incubations at 37° (0.23 (SE 0.01) nmol glucose/mg per h). The stimulatory effect of non-glycated GIP on glycogenesis was reduced by 41% following glycation of the peptide (10^{-8} M, $P<0.01$, n 13).

This study indicates that the gut hormone GIP promotes glucose uptake, glucose oxidation and glycogenesis in muscle tissue. The effects of 10^{-8} to 10^{-10} M GIP were equivalent to those of 10^{-8} M insulin. This action of GIP complements the well known role as an insulinotropic peptide. Furthermore, these data indicate that GIP can be glycated *in vitro* and that glycation decreases the biological activity of this hormone in isolated muscle tissue. The significance of extra-pancreatic GIP action and its potential glycation in diabetes merits further investigation.

O'Harte, F.P.M., Boyd, A.C., Abdel-Wahab, Y.H.A., Barnett, C.R. and Flatt, P.R. (1994). *Biochemical Society Transactions* **22**, 239S.

Mono- and di-glycated insulin exhibits reduced cellular glucose uptake and glucose lowering activity in mice. By A.C. BOYD, F.P.M. O'HARTE, A.M. GRAY, Y.H.A. ABDEL-WAHAB, H. McNULTY, C.R. BARNETT and P.R. FLATT, *School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

Hyperglycaemia encountered in diabetes is responsible for the non-enzymic glycosylation (glycation) of proteins (Brownlee, 1991). Previous studies illustrated that insulin is glycated within pancreatic ß-cells isolated from animal models of diabetes (O'Harte *et al.* 1995). It has been postulated that glycation of proteins may alter their biological potency. Thus, the aim of this present study was to evaluate the biological activity of mono-glycated and di-glycated human insulin on the regulation of glucose homeostasis in mice.

Mono- and di-glycated insulin were prepared under hyperglycaemic conditions *in vitro*. Briefly, 220 mM-D-glucose was incubated with human insulin (*E. coli* recombinant, Sigma; 1 mg/ml) in the presence of $NaBH_3CN$ (1000 molar excess) for 24 h at 37°, and purified by reversed-phase HPLC (O'Harte *et al.* 1994). Matrix-assisted laser desorbtion mass spectrometry revealed single (mono-glycated, MW 5971.8 Da) and double (di-glycated, MW 6135.1 Da) glucitol adducts attached to insulin. The glucose lowering ability of both mono- and di-glycated insulin was assessed using four groups of lean (OB/+) female mice (*n* 6), aged 15-18 weeks. Intra-peritoneal injection of 400 g/L glucose (2 g/kg body weight) raised plasma glucose levels from 7.5 (SE 0.4) mmol/l to 13.0 (SE 0.5) mmol/l within 30 min ($P<0.001$). Simultaneous administration of non-glycated insulin at a dose of 1.0 U/kg body weight produced a hypoglycaemic response, resulting in a 23% reduction to 5.7 (SE 0.2) mmol glucose/l after 30 min ($P<0.01$). Mono-glycated insulin (1.0 U/kg) displayed a decreased biological activity, reducing plasma glucose levels by only 5% after 30 min to 7.1 (SE 0.4) mmol/l from basal, representing a 22% reduction ($P<0.01$) in glucose lowering ability compared with non-glycated insulin. Furthermore, administration of di-glycated insulin (1.0 U/kg) did not significantly reduce plasma glucose concentrations at 30 min compared with basal.

The ability of mono-glycated and di-glycated insulin to stimulate 2-deoxy-D-[1-^{3}H]glucose uptake was assessed *in vitro*, using isolated abdominal muscle from lean mice aged between 3 and 5 weeks (*n* 8). Non-glycated insulin at 10^{-9} and 10^{-8} mol/l resulted in a dose-dependent increase (32-77%) in 2-deoxy-D-[1-^{3}H]glucose uptake ($P<0.001$) above control (no insulin) (245 (SE 7) dpm/mg per h). Mono-glycation of insulin decreased 2-deoxy-D-[1-^{3}H]glucose uptake by 23-19% compared with non-glycated insulin at 10^{-9} and 10^{-8} mol/l ($P<0.05$). Di-glycated insulin stimulated 2-deoxy-D-[1-^{3}H]glucose uptake above basal (no insulin) at 10^{-7} and 10^{-8} mol/l ($P<0.01$ and $P<0.05$), but exhibited a 27% reduction in activity compared with mono-glycated insulin (10^{-7} mol/l, $P<0.01$) and a 42-31% reduction when compared with non-glycated insulin (10^{-7} and 10^{-8} mol/lz, $P<0.01$).

These present data indicate that glycation of insulin reduced its biological activity in mice, with the di-glycated form appearing to be less potent than mono-glycated insulin. This suggests that glycation of insulin *in vivo* may contribute to insulin resistance and the pathogenesis of non-insulin-dependent diabetes mellitus.

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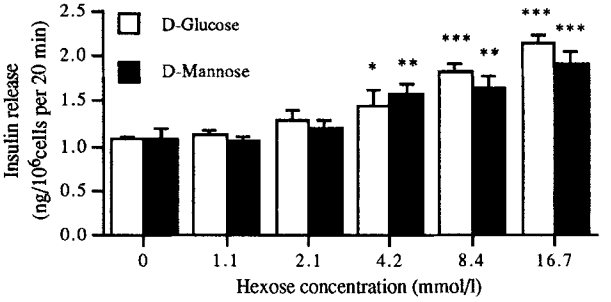
Molecular mechanisms of hexose recognition in insulin-secreting pancreatic BRIN-BD11 cells. By NEVILLE H. McCLENAGHAN, CHRISTOPHER R. BARNETT and PETER R. FLATT, *School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

Much of the current understanding of nutrient-induced insulin secretion has been achieved from studies utilizing insulin-secreting cell lines. We have recently cloned a number of novel pancreatic β-cell lines following the electrofusion of New England Deaconess Hospital (NEDH) rat β-cells with the RINm5F cell line (originally derived from an NEDH rat insulinoma). Two of these, BRIN-BG5 and BRIN-BG7, have been characterized in detail (McClenaghan *et al.* 1996). The aim of the current study was to investigate hexose recognition, and the secretory responsiveness of clonal BRIN-BD11 cells to glucose and other sugars.

Western blotting analysis of cell membrane preparations using a highly specific antibody, revealed that BRIN-BD11 cells express high levels of the glucose transport protein, GLUT-2. Spectrophometric analysis of cytoplasmic cell fractions revealed that BRIN-BD11 cells had a high glucokinase:hexokinase ratio, with glucokinase and hexokinase activities of 5.53 (SE 0.06) and 2.13 (SE 0.25) mU/mg protein respectively (n 6). GLUT-2 is known to act in conjunction with glucokinase to form the 'glucose sensing' mechanism of the pancreatic β-cell (Tiedge & Lenzen, 1994). Indeed, glucose insensitivity of parental RINm5F cells is linked to the absence of the GLUT-2 and a low glucokinase:hexokinase ratio (Halban *et al.* 1983; Tiedge *et al.* 1993).

During acute 20 min incubations (n 6) with Krebs-Ringer Bicarbonate buffer, a range of hexose sugars (glucose, mannose, galactose, fructose, sorbitol, sucrose and mannitol) were tested at 0, 1.1, 2.1, 4.2, 8.4 and 16.7 mmol/l. Of these, only glucose and mannose were effective in evoking an insulin-secretory effect. As shown in the Figure, glucose induced a stepwise 1.3-2-fold increase (P<0.05) in insulin release with a threshold at 4.2 mmol/l. Likewise, mannose caused a significant 1.4-1.7-fold (P<0.05) increase in insulin output indicating that BRIN-BD11 cells transport and metabolize these hexose sugars resulting in insulin secretion.



Values are means with their SE (n 6). *P<0.05, **P<0.01 ***P<0.001 compared with 0 mmol/l hexose (unpaired t test).

In order to study the ability of sugars to potentiate glucose-induced insulin secretion, acute incubations were also performed with 16.7 mmol/l of each hexose in the presence of 5.6 mmol/l glucose. Of these, only glucose and mannose caused respective 2.2 and 1.2-fold increases (P<0.01) of insulin secretion, over that seen with 5.6 mmol/l glucose (1.65 (SE 0.12) ng/10^6 cells per 20 min). These data clearly demonstrate the ability of glucose and mannose to initiate insulin secretion in BRIN-BD11 cells which represent a novel pancreatic B-cell line suitable for further detailed studies of nutrient-induced insulin secretion.

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Evaluation of the antidiabetic effects of coriander (*Coriandrum sativum*). By ALISON M. GRAY and PETER R. FLATT, *School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

Before the discovery of insulin in 1922 and the later development of oral hypoglycaemic agents, treatments for diabetes mellitus relied mainly on dietary measures including traditional medicines derived from plants. Although several hundred traditional plant treatments for diabetes mellitus are documented, few have received scientific or medical scrutiny (World Health Organization, 1980). Coriander (*Coriandrum sativum*) has in the past been used as a traditional plant treatment for diabetes. Here, the antidiabetic potential of *C. sativum* (ground seed) was investigated using streptozotocin (STZ)-induced diabetic mice. The effect of aqueous extract of *C. sativum* on glucose uptake and metabolism was examined using isolated mouse abdomen muscle. The effects of *C. sativum* extract on insulin secretion were also studied using the clonal insulin-secreting BRIN-BD11 pancreatic ß-cell line.

C. sativum was incorporated into the diet (62.5 g/kg) and drinking water (1 g/400 ml prepared by decoction; plant material placed in cold water, covered, brought to the boil then removed from heat source and allowed to stand for 15 min before being filtered) of adult male mice (21-24 weeks, *n* 6-7) fed *ad libitum* for 21 d. STZ (200 mg/kg body weight) was administered by intraperitoneal injection on day 5. Administration of *C. sativum* countered the hyperglycaemia induced by STZ and glycaemic levels did not differ from those of normal mice by 14 d after the administration of STZ, confirming the findings of Swanston-Flatt *et al.* (1990).

Using isolated (normal) mouse abdomen muscle (*n* 6-10), aqueous extract of *C. sativum* (1 mg/ml, prepared by 15 min decoction) increased 2-deoxy-glucose transport (244 ± 37 *v* 397 ± 44 dpm/mg per hr, *n* 6, $P<0.05$), CO_2 production (0.41 ± 0.04 *v* 0.59 ± 0.05 nmoles/mg per hr, *n* 10, $P<0.05$) and incorporation of glucose into glycogen (0.23 ± 0.03 *v* 0.386 ± 0.06 nmoles/mg per h, *n* 10, $P<0.05$). These effects were comparable with those of insulin (10^{-8}M).

During 20 min incubations, *C. sativum* extract (0.25 - 10 mg/ml, *n* 4-6) evoked a dose-dependent 1.3 - 5.7-fold increase in insulin secretion ($P<0.05$ at 0.5 mg/ml) by BRIN-BD11 cells in the presence of low glucose (1.1mM). The effect of extract was abolished by 0.5 mM-diazoxide ($P<0.001$). Extract potentiated ($P<0.05$) insulin secretion induced by 16.7 mM-glucose (3.4-fold), 10 mM-L-alanine (5.9-fold) and by completely depolarized cells (16.7 mM-glucose + 25 mM-KCl, 1.5-fold), but its stimulatory action was not enhanced by 1 mM-3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor.

These results confirm the antihyperglycaemic properties of *C. sativum* and point to the presence of water-soluble component(s) which have both extrapancreatic and pancreatic actions.

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Vitamin C supplementation ameliorates aspects of the diabetic syndrome in obese hyperglycaemic (ob/ob) mice. By YASSER H.A. ABDEL-WAHAB, FINBARR P.M. O'HARTE, CHRISTOPHER R. BARNETT, and PETER R. FLATT, *School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

The effects of vitamin C supplementation on food intake, plasma glucose homeostasis and pancreatic insulin content were examined in groups of six 18-25-week-old lean and obese hyperglycaemic (ob/ob) male Aston mice.

In lean mice, supplementation of the drinking water with vitamin C (25 g/l) did not affect food intake, fluid intake, glycated haemoglobin, plasma glucose or plasma insulin concentrations. Total pancreatic insulin content (167 (SE 17) μg/g wet weight) and the percentage of glycated pancreatic insulin (5.6 (SE 0.4) %) were also similar to control mice (160 (SE 16) μg/g wet weight and 5.3 (SE 0.3) % respectively). In ob/ob mice, vitamin C supplementation caused significant reductions ($P<0.001$) in food intake (8 (SE 4) *v* 11 (SE 1) g/mouse per d), and fluid intake (11 (SE 1) *v* 18 (SE 1) ml/mouse per d) compared with unsupplemented controls. After 14 d supplementation with vitamin C, ob/ob mice exhibited significantly lower ($P<0.001$) plasma glucose (10 (SE 2) *v* 20 (SE 2) mM), glycated haemoglobin (8 (SE 1) *v* 13 (SE 1) %) and plasma insulin concentrations (10 (SE 1) *v* 15 (SE 2) ng/ml) compared with untreated control ob/ob mice. The pancreatic insulin content (98 (SE 9) *v* 179 (SE 13) μg/g wet weight) and extent of insulin glycation (12 (SE 2) *v* 21 (SE 2) %) were also significantly lower ($P<0.001$) in vitamin C supplemented ob/ob mice. In addition the percentage glycated insulin in the circulation was significantly ($P<0.001$) lower compared with control ob/ob mice (2 (SE 1) *v* 9 (SE 2) % respectively).

These data demonstrate that vitamin C supplementation can ameliorate aspects of the obesity-diabetes syndrome in ob/ob mice. Since glycated insulin exhibits impaired glucose-lowering activity (Abdel-Wahab *et al.* 1994), part of this effect might be due to the action of vitamin C as an inhibitor of glycation (Stolba *et al.* 1991).

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Stolba, P., Streda, M., Vondra, K., Hartle, K.& Adam, M. (1991). *Diabetologia* **31**, 546A.

Effect of vitamin C supplementation on metabolic variables and hepatic cytochrome P450 mixed-function oxidase activity in streptozotocin - diabetic rats. By JACQUELINE CLARKE[1], JACQUIE SNELLING[2], COSTAS IOANNIDES[2], PETER R. FLATT[1] and CHRISTOPHER R. BARNETT[1]. [1] *School of Biomedical Sciences, University of Ulster, Coleraine, BT52 1SA,* [2] *school of Biological Sciences, University of Surrey, Guildford, GV2 5XH.*

The P450 - dependent mixed function oxidase system is one of the most important enzyme systems involved in the metabolism of xenobiotics. Its activity may be modulated by a number of factors, including pathological conditions such as insulin - dependent diabetes mellitus (IDDM). IDDM is associated with decreased levels of vitamin C and increased oxidative stress.

The present study investigated the effects of vitamin C supplementation on metabolic variables and hepatic cytochrome P450 expression in streptozotocin (STZ)- diabetic male Wistar Albino rats. The STZ rats displayed the usual characteristics of IDDM including; hyperphagia, polydipsia, decreased body -weight gain, and increased expression and activity of hepatic CYP 1A, 2B, 2E, 4A proteins, as previously demonstrated (Ioannides *et al.* 1995) .

	Control (n 5)		Control+ vitamin C (n 5)		STZ- diabetic (n 5)		STZ- diabetic+ vitamin C (n 5)	
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Plasma vitamin C (μmol/l)	128.3	13.3	151.2*	8.3	95.1	20.5	220.4***†††	14.4
Plasma glucose (mmol/l)	8.1	1.2	10.1	1.4	29.9***	0.5	22.3***†	2.4
Glycated haemoglobin (%)	3.8	0.2	3.4	0.3	15.6***	2.4	12.1***†	1.4
Plasma triglycerides (mmol/l)	1.2	0.2	1.3	0.1	4.3***	0.8	1.8††	0.3
Plasma ketone bodies (mmol/l)	1.6	0.1	1.7	0.2	7.3***	0.1	3.0***†††	0.1
P - nitrophenol hydroxylase (nmol/min per mg protein)	0.61	0.1	0.69	0.1	1.45***	0.1	0.21**†††	0.1

Mean values were significantly different from those for controls *P<0.05,***P<0.001. Mean values were significantly different from those for STZ-diabetic †P<0.05, ††P<0.01, †††P<0.001. Statistical analysis performed using student's t-test

As shown in the Table vitamin C adminstration in drinking water (20g/l) was associated with significant decreases in the levels of hyperglycaemia, glycated haemoglobin, hyperlipidaemia and hyperketonaemia compared with the STZ diabetic group receiving no vitamin C. Vitamin C - treatment significantly and selectively reduced the diabetes induced increase in P- nitrophenol hydroxylase activity, an activity specifically catalysed by CYP 2E. Vitamin C adminstration did not affect these variables in control rats.

The reduction of CYP 2E activity by vitamin C, could be a consequence of reduced circulating ketone body concentrations, although a direct effect of vitamin C on CYP 2E expression in STZ - diabetic rats cannot be discounted.

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Weight perceptions and weight loss practices of Irish teenage girls. By Y.M. RYAN[1], M.J. GIBNEY[2], H. JOHNSON[3], and M.A.T. FLYNN[1], [1]*Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland.* [2]*Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland.* [3]*Community Care Area 2, Eastern Health Board, Ireland.*

A 'desire for thinness' resulting in inappropriate dieting and weight loss practices is a well-recognised phenomenon amongst adolescent females (Hill *et al.* 1992). The present study was designed to investigate the level of dissatisfaction with body weight and the weight loss practices of a group of Irish teenage girls.

Subjects (*n* 420, mean age 15 years) were recruited from the transition year of five single-sex, secondary schools (two fee-paying and three public) in Community Care Area 2 of the Eastern Health Board (South Dublin). A self-report questionnaire was administered in a classroom setting to assess the perceived weight and slimming practices of the group. The height and weight of a random sample of 201 subjects was measured. The growth standards chart as devised by Hoey *et al*, (1986) was used to determine the ideal weight for height, age and sex for each subject, percentage relative weight was calculated and each subject was assigned to one of three categories of relative weight i.e. underweight, normal weight or overweight, as defined by the National Center for Health Statistics (1973).

A significant disparity was found to exist between actual relative weight and perceived weight categories and a high level of body weight dissatisfaction was identified (see Table).

	Actual relative weight (*n* 201)		Perceived weight (*n* 201)		Satisfaction with perceived weight: Satisfied with perceived weight (*n* 53)		Satisfaction with perceived weight: Dissatisfied with perceived weight — 'Want to be lighter' (n 120)		'Want to be heavier' (*n* 7)		Don't know (n 19)	
	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*
Underweight	14	29	9	18*	44	8	11	2	33	6	11	2
Normal weight	62	125	44	89	48	43	34	30	1	1	15	13
Overweight	23	47	45	90	1	1	96	86	0	0	3	3
Don't know	0	0	2	4	25	1	50	2	0	0	25	1

* Significant difference between actual relative weight and perceived weight categories, Chi-square=52.16, $P<0.0001$.

Of 420 subjects, 247 (59%) reported that they were not satisfied with their weight and wanted to be lighter; although 67 (27%) of these perceived themselves as underweight or normal weight.

Of the total group, 286 (68%) reported that they had tried to lose weight in the past and of these 194 (68%) said that they did lose weight. Slimming methods reported included 'avoiding sugary foods' 81% (*n* 232); 'exercising' 80% (*n* 228); 'skipping meals' 52% (*n* 148); 'dieting' 44% (*n* 125); 'smoking' 19% (*n* 55); 'inducing vomiting' 15% (*n* 42); 'avoiding red meat' 13% (*n* 38); 'avoiding snacks' 7% (*n* 19); 'drinking water to decrease hunger' 6% (*n* 16); 'using laxatives' 5% (*n* 15) and 'using diet pills' 4% (*n* 10). In conclusion irrespective of body weight, a high proportion of the teenage girls surveyed wanted to be slimmer than their perceived weight and have engaged in many inappropriate weight loss strategies.

We gratefully acknowledge support from the SRD programme, Dublin Institute of Technology and An Bord Bia, Dublin.

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Meat avoidance, weight perceptions and nutrient intakes of Irish teenage girls. By Y.M. RYAN[1], M.J. GIBNEY[2], H. JOHNSON[3], and M.A.T. FLYNN[1], [1]*Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland.* [2]*Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland.* [3]*Community Care Area 2, Eastern Health Board, Ireland.*

It has been hypothesised that the increased popularity of meat avoidance by teenage girls may be associated with a desire to reduce body weight. Many authors have questioned the nutritional adequacy of a 'meatless' diet during the teenage years, a time of high nutrient requirements (British Nutrition Foundation, 1995). The present study examines meat avoidance by a group of Irish teenage girls and investigates the nutritional implications of a reduced meat intake.

Subjects (*n* 420, mean age 15 years) were recruited from the transition year of five single-sex, secondary schools (two fee-paying and three public) in the Eastern Health Board (South Dublin). Each subject completed a self-report questionnaire that assessed meat avoidance and reasons for avoiding meat, weight perceptions and slimming practices. The nutrient intake of a random sample of 201 subjects was determined using the 7 day diet history method of the INNS (Lee & Cunningham, 1990).

Of 201 subjects, 21 (10%) avoided eating meat. A further 42 (21%) subjects said that they 'would like to avoid meat'. In the Table the nutrient intakes of 'meat eaters' are compared with those of subjects who 'would like to avoid meat' and vegetarians. The Fe intake of the vegetarians was well below the estimated average requirement of 11.4mg/ day for teenage girls (DOH, 1991).

	'Meat eaters' (*n* 138)		'Reduced meat eaters' 'Would like to avoid meat' (*n* 42)		Vegetarians (*n* 21)	
	Mean	SD	Mean	SD	Mean	SD
Energy (MJ)	9.0	2.2	8.5	2.6	8.0*	2.0
EI:BMR (MJ/ 24hours)	1.47	0.39	1.38	0.45	1.36	0.36
% Fat	37.5	5.4	36.3	5.1	37.2	6.2
% Protein	13.9	1.9	13.7	2.1	11.4*** †††	2.3
% Carbohydrate	48.0	4.7	48.8	4.7	50.6*	5.6
Fibre (g)	19.3	5.0	18.9	6.1	19.0	7.2
Total iron (mg)	11.3	3.2	10.6	3.5	9.6*	2.9
Haem iron (mg)	2.1	1.1	1.6††	0.9	0.10*** †††	0.2
Non-haem iron (mg)	9.2	3.0	9.0	3.3	9.5	2.9
Vitamin C (mg)	118.8	56.0	123.2	79.3	120.7	83.3
Vitamin B_{12} (μg)	3.7	3.5	3.4	2.1	1.9*	0.9
Zinc (mg)	8.6	2.1	8.1	2.9	5.6***	1.9

* Mean value was significantly different from 'meat eaters'; * $P < 0.05$, *** $P < 0.001$. † Mean value was significantly different from 'meat eaters'; †† $P < 0.01$. ‡ Mean value was significantly different from subjects who 'would like to avoid meat'; ††† $P < 0.001$ (unpaired *t* test).

The proportion of subjects who 'wanted to be slimmer' was higher among the 'reduced meat eaters' compared with 'meat eaters' (75 *v.* 61%, P=0.003) and more of the 'reduced meat eaters' compared with 'meat eaters' reported that they had tried to lose weight (80 *v.* 62%, P=0.002). In conclusion, meat avoidance by teenage girls may increase the risk of having an inadequate dietary iron intake and appears to be linked with a desire to reduce body weight.

We gratefully acknowledge support from the SRD programme, Dublin Institute of Technology and An Bord Bia, Dublin.

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Department of Health (1991). *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom.* Report on Health and Social Subjects No. 41. London: HMSO.

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Meat avoidance and dietary calcium intakes of Irish teenage girls. By Y.M. RYAN[1], M. O'DONOGHUE[1], M. CANTWELL[1], H. JOHNSON[2], and M.A.T. FLYNN[1], [1]*Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland.* [2]*Community Care Area 5, Eastern Health Board, Ireland.*

Ca consumption during adolescence may influence the risk of osteoporosis in later life. Optimal Ca intake is estimated to be 800mg/ d for adolescent girls (DOH, 1991). Girls on energy-restricted diets may be at particular risk of inadequate Ca intakes (Portsmouth *et al.* 1994). The purpose of the present study was to assess the weight perceptions and dietary Ca intakes of adolescent girls in transition year in two Dublin schools (West Dublin).

Subjects (*n* 40, mean age 15 years) were randomly selected from the two schools. Weight perceptions, attitudes towards milk and knowledge about Ca requirements were assessed using a self-report questionnaire. The nutrient intake of the subjects was determined using the 7 day diet history method of the INNS (Lee & Cunningham, 1990) and weight and height were measured. Based on their weight concerns, the girls were divided into two groups; those who were 'dissatisfied with their weight and wanted to weigh lighter' and those who were 'satisfied with their weight'. Within the group of girls who 'wanted to weigh lighter' two further subgroups were identified; the 'Dieters' whose reported energy intake(EI) : BMR ratio was less than 1.35 and who claimed to be dieting at the time of the study and the 'Non-dieters'. In the Table the nutrient intakes and nutritional knowledge of the three groups of subjects are compared.

Adolescent girls†	'Dissatisfied with weight and want to weigh lighter' 'Non-dieters' (*n* 16)		'Dieters' (*n* 7)		'Satisfied with weight' (*n* 15)	
	Mean	SD	Mean	SD	Mean	SD
Energy (MJ)	8.5	1.2	5.2*	1.3	8.3	1.4
EI:BMR (MJ/ 24hours)	1.36	0.2	0.84*	0.2	1.43	0.3
Calcium (mg)	885	253	389*	177	777	250
Vitamin D (μg)	2.7	1.6	1.4	0.7	2.31	1.2
Fibre (g)	16.1	4.0	10.6	3.6	17.5	4.0
Nutritional knowledge	%	*n*	%	*n*	%	*n*
Perceive milk as fattening	56	9	86	6	73	11
Aware of calcium requirements in food terms	81	13	57*	4	100	15

* Significantly different from subjects 'satisfied with weight', $P<0.05$ (Mann-Whitney U).

† Excluded from this table are subjects (*n* 2) who 'want to be heavier'.

The reported energy intake of the 'Dieters' reflects intake while dieting and could not represent habitual energy consumption. The low energy intake of the 'Dieters' was associated with Ca intakes which were less than half of the current recommendations for teenage girls. Of the 'Dieters', 95% had Ca intakes below the lower reference nutrient intake of 480mg/ day compared with 20% of the total group (DOH, 1991). Moreover, the 'Dieters' were less aware of how to achieve their Ca requirements in food terms. Milk provided 12% of the 'Dieters' Ca intakes compared with 27% for the total group. Of the total group, 68% perceived milk to be a fattening food. In conclusion, teenage girls who are dieting may not achieve an adequate Ca intake. A lack of knowledge about Ca requirements among 'Dieters' and the general perception that 'milk is fattening' may contribute to their low Ca intakes.

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Food choices of primary-school children: initial observations. By ANITA EVES[1], MICHAEL CORNEY[1], MICHAEL KIPPS[1] ,CAROL NOBLE[2] and MARGARET LUMBERS[1], [1]*Department of Management Studies, University of Surrey, Guildford GU2 5XH;* [2]*Applied Technology and Computing Department, Roehampton Institute, Whitelands College, West Hill, London SW15 3SN*

School meals provide the main meal of the day for many children, and thus the composition of the meal is of nutritional significance. Previous studies have found that school meals can contribute significantly to the fat intake of children, especially those selecting foods from self-service-style canteens (Noble & Kipps, 1994). Primary school children (*n* 105), aged 10-11 years, were asked to make meal choices (their preferred meal and the meal they perceived to be most healthy) from a range of high quality photographs of components of school meals. They were asked to explain their choices.

	Food category	Item most often selected	% selecting item
Preferred meal	Main item	Pizza	39.0
	Starch source	Chips	39.8
	Vegetable	Baked beans	34.1
	Pudding	Ice-cream with chocolate sauce	29.3
'Healthy' meal	Main item	Sliced gammon	32.5
	Starch source	Rice	39.0
		Boiled potatoes	39.0
	Vegetable	Salad	60.2
	Pudding	Apple pie with cream	61.8

Pupils were presented with the four categories of foods shown in the Table, as this is how they are required to make their choices in the canteen. The Table shows the item in each category selected by the greatest number of children, it does not indicate that the items constitute the most commonly selected combination of foods. It is clear that the items chosen in the 'preferred' and 'healthy' meals differ. Reasons for selection of items in the 'preferred' meal included taste and the presence of specific ingredients such as cheese, with some consideration being given to appropriate food combinations. 'Healthy' was rarely given as a reason for choosing items in the 'preferred' meal. Reasons for selecting items for the 'healthy 'meal included the presence of cheese, eggs, meat, fruits or vegetables, which were almost always considered to relate to healthiness; and the absence of fat. Thus it appears that some of the messages relating to healthy eating have reached children, but are not acted on by most. In addition, the reason given for a 'healthy' choice was not always consistent with the food chosen. The main source of information on healthy foods was family members. It is clear that the quality of this information was variable, suggesting the need for classroom education to guide food choices. The nutritional implications of the meal choices are to be assessed and the reasons for meal choices explored further. Previously work has focused on single items, ignoring the influence of food combinations in meal choices.

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Leeds High Fat Study: Behavioural characterization of high and low fat consumers. By JENNIE I. MACDIARMID[1], VIKKI HAMILTON[1], JANET E. CADE[2] and JOHN E. BLUNDELL[1], [1]*BioPsychology group, Department of Psychology and* [2]*Nuffield Institute for Health, University of Leeds, Leeds LS2 9JT*

One of the major health concerns in Western society is the high intake of dietary fat. Despite goals to reduce consumption, the percentage of fat in the diet has remained well above the recommendations. In a climate where a principal aim of health promotion is to reduce fat intake, only little is known about the characteristics of people consuming a high fat diet. Characterization of these individuals may help understanding of a group at which health promotion may be targeted.

The Leeds High Fat Study (LHFS) was designed to identify and characterise high fat (HF) consumers and compare them with a group of low fat (LF) consumers (who meet the current dietary recommendations for fat). The LHFS differs from many large cross-sectional studies in having identified high and low fat consumers, these individuals were then followed up in detail and further characterized. In phase I of the LHFS, high and low fat consumers were identified from 3000 randomly selected individuals in the Leeds postal area using a sixty-three item food frequency questionnaire (FFQ). The 177 HF and 472 LF consumers were classified as consuming >45% and ≤35% food energy from fat respectively. Fifty-four HF, with both a high proportion (% energy) and absolute intake (g/day) of fat in their diet (Macdiarmid *et al* 1995), and fifty-seven LF consumers were followed up in phase II of the study. Individuals completed a 7d weighed food record, a series of questionnaires (Three Factor Eating Questionnaire, TFEQ) (Stunkard & Messick 1985), fat preferences, nutritional knowledge and a further FFQ) and an in-depth interview which explored a number of physical, behavioural and social characteristics.

HF and LF consumers were re-classified on the basis of the 7d weighed food record and both FFQ, and those with consistently high or low fat intakes were included in the analysis. Logistic regression analysis was used to identify characteristics associated with high fat consumption (all models included age, sex and BMI). The models distinguished the two groups on a number of characteristics which could be perceived as risk taking behaviours. High fat consumption was significantly associated with smoking ($P<0.01$), 52% of the HF consumers compared with only 3% of the LF consumers were regular smokers. Regular beer, but not wine, drinking (> once a week) was associated with HF intakes ($P<0.01$). HF consumers also tended to eat fewer meals per day which was explained by significantly fewer of them eating breakfast (only 44% compared with 96% of LF; $P<0.01$). Assessing nutritional knowledge between the two groups, HF consumers had a significantly poorer knowledge ($P<0.01$). HF consumers were less dietary restrained than the LF consumers as measured on the TFEQ. Although HF consumers had a greater preference for high fat foods, LF consumers also scored highly on preferences for high fat foods. These distinct differences found between high and low fat consumers have some implications for the promotion of health messages.

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Honest but invalid: consumer views on recording their food intake. By DAVID J. MELA and JACQUELINE I. AARON, *Consumer Sciences Department, Institute of Food Research, Reading RG6 6BZ*

There is little information about the subject characteristics which might predict the accuracy or validity of self-reported food intake records. Many studies suggest that the reporting of implausibly low total energy intakes may be more likely amongst overweight and obese subjects, but this is common in records from normal-weight subjects as well (Schoeller, 1995). Furthermore, it is not generally possible to distinguish between honestly recorded but atypical intakes during the recording period (under-eating), and truly fraudulent or incomplete records (under-reporting). As part of a more extensive examination of these issues, we have assessed consumer perceptions of different diet recording methods and beliefs about their own likely behaviour in undertaking such a task.

Individuals attending open days and fairs in the Reading area were asked to complete questionnaires eliciting demographic information, with single YES/NO questions to establish current practice of dietary restraint and current and past dieting for weight control. These were followed by a set of 10 cm line scales for rating the degree of embarrassment they would feel at having their weight measured, the perceived difficulty, inconvenience, etc of keeping diet records, how accurately they believed they would record their intake, the ways and extent to which recording their intake would cause them to eat differently, and the likelihood that their records would be complete and honest. Each subject was given a single questionnaire, in which all questions on diet records specifically referred either to filling out a food frequency questionnaire (FFQ), or to recording estimated (EST) or weighed intakes (WEIGH). Responses here are from 240 subjects aged ≥18 years (eighty-two male, 158 female), with selected outcome measures analysed in relation to sex, restraint, dieting, and normal- *v.* overweight (BMI ≤25 *v.* >25 kg/m² respectively).

Taking all recording methods together, scores for predicted accuracy and honesty were generally moderate to high, and did not differ in relation to subject characteristics. However, reported likelihood of eating differently, eating less overall, and eating less of certain foods were all significantly greater for restrained *v.* unrestrained eaters (*t* test, all $P<0.001$) and for dieters *v.* non-dieters (all $P<0.05$), but did not differ in relation to sex or normal *v.* overweight status. In addition, restrained eaters indicated they would also be more likely to eat more of certain foods during the recording period ($P<0.001$). It is particularly notable that all of these effects of restraint remained robust even when the analysis was limited to subjects of normal weight. Embarrassment about weight was the single continuous variable most closely and consistently correlated with measures of anticipated dishonesty and difficulty in recording intake. This, too, was true even amongst subjects of normal weight.

Anticipated difficulty was generally low for all three recording methods, although EST was viewed as likely to be more difficult than FFQ or WEIGH ($P<0.05$, one-way ANOVA), FFQ was generally seen as less inconvenient and less time-consuming than other methods, and WEIGH more accurate. Scores for honesty were similarly high for all methods, although the rated likelihood of eating differently, and eating less and more of certain foods were all lower for FFQ *v.* EST and WEIGH.

These data indicate that while consumers largely believe they would keep honest diet records, certain consumer subgroups explicitly acknowledge that they would alter their food intake behaviour during the recording period. Subjects who are practising dietary restraint or embarrassed about their weight appear much more likely to generate records which would not be a valid reflection of their habitual diet, even if those subjects are of normal weight. Assessment of these and related subject characteristics, and of subject views about dietary intake recording, may be a useful adjunct to the selection of study participants and/or analysis of resultant nutrient intake data.

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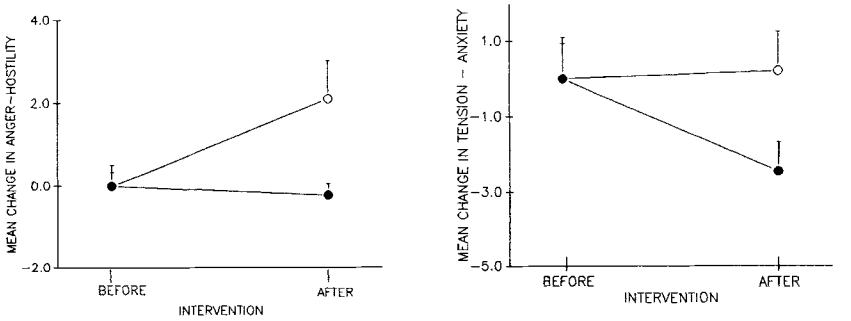
Adverse alterations in mood after changing to a low fat diet. By A. S. WELLS[1], N. W. READ[1], J. D. E. LAUGHARNE[2] and N. S. AHLUWALIA[2], [1]*Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Herries Road, Sheffield S5 7AU,* [2]*University Department of Psychiatry, Northern General Hospital, Herries Road, Sheffield S5 7AU*

For the past few decades health experts have recommended a reduction in the amount of fat in the British diet with the aim of lowering plasma cholesterol levels and thereby reducing mortality from ischaemic heart disease. There are now some suggestions that this policy may be associated with adverse behavioural and psychological effects (Muldoon *et al.* 1990; Kaplan *et al.* 1991).

The effects on mood of a reduction in dietary fat while keeping the energy constant were examined in ten healthy male and ten healthy female volunteers aged between 20 and 37 years. Subjects were excluded if they had abnormal concentrations of blood lipids, a BMI less than 20 or greater than 31 kg/m^2, smoked cigarettes, consumed more than 21 units of alcohol a week, had previously suffered from any psychiatric disorder, or were likely to experience a stressful life event during the months of the study. Alcohol consumption and exercise patterns were controlled during the study. Each volunteer consumed a medium fat diet (41% energy fat) for 1 month. For the second month half of the subjects changed to a low fat diet (25% energy fat) and the remainder continued to eat the medium fat diet. All diets were supplied to volunteers' homes. Ratings of mood were assessed using the profile of mood states questionnaire (McNair *et al.* 1971).



○ Low fat diet (25% energy fat) ● Medium fat diet (41% energy fat)

Scores for anger-hostility increased in the intervention group after 1 month on the low-fat diet, while during the same time there was virtually no change in anger-hostility in the subjects consuming the medium-fat diet (group x time (F 4.58; df 1,15; P=0.049)). Tension-anxiety declined in the group consuming the medium-fat diet but did not change in the group consuming the low-fat diet (group x time F 5.94; df 1,15; P=0.028). There was a trend for ratings of depression to increase after one month on the low-fat diet and to decline slightly after the medium-fat diet, however, this group x time interaction just failed to reach statistical significance, (F 4.23; df 1,15; P=0.058). Fasting HDL-cholesterol concentrations declined significantly after the low fat diet (time x group F 5.21; df 1,13; P=0.040) (mean change, low-fat -0.122 (SE 0.043) medium-fat 0.050 (SE 0.053) mmol/L), but in contrast to previous epidemiological observations, there were no significant correlations between the changes in any of the blood lipid measurements and alterations in mood. There were no significant differences in palatability ratings of the two diets.

In summary, there are indications that changing to a low fat diet may have undesirable effects on mood. The subjects in the present study were psychologically healthy, and the effects that a change in diet may have on a less healthy or a more susceptible group of subjects may be more pronounced. The results of this preliminary study indicate the need for further investigations into the effects of a change of diet on mood.

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An investigation of the beliefs, attitudes and practices of dietitians in relation to the use of antioxidant vitamins in the treatment of coronary heart disease (CHD) and cancer. By DIANE F. SMITH* and TERRY KIRK, *Queen Margaret College, Clerwood Terrace, Edinburgh EH12 8TS.*

As evidence continues to increase from epidemiological studies and from clinical trials for a possible protective role for antioxidant vitamins against CHD and cancer, there may be a need for more education of dietitians to consider recommending antioxidant vitamins as part of the treatment of these diseases. With this in mind, a questionnaire was designed according to the methodology of Oppenheim (1968) to investigate the beliefs, attitudes and practices of dietitians in relation to the use of antioxidant vitamins (α tocopherol, β carotene and vitamin C) in the treatment of CHD and cancer.

The study was carried out by eighty postal and twelve telephone questionnaires. Fifty-four dietitians completed the postal questionnaire and twelve completed the telephone questionnaire. The total response rate was 72%. All were based in hospitals throughout Great Britain.

The number of dietitians covering CHD and cancer was thirty-six, and of these, 69% reported giving advice on all three of the antioxidant vitamins to CHD patients with 33% giving advice to cancer patients. Of dietitians (*n* 17) covering CHD alone, 65% gave advice on antioxidant vitamins. Of dietitians (*n* 13) covering cancer alone, 15% gave advice on antioxidant vitamins. No significant differences were found between the strengths of beliefs and attitudes for the three antioxidant vitamins in the context of prevention and treatment. The majority of the sample had moderately strong beliefs in the context of prevention in CHD and cancer (i.e. for CHD typical rating being 66% for vitamin C, 68% for β carotene and 70% for α tocopherol 'agree', for cancer 37%, 45% and 45% respectively). None disagreed. However, dietitians are slightly less convinced that CHD patients with high plasma antioxidant levels and patients with certain cancers undergoing active treatment, will benefit from dietary advice on antioxidant vitamins as more rated 'undecided' to questions about these (40% to 51% for CHD and 37% to 39% for cancer). The majority had moderately strong beliefs and attitudes against supplementation in CHD patients with high plasma antioxidant levels (55% to 59%) and moderately strong beliefs and attitudes regarding supplementation when plasma levels are low (38% to 43%). The majority (49% to 51%) were 'undecided' regarding supplementation for patients with certain cancers undergoing active treatment.

Results indicate that journal articles, conferences and work colleagues are major influences on beliefs and attitudes and hence, practice. A Spearman-Rank correlation test found a significant difference between median rank order of different sources of information on antioxidant vitamins in relation to importance in influencing beliefs and attitudes towards antioxidant vitamins and quantity of information obtained (P=0.04). Therefore, the more information received, the more influence it has. This provides information for educators of dietitians on how best to influence practice and thus potentially improve the health of the patient.

However, the study suggests that strong beliefs and attitudes towards antioxidant vitamins do not necessarily translate into practice. Practicalities, such as patients' understanding, prognosis, priority for other advice and time available, all appear to influence practice.

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Applying stages of change and motivational interviewing to dietary counselling. By C. NI MHURCHU and B.M. MARGETTS, *Wessex Institute of Public Health Medicine, University of Southampton, Southampton SO16 6YD*

There are many initiatives currently underway in the UK to reduce the prevalence of coronary heart disease (Department of Health, 1992). Hyperlipidaemia is considered to be a major modifiable risk factor for this disease.

Recently, intervention trials have been undertaken in primary care (OxCheck Study Group, 1995) to reduce the prevalence of CHD but dietary advice has only achieved small reductions in serum cholesterol levels. Consequently, there is doubt about the effectiveness of dietary treatment in this area (Haq *et al*, 1995).

However, behaviour change is a complex process as demonstrated by behavioural theories such as the stages of change (Prochaska & DiClemente, 1982) and motivational interviewing (Miller & Rollnick, 1991). The primary care studies have failed to adequately assess or quantify the dietary interventions in terms of content and motivation.

In the present study, these theories are being applied to the process of dietary counselling. Patients with hyperlipidaemia (n 130) have been randomized to either standard dietary intervention or to motivational interviewing. The study has involved applying the stages of change to reductions in dietary fat intake, and also the development of a motivational interviewing menu for use in dietary counselling. Outcomes being evaluated include changes in serum lipid levels, BMI, stage of dietary change, and dietary intake (using seven day food records).

Preliminary analysis has been carried out on the baseline characteristics of a sub-sample (n 87). No statistically significant differences were found between groups at baseline. Mean percentage energy intake from fat (32.7%) was lower than the UK population average. 85% of the sample were categorised in the "action" or "maintenance" stages of dietary change (i.e. already involved in reducing fat intake) prior to receiving any dietary advice. This is a higher percentage than is generally seen in population samples. This may explain the low mean percentage energy intake from fat. It would also suggest that little further significant dietary change will occur in this group of subjects.

There are indications at present that the current stages of change model and the means of classifying people within stages (Prochaska *et al*, 1992) may need to be further developed to adequately characterise a complex behaviour such as diet.

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Effect of nutrition education on the attitudes of caterers towards the provision of healthy meals.

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A number of catering companies offer employees nutrition education courses to aid them in the provision of more healthy meal choices. These courses include elements of nutrition theory, as well as covering recipe modifications or changes in preparation and cooking methods. The present study aimed to determine how attendance of such courses influenced attitudes and intentions towards the provision of more healthy meal choices. A questionnaire was designed according to the requirements of the reasoned action model (Ajzen & Fishbein, 1980) and sent to 200 industrial caterers (85% response rate). Ninety-seven respondents had attended an in-house nutrition training course, sixty-nine had not. Similar questionnaires were also sent to school caterers, and their attitudes and intentions compared with their level of nutritional knowledge.

Belief	Score		Significance of difference $P =$
	Training not attended n 69	Training attended n 97	
* Providing fibre-rich foods means customers can make healthy choices [a]	5.93	7.48	0.01
* Customers think that I should provide starchy carbohydrates [b]	2.12	5.13	0.05
* Customers think that I should provide vegetables [b]	12.87	16.26	0.001
* Customers think that I should provide fruit and fruit juice [b]	13.44	16.67	0.001
* Customers think that I should provide fibre-rich foods [b]	2.63	6.82	0.01
Overall intention to provide more healthy choices [c]	8.65	10.80	0.001

Possible range of scores: [a] -9 to +9; [b] -21 to + 21; [c] -15 to + 15

The Table shows significant differences between the beliefs and intentions of those who had and had not attended the in-house nutrition training course. A higher score in all cases indicates a more positive response. Those who had attended the course were significantly more positive in their beliefs towards providing more healthy meal choices (in relation to the provision of particular food groups). They were significantly more likely to believe that their customers wanted the foods, and overall had a more positive intention to provide the foods. Results suggest that nutrition training can have a positive effect on caterers' beliefs and intentions to provide more healthy meal choices. The provision of practical training may also give caterers the confidence to prepare and serve more healthy items. Results comparing aspects of nutritional knowledge with attitudes also showed a positive relationship for all types of caterer studied.

The authors gratefully acknowledge funding by MAFF.

Ajzen, I. & Fishbein, M. (1980). *Understanding Attitudes and Predicting Social Behaviour.* Englewood Cliffs: Prentice-Hall.

The teaching of nutrition in catering-related courses in England and Wales. By ANITA EVES[1], MICHAEL CORNEY[1], MICHAEL KIPPS[1] and CAROL NOBLE[2], *[1]Department of Management Studies, University of Surrey, Guildford GU2 5XH; [2] Applied Technology and Computing Department, Roehampton Institute, Whitelands College, West Hill, London SW15 3SN0*

The *Health of the Nation* white paper (Department of Health, 1992) specifically identified caterers as having a role in the provision of a healthy diet. This is particularly pertinent for caterers who supply meals for regular clientele (e.g. in schools or workplace canteens), where the meal may constitute the main meal of the day. Previous studies (Noble & Kipps, 1994; Ministry of Agriculture, Fisheries and Food, 1995) have indicated, for example, that such meals contribute significantly to daily fat intakes. If caterers are to provide healthy meal options, they must understand the basis of a healthy diet and how to prepare healthy meals. This requires nutrition education. All institutions (*n* 244) offering catering-related courses in England and Wales were surveyed to determine the extent and nature of nutrition education offered to catering students. Responses were received from seventy-one colleges, giving a response rate of 29% (which is not unusual for a postal questionnaire). Non-respondents were followed up on two occasions.

Proportion of colleges teaching nutrition and specific elements of nutrition (of those that do teach nutrition)

Course	Teach nutrition	Nutrients -function in body	Nutrients in foods	Effect of processing and storage	Needs of different age groups	Implications of food on health	Current dietary goals and guidelines	Menu planning
Degree	100	67	75	75	83	75	75	83
HCIMA/A	93	43	36	43	36	36	36	43
HCIMA/B	90	11	11	22	22	22	22	22
BTEC-CD	88	67	63	67	67	57	60	63
'' HC/HD	95	62	76	59	67	62	62	71
NVQ1	75	47	47	47	27	23	20	40
NVQ2	72	50	61	67	48	39	33	65
GNVQ	83	76	82	82	84	79	68	84
RSH-CD	85	50	67	67	67	67	67	67

HCIMA A/B, Hotel and Catering International Management Association professional qualifications; BTEC C/D, Business and Technology Education Council certificate/diploma (Hotel, Catering and Industrial Operations); BTEC HC/HD, Business and Technology Education Council higher certificate/higher diploma (as above); NVQ 1/2, National Vocational Qualification levels 1 and 2 (Catering and Hospitality); GNVQ, General National Vocational Qualification (Hospitality and Catering); RSH -CD, Royal Society of Health certificate/diploma in nutrition (aimed at those working in the catering sector).

The Table indicates that nutrition was not taught on all catering courses, notably not on NVQ courses (there was no requirement to teach nutrition at the time). NVQ courses were taken by the largest proportion of students. Most food preparation lecturers felt that the amount of nutrition taught on such courses was insufficient, some expressing great concern. The nature of nutrition education also varied, with a notable proportion of colleges offering very limited courses. Of respondents, however, 94% agreed that caterers have a responsibility to modify food habits and 92% that it was relevant to teach the implications of diet on health. Data suggest that a large number of students enter catering careers ill-equipped to provide healthy meal options.

The authors gratefully acknowledge funding by MAFF.

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Healthier eating: income, difficulty and food intake. By RICHARD SHEPHERD[1], CLAIRE M. PAISLEY[1], SUSAN ELEY[2], PAUL SPARKS[1], ANNIE S. ANDERSON[2] and MIKE E.J. LEAN[2], *[1]Institute of Food Research, Earley Gate, Whiteknights Road, Reading RG6 6BZ, [2]Department of Human Nutrition, University of Glasgow, G31 2ER*

To investigate the association between income and healthier eating, 400 adults from five different income groups in England and Scotland completed a structured questionnaire. The theory of planned behaviour (Ajzen, 1988) was used to assess the factors influencing the likelihood of eating a healthier diet in the next 6 months. Current food intake was assessed by means of a food frequency list. Potential barriers (including the cost and value for money of eating a healthier diet), nutrition knowledge, the amount of money spent on food and income from several sources were also measured.

The higher income groups spent more on food but less as a proportion of their income than did those in the lower income groups ($p<0.001$). The lower income groups consumed significantly ($p<0.05$) less brown bread, fruit juice, fresh vegetables and semi-skimmed milk than the higher income groups, but more white bread, chips, tinned vegetables and whole milk.

Those in the higher income groups were more likely to think that their current diets were healthy (74% of highest income group compared with 44% of lowest income group, $p<0.05$) but there was no difference in perceived need to eat a healthier diet between the different income groups. A healthy diet was perceived as good value for money by more people in the highest income group (81%) compared with the lowest income group (54%) ($p<0.05$). While there were no differences between income groups in the 'likelihood of eating a healthier diet', the lower income groups saw greater difficulty in consuming a healthier diet ($p<0.01$).

Multiple regression showed that perceived difficulty was the most important predictor of likelihood of eating a healthier diet in the future for all income groups, with perceived need also important for all groups. However, perceived value for money of a healthy diet was a significant predictor of likelihood for the two lowest income groups but not for the remaining three income groups.

Although there were some differences between income groups in nutrition knowledge, generally knowledge did not differ greatly between the groups. There were misconceptions about healthy eating in all income groups, with only 20% agreeing that people in Britain should eat more starchy carbohydrate.

Food consumption differs between people at different levels of income. If people are to adopt healthier eating practices it is necessary to understand the beliefs and concerns that they have and the barriers they see to making changes. In the present study, the difficulty people anticipate in making changes was a major determinant of likelihood of eating a healthier diet in the future and this perceived difficulty was greater for those on a lower income.

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

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Dietary education of free-living volunteers participating in a 4-month dietary intervention study.

By J.M.E. KNAPPER[1], D.H. WEBB[1], C.N. BROOKS[1], J.A. TREDGER[1], L.M. MORGAN[1], J. WRIGHT[1] and C.M. WILLIAMS[2]. [1] *Centre for Nutrition and Food Safety, University of Surrey, Guildford GU2 5XH and* [2]*Hugh Sinclair Unit of Human Nutrition, University of Reading, Whiteknights, Reading RG6 6AP*

We have recently conducted a single-blind, randomized, controlled crossover dietary intervention study in a total of forty-three men; age range 24-57 years. Of these, 86% were married and 70% had children. The dietary aim of the study was to increase the percentage of energy derived from monounsaturated fatty acids (MUFA) from 12% to 20-22% at the expense of saturated fatty acids (SFA) whilst keeping total energy intake and percentage energy from fat constant. In the test diet MUFA intake was increased by using MUFA-enriched margarines, cooking fats (provided by Van den Berghs, UK), four varieties of biscuits (provided by Diet & Health Ltd, UK) and nine frozen recipe meals (provided by Sainsburys, UK). It was not feasible for all foods to provide the same quantity of MUFA in an acceptable portion size. A lipid unit system was thus devised where a lipid unit represented 5 g MUFA and the number of lipid units contained within a single portion of food ranged from 1-4. A daily lipid unit intake was set for each subject based on calculated individual dietary requirements. An identical range of foods and the same lipid unit system was used to devise the control diet, but spreads and cooking oils based on SFA were used. Subjects were asked to consume their designated number of lipid units for 5 d of the week. Daily intakes ranged from 7-10 lipid units. Due to the high ratio of volunteers to dietetic staff we needed to develop educational material that would standardize the dietary information whilst allowing individual dietary flexibility. One approach used was to develop and produce a dedicated 15 min video explaining the practical issues of the study including: the diet; keeping a diet diary, and monitoring body weight. Subjects were each given verbal and written guidance on the diet and this was summarized on video. The video complemented the written material by providing guidance on how to achieve the required daily lipid unit intake whilst altering the foods eaten each day. All volunteers watched the video with a dietitian and this was followed by a 20 min discussion of its contents. Volunteers were each provided with a copy of the video for their home viewing. Most subjects (83%) found the video to be helpful and 53% reported watching the video at home. The main advantage of the video was the standardizing of the educational message the investigators were trying to impart.

Regular contact with volunteers and monitoring of body weight helped to ensure that subjects were consuming the appropriate number of lipid units. Subject assessment of ease of using the lipid unit system was performed using a linear analogue score where 1 = difficult and 10 = very easy. A mean score of 7.46 (SD 1.80, *n* 34) was reported during the control period and a mean of 7.57 (SD 1.77, *n* 38) during the test period. Subjects reported achieving their daily lipid unit target for a mean 4.7 (SD 0.5, *n* 33) d out of 5 during the control period and 4.7 (SD 0.4, *n* 39) d during the test period. Body weights were checked by investigators at 0, 2, 4 and 8 weeks of the first arm of the intervention. There was a small but significant increase in weight over this time (mean 0.8 (SD 1.4) kg $P<0.01$ on the control diet and mean 1.3 (SD 1.4) kg $P<0.01$ on the test diet).

We conclude that, for research purposes, the use of video in dietary education is acceptable to both investigators and to volunteers and the use of an exchange unit system is a convenient easily understood means of implementing dietary fat substitution in a free-living population. Further analysis will include correlating subjects' perceived dietary compliance with analysis of dietary intakes.

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Stages of change towards healthy eating among Irish and UK adults. By M. KEARNEY, B. MARGETTS, M.J. GIBNEY and J. KEARNEY. [1]*Institute European Food Studies, University of Dublin, Trinity College, Dublin 2, Republic of Ireland,* [2]*Wessex Institute of Public Health Medicine, University of Southampton, Southampton SO16 6YD.*

The development of effective public health nutrition interventions will be facilitated by a clear understanding of the beliefs, attitudes, knowledge and behaviour in the general population. Quota-controlled nationally-representative samples of Irish (*n* 1009) and UK (*n* 961) adults completed an interview-assisted questionnaire as part of a pan-European Union survey on consumer attitudes to food, nutrition and health. The instrument included questions on the following : influences on food choice; definition of healthy eating; sources of healthy eating information; beliefs about certain foodstuffs; barriers to healthy eating and dietary changes. Prochaska & DiClemente's (1983) stage model of behaviour change is a useful model for understanding the dietary changes process in the general population. The assumption upon which this model is built is that behaviour change is a dynamic, non-linear process, which involves several distinct phases : precontemplation (not even thinking about changing behaviour); contemplation (thinking about it); decision (making definite plans to change); action (beginning behaviour change) and maintenance (following initial change). Curry et al. (1992) validated the use of this model for classifying individuals with regard to dietary fat reduction. In the present study, subjects were questioned about their stage of change with regard to healthy eating in general. In both countries, between 80 and 90% of subjects were classified into either the precontemplation or maintenance stages of dietary change. These results are shown in the Table (don't knows excluded from analysis) for each country by sex.

	Total	Ireland			United Kingdom		
		Total	Male	Female	Total	Male	Female
n	1763	885	445	441	878	420	458
Precontemplation (%)	48	59	70	49	36	44	29
Maintenance (%)	38	29	21	37	47	42	53

There were significantly more UK than Irish adults, and within each country more women than men in the latter stages of behaviour change. This was true when the total samples were examined ($P<0.0001$) and when each sex was examined separately ($P< 0.0001$ in both cases). Within both countries there were more subjects from the higher than from the lower social classes in the latter stages of behaviour change (Ireland $P<0.01$; UK $P<0.001$) with the differences between the social classes greater in the UK than in Ireland. The results clearly suggest that different nutrition intervention strategies are required in the two countries and among different groups within countries. Interventions for creating awareness and improving motivation may be needed for individuals or groups at the early stages, while more specific information and skills training would be suitable for individuals who have already decided to change their diets. The results further strengthen the argument that the promotion of healthy eating needs to be targeted more specifically.

This work was funded by the Institute of European Food Studies.

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Prochaska, J.O. & DiClemente, C.C. (1983). *Journal of Consulting and Clinical Psychology* 51, 390 - 395.

Consumer strategies for increasing fruit and vegetable consumption in the UK. By DAVID N. COX[1], ANNIE S. ANDERSON[2], JOANNA REYNOLDS[1], SUSAN McKELLAR[2], DAVID J. MELA[1] and MICHAEL E. J. LEAN[2], [1]*Consumer Sciences Department, Institute of Food Research, Reading RG6 6BZ;* [2]*Department of Human Nutrition, University of Glasgow, G31 2ER*

In order to improve understanding of salient barriers and effective strategies relating to increased fruit and vegetable (F & V) intakes, randomly approached adult consumers from Glasgow and Reading, were screened and recruited if eating less than the recommended five (80 g) portions/d of F&V and contemplating increasing consumption. Those fulfilling these criteria (*n* 125) participated in an 8 week intervention study. Two intervention groups (A, weighed intakes; B, unweighed measures) and a control group (C, weighed intakes) at each site provided baseline measures of total dietary intake. Groups A and B attended a "F&V for health" lecture and were given definitions of F&V, portions (Williams, 1995), recipes for and tasting of vegetable-based-dishes, and charts for recording a "strategy measure", consisting of a diary record of portions eaten, occasions and strategy used, e.g. "fruit as a snack", "two portions of vegetables with main meals", etc. The target consumption was > five portions/d (i.e., > 400 g) total F&V (excluding potatoes and a maximum one portion of fruit juice). Subjects recorded the strategy measure in weeks 1 (7 d), 4 (4 d) and 8 (7 d); groups A and C also provided weighed total dietary intake at weeks 4 (4 d) and 8 (7 d).

The strategy measure for the intervention groups (*n* 101) indicated that the recommended target was achieved by 74%, 71%, and 65% of subjects in weeks 1, 4 and 8 respectively. Correlations between weighed and diary (strategy) measures of F&V eaten were highly significant (week 4, *r* 0.69 and week 8, *r* 0.73) indicating that portions can act as proxy measures for weighed intakes. Recording of portions tended to underestimate F&V consumption relative to weighed intakes suggesting that a target of five-a-day may encourage consumption > 400 g.

The Table reveals significantly greater F&V consumption for group A (*v.* C) after intervention.

	F & V consumption from weighed intake records† (g/d)					
	Baseline		Week 4		Week 8	
	Mean	SE	Mean	SE	Mean	SE
Intervention group A (*n* 42)	324	25	590***	27	557***	31
Control group C (*n* 24)	344	28	337	35	305	31

***Mean values were significantly different from control, $P < 0.001$.

† Only for subjects with (recorded energy intake/estimated BMR) > 1.1 at baseline (Goldberg *et al.* 1991).

The strategies chosen most frequently by achievers, in order of contribution to intakes, were: fruit as a snack, vegetables with a main meal, fruit juice, fruit as a dessert, and salad. Seven other strategies were used but it was clear that most of the additional F&V was consumed within conventional eating habits.

Along with other data on the acceptability and characterization of achievers and non-achievers the results of this intensive intervention suggest that future F&V public health measures should focus on conventional eating habits, utilizing defined portions. A validation study using an educational package incorporating these findings is presently underway.

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Williams, C. (1995). *British Medical Journal* **310**, 1453-1455.

Smoking status and knowledge, attitudes and behaviour in relation to fruit and vegetable consumption. By R.L. THOMPSON[1], B.M. MARGETTS[1], V. SPELLER[1] and D. McVEY[2], [1]*Wessex Institute of Public Health Medicine, University of Southampton, Southampton SO16 6YD and* [2] *Health Education Authority, London WC1H 9TX*

Cigarette smokers consume less fruit and vegetables than non-smokers although their requirements for micronutrients may be higher (Margetts & Jackson, 1993). We have used data from the Health Education Authority's *Health and Lifestyle Survey 1993* (B. Margetts, D. McVey, K. Oldfield, E. Rogers, J. Royle, V. Speller, R.Thompson & N. Woodward unpublished) to determine whether the difference in fruit and vegetable consumption between smokers and non-smokers is due to knowledge or attitudes about fruit and vegetables. A questionnaire was administered, by a trained interviewer, to a random sample of 5553 people, aged between 16 and 74 years, in England (response rate 70%). Data were weighted by gender, age, region and household type to ensure the sample was representative of the English population. The present analysis has been restricted to subjects aged 25-44 years.

	Men				Women			
	Manual (%)		Non-manual (%)		Manual (%)		Non-manual (%)	
	S	NS	S	NS	S	NS	S	NS
	(*n* 228)	(*n* 314)	(*n* 125)	(*n* 398)	(*n* 142)	(*n* 233)	(*n* 158)	(*n* 526)
Eat fruit and vegetables daily	29	42*	34	51*	38	57*	48	67*
Should eat more fruit and vegetables	47	38*	54	40*	45	33*	33	33
Fruit is part of a healthy diet	63	66	61	67	77	78	75	82
Care about what they eat	53	69*	75	79	71	86*	82	88*
Healthy foods are expensive	54	43*	28	25	57	45*	39	35

S, smokers; NS, non-smokers.
* Mean values were significantly different from those for smokers, $P<0.05$ (Chi squared test).

The Table shows that fewer smokers than non-smokers consumed fruit and vegetables every day. When subjects were asked whether they felt they ate too little fruit and vegetables, statistically significantly more smokers than non-smokers in all occupation groups, except women with non-manual occupations, answered yes. About two thirds of men and three quarters of women included fruit as part of a healthy diet irrespective of smoking or occupation group. For each occupation group, except non-manual men, non-smokers cared more about what they ate than smokers, with larger differences shown between smokers and non-smokers in manual occupations. More than 50% of smokers in manual occupations thought eating a healthy diet was expensive compared with about 30% of non-smokers with non-manual occupations. These results are in contrast to the Scottish Heart Health Study carried out between 1984 and 1986 which showed that smokers have a poorer dietary knowledge than non-smokers (Woodward *et al.* 1994).

Smokers although consuming less fruit and vegetables than non-smokers were aware of the health message to increase fruit and vegetable consumption. For smokers with manual occupations the difference in fruit and vegetable consumption appeared more to be due to attitudes such as being less concerned about what they ate or regarding healthy foods as expensive than level of knowledge. Emphasis needs to be placed on ways to translate knowledge into behaviour change.

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The UK Women's Cohort Study: background and obtaining local ethical approval. By AMANDA WOODHOUSE, CLAIRE CALVERT and JANET CADE, *Public Health Division, Nuffield Institute for Health, University of Leeds, 71-75 Clarendon Road, Leeds LS2 9LN and the UKWCS Steering Group**

The UK Women's Cohort Study (UKWCS) aims to study the relationships between diet and cancer incidence and mortality (from selected causes) in a group of middle-aged women with a range of nutrient intakes. Currently, 26 557 women have been identified from responders to the World Cancer Research Fund's mail survey which includes people living in England, Wales and Scotland. The women are aged 35-69 years and are vegetarians and/or vegans, fish eaters or meat eaters. Food, nutrient and lifestyle information will be collected from the whole cohort at baseline, using a questionnaire which includes a detailed food frequency questionnaire (FFQ) adapted from the EPIC Study questionnaire (Riboli, 1992) for use amongst vegetarians. This was done by developing and pilot testing the FFQ on 55 vegetarian women who had completed a 7 d weighed intake. The cohort will be followed up over 10 years and subjects will be asked to complete further FFQ and a food diary. Permission has been obtained from the National Health Service (NHS) Central Register for the subjects to be flagged for deaths and cancer registrations. To obtain this permission, and before any data could be collected, it was necessary to get approval for the study from Local Research Ethical Committees (LREC) in all the areas covered by the study.

Contacting LREC started in October 1994, a comprehensive list of all committees was not available at this time. In all, 174 LREC were identified, and chairs of each committee were sent an explanatory letter, a summary of the study, a reply proforma to allow approval by Chairman's action or request further information. Of the 174 LRECs contacted, 147 (84%) had responded after 3 months of follow up; 101 (58%) had given approval, the median time to response was 4 weeks. A further forty-six had responded but not given approval, one of whom initially refused to give ethical approval. A telephone reminder to committees after 3 months showed that most had not received the initial mailing, due to the merging of some committees during recent NHS reorganization. As of March 1996, responses from eleven committees were still outstanding, despite attempts to contact them. It was considered that these committees may no longer exist. Most committees requested more information for each member (table). The substantial workload required to obtain this permission delayed the start of the study, since certain postcode areas could not be mailed until permission had been obtained.

	LREC approvals so far	**160**
of which:	Questionnaire(s) requested	37%
	Additional information requested	39%
	LREC form to be completed	23%
	Protocol requested	21%
	Progress reports requested	29%

The UKWCS is overseen by a Steering Committee of experts, who considered that the use of a postal questionnaire on diet and lifestyle did not expose subjects to risk. The problems encountered in obtaining LREC approval constitute an excessive burden on researchers. The situation could be improved by the establishment of a regional committee or a single national body for multicentre studies of this nature. Researchers contemplating studies with a widely spread population need to allow time and financial resources to meet the demands of each LREC concerned. Guidelines for ethical committees state that they 'should remember that research benefits society and that they should take care not to hinder it without good cause.' (Royal College of Physicians of London, 1990).

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*Rhys Williams, Jennifer Barrett *University of Leeds*; Barrie Margetts, *University of Southampton*; Margaret Thorogood, *London School of Hygiene and Tropical Medicine.*

Dietary changes: association with lifestyle, socio-demographic circumstances and health variables. By A. TOBY PREVOST[1], MARGARET J. WHICHELOW[2] and BRIAN D. COX[2], [1]*Centre for Survey Data Analysis, Southampton University, Southampton SO17 1BJ,* [2] *Department of Community Medicine, University of Cambridge CB2 2SR*

Previous communications have described the four main dietary patterns, identified by principal component analysis, from thirty-nine food items in the 1984-5 Health and Lifestyle Survey (HALS1) (Whichelow & Prevost, 1996*a*), and the considerable changes in scores on those components between respondents taking part in both HALS1 and the follow-up study in 1991-2 (HALS2) (Whichelow & Prevost, 1996*b*).

Overall the scores on component 1 (high in fruit, salads and high-fibre foods and low in fatty foods) and component 4 (high in confectionery and other sweet foods, but low in vegetables) had risen, indicating closer compliance with those dietary patterns. Those of component 2 (high in high-carbohydrate foods and carcass meat) had fallen, and there was little change in the component 3 scores (high in fatty and convenience foods).

These changes have been examined in relation to socio-demographic, lifestyle and health variables by ANOVA using the GLIM software (Payne, 1985). Each score change was adjusted for the HALS1 value, and the analyses included age, socio-economic group, household size, region of residence, ethnicity, smoking, alcohol consumption, being on a prescribed diet, current physical symptoms, psycho-social symptoms, handicap and self-assessed health. The most marked changes , $P<0.01$ unless otherwise stated, are listed below.

For component 1 (high fruit etc.) scores rose most for non-manual men and women ($P<0.001$), men and women who remained non-smokers or who gave up or reduced smoking ($P<0.001$), men who were 'sensible' drinkers at HALS1 compared with non and heavy drinkers, men and women who had started a prescribed diet by HALS2 ($P<0.001$), women with few physical symptoms at HALS1 ($P<0.001$) and men with 'good' self-assessed health at HALS1. Those who showed the greatest decrease on component 2 (high carbohydrate etc.) scores were: non-manual compared with manual men, men and women who were living alone at HALS2 ($P<0.001$), men who were heavy drinkers at either survey ($P<0.001$) and men and women who had begun a prescribed diet by HALS2 ($P<0.001$). Component 3 (high fat etc.) was remarkably stable except for a greater rise in score for women who were heavy drinkers by HALS2. For component 4 (high confectionery etc.) the greatest increases in score were for men in the non-manual group, women living in Scotland, men ($P<0.001$) and women not smoking at HALS2, men ($P<0.001$) and women not drinking at HALS1, women ($P<0.001$) not starting a prescribed diet and women with few psycho-social symptoms at HALS1.

The longitudinal associations between dietary change and individuals' circumstances, whether altered or stable, reinforce the earlier cross-sectional findings (Whichelow & Prevost, 1996*a*). Moreover, they suggest a polarisation of dietary habits in the British population.

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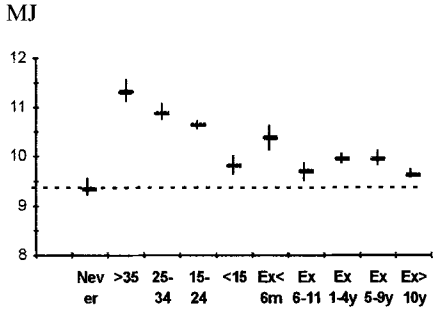
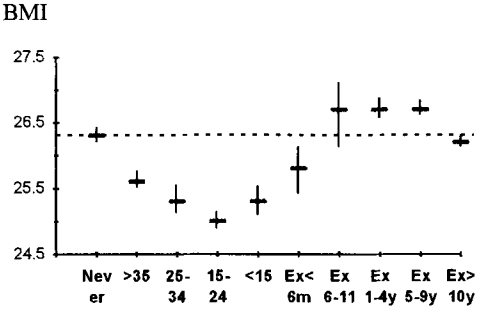
Trends in energy intake and body mass index across smoking-habit groups for men. By C. BOLTON-SMITH[1] and MARK WOODWARD[2], [1]*Cardiovascular Epidemiology, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY,* [2]*Department of Applied Statistics, University of Reading, PO Box 240, Earley Gate 3, Whiteknights Road, Reading RG6 6FN*

Tobacco smoking appears to modify body weight, and whether a rise in BMI (kg/m^2) is a necessary and/or permanent consequence of quitting smoking is of major interest to all potential quitters, and to health professionals.

Smoking (World Health Organisation questionnaire and plasma cotinine) and dietary (food-frequency questionnaire) information was collected from 4051 men aged 40-59 years as part of the baseline Scottish Heart Health Study in 1984-6 (Smith *et al.* 1989). Body height and weight were measured, and the level of physical activity assessed by subjective and objective questions for work and leisure time. Energy intake and BMI values were plotted for each smoking group (never, <15, 15-24, 25-34, ≥35 cigarettes/d and <6 months, 6-11 months, 1-4 years, 5-9 years, ≥10 years ex-smoking) before and after adjustment for physical activity, age, and the percentage energy from alcohol and fat, using general linear models procedure in SAS. The unadjusted plots (means with SE bars) are shown below. Adjustment did not alter the shape of the plots, but curtailed the extremes and widened the confidence limits.





These results may be influenced by biased dietary reporting since the mean energy intake to basal metabolic rate (EI:BMR) was greater in current than ex and never smokers (EI:BMR 1.48, 1.29,1.25 respectively), and the percentage of dietary energy from protein was significantly lower in ≥35 cigarettes/d group than never smokers (13.9 % v 15.3 %). However, biased reporting is unlikely to explain the observed trends. These data appear to support the hypothesis that smoking re-sets the body's "set-point" for weight, since BMI was lower in all the current smoking groups than in the never-smokers, while energy intake was greater, even after adjustment for confounders. An over-shoot of BMI was seen in the short-duration quitters (Lund-Larsen & Tretli 1982), and this occurred with an energy intake lower than that of current smokers of >15 cigarettes/d, but still higher than that of never smokers.

It is important not to over-interpret these cross-sectional results. Longitudinal studies are needed to confirm or refute these trends which show no long-term difference in BMI between never and ex-smokers.

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Influence of preceding physical activity on the metabolic partitioning of an oral glucose load using naturally enriched [^{13}C]glucose. By S.A. WOOTTON, N. LODGE, S. MARSH and J.L. MURPHY, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

The improved glucose tolerance and increased insulin sensitivity associated with regular exercise appear to be the result, in large part, of the residual effects of the last bout of exercise (Heath *et al.* 1983). Potential mechanisms include increased tissue sensitivity to insulin, persisting increased muscle cell permeability to glucose brought about by the insulin-like effect of muscle contractile activity and the depletion of muscle and liver glycogen making available "glucose-storage space" (Fell *et al.* 1982). What remains unclear, is the extent to which preceding physical activity alters the partitioning of exogenous glucose towards either oxidation or storage following administration of an oral glucose load. The aim of the present study was to use naturally enriched [^{13}C]glucose, in the form of maltodextrin, to examine the disposal of an oral glucose load following both a modest exercise bout on the day of the test or prolonged activity on the day before the test.

Six healthy young men who were recreationally active (aged 21-27 years; BMI 20-25 kg/m^2) performed an oral glucose tolerance test (OGTT) on three separate occasions at the same time of day after an overnight fast: (1) after a day of inactivity and at least 48 h after their last exercise session (CONTROL); (2) as control, but with 45 min of cycle ergometer exercise at 65% maximal heart rate ending 60 min before the OGTT (ACUTE) and (3) 6 h of continuous activity including low and high intensity exercise followed by a low carbohydrate (<25 g), high-protein evening meal ending 12 h before the OGTT (PROLONGED). The OGTT consisted of 75 g maltodextrin (Maxijul Powder, SHS Ltd, Liverpool: -7 ‰ ^{13}C) in 500 ml water. Breath samples were collected before and at hourly intervals for 6 h. Whole body breath CO_2 excretion and carbohydrate (CHO) utilisation was measured by indirect calorimetry (GEM, Europa Scientific Ltd., Crewe) at the same time points. Enrichment of $^{13}CO_2$ on breath was analysed by Isotope Ratio Mass Spectrometry (ABCA, Europa Scientific Ltd., Crewe). The results are shown in the Table.

	Control		Acute		Prolonged	
	Mean	SE	Mean	SE	Mean	SE
Total CHO oxidation (g / 6 h) †	89.4	7.8	71.7	6.5	62.0 *	6.6
Exogenous CHO oxidation (g / 6h)	16.6	0.7	14.3 *	1.2	11.3	2.3
Endogenous CHO oxidation (g / 6 h) §	72.8	7.8	57.4	5.7	50.7 *	7.8

† Calculated using Frayn, 1983; ‡ From breath $^{13}CO_2$ excretion; § Total CHO -exogenous CHO oxidation;
* mean values were significantly different from contro, P <0.05 (paired t test).

Total CHO oxidation over the 6 h OGTT tended to be lower in both activity trials than CONTROL, with the greatest reductions observed in the PROLONGED trial (P<0.05) and was associated with a reduction in oxidation of carbohydrate derived from both endogenous and exogenous sources. These results support the view that preceding activity induces a pronounced glucose-sparing effect, principally by suppressing endogenous carbohydrate oxidation and promoting exogenous glucose storage.

The support of Scientific Hospital Supplies Ltd is gratefully acknowledged.

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The effect of intense physical activity on profiles of hunger and energy intake in free-living individuals. By NEIL A. KING[1], CLAIRE L. WATSON[1], R. JAMES STUBBS[2], and JOHN E. BLUNDELL[1], [1]*BioPsychology Group, Department of Psychology, University of Leeds, Leeds LS2 9JT, and* [2]*The Rowett Research Institute, Aberdeen AB2 9SB*

An increase in physical activity has been proposed as one method of creating a negative energy balance and hence treating obesity. Therefore, the relationship between physical activity and energy intake (EI) is important. However, the effect of physical activity on EI and energy balance is not well understood. It is a common sense view that a physical activity-induced increase in energy expenditure (EE), giving rise to an energy deficit (i.e. a physiological need), will generate a drive of hunger (H), and in turn increase EI. Therefore, one major issue is: do individuals eat more to match the increased EE?

In a repeated-measures design, healthy lean males (*n* 8) were used to examine the effects of two high-intensity, long-duration (70% VO_2max, 50 min) exercise sessions in 1d on diurnal H profiles and EI. The effects of the high dose of exercise on 1d (Ex1), were compared with the effects on the day immediately after exercise (Ex2), and with two consecutive days of no exercise (Res1 and Res2). On days Ex2, Res1 and Res2, individuals were asked to refrain from undergoing any exercise. Hunger profiles were tracked with a new electronic appetite rating system (EARS) using a hand-held computer (Delargy *et al.* 1996). Energy and macronutrient intake was monitored using self-recorded food diaries. Heart rate was continuously monitored using the POLAR heart rate tester.

	Res1		Res1		Res1		Res1	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Energy intake (MJ)	12.1	2.45	12.3	4.19	12.5	2.08	11.4	2.25
Feeling of hunger (mm)	41	12	40	10	38	11	45	9

The mean total energy cost of the two exercise sessions was 5.0 MJ. Hunger was not driven up by the large increase in EE on the day of exercise (Ex1) or on the day after exercise (Ex2). In fact, H profiles on Ex1 were significantly lower compared with Ex2 ($P<0.05$), but not Res1 or Res2. Despite manipulations in habitual daily EE, energy and macronutrient intakes were not different on Ex1, Ex2, Res1 or Res2. Therefore, individuals appeared to eat to a habitual uniform level on all 4d, which was independent of EE. A similar phenomenon can be seen by comparing separate studies on different subjects in a calorimeter (Stubbs *et al.* 1995*a*), with free-living conditions (Stubbs *et al.* 1995*b*).

The results of the present study show that substantial EE due to intense exercise does not increase H or EI either during or afterwards. This suggests that EI (the pattern of eating behaviour), may be influenced more by social convention, enduring habits and physiological limits, rather than by a metabolic drive induced by EE (at least in the short-term).

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Effect of caffeine on muscle tremor and exercise performance in healthy men. By L. J. ARCHIBALD, M. BEROVIC, N. McCATHIE, A. REES, P. L. GREENHAFF and I. A. MACDONALD, *Department of Physiology and Pharmacology, Nottingham University Medical School, Queen's Medical Centre, Nottingham NG7 2UH*

It is widely believed that caffeine improves performance during prolonged exercise, but the only studies addressing this objectively (Costill *et al.* 1978; Graham & Spriet, 1991) used high doses (3-9 mg/kg body weight) which exceed the amounts obtained from normal intakes of beverages. It is also unclear whether any effects of caffeine are due to primary effects on skeletal muscle contractile function, or are secondary to metabolic or hormonal responses. The present study compared the effects of caffeine and placebo on resting muscle tremor, plasma metabolites and catecholamines and exercise performance.

Eight healthy men (20-22 years, 1.80 (SE 0.02) m, 76.9 (SE 2.4) kg, habitual caffeine intake 172 (SE 45) mg/day) gave written informed consent before being studied on three occasions after fasting for at least 5 h and abstaining from caffeinated products for 48 h. Resting finger tremor was measured with an accelerometer before and 1 h after ingestion of capsules containing placebo, 1.5 mg or 6 mg caffeine/kg body mass, in a randomized, double-blind design. Subjects then exercised to exhaustion on an electrically braked cycle ergometer at 70% of their individual maximum O_2 uptake. Venous blood samples were obtained at rest and during exercise for measurement of plasma non-esterified fatty acids (NEFA), glycerol, lactate and catecholamines. Expired air was collected during exercise for the determination of respiratory exchange ratio (RER). Data were assessed statistically using ANOVA.

There was no difference between the effects of placebo and the low dose of caffeine on resting finger tremor (0.25 (SE 0.04) *v.* 0.22 (SE 0.04) m/s^2) or exercise time (81(SE 27) *v.* 83 (SE 28) min), but the low dose of caffeine increased pre-exercise plasma NEFA (by 0.27 (SE 0.07) mmol/l, $P < 0.01$) and glycerol (by 15.8 (SE 4.8) μmol/l, $P < 0.01$) compared with placebo. The high dose of caffeine increased resting finger tremor by 0.11 (SE 0.04) m/s^2 ($P < 0.05$), pre-exercise plasma NEFA (by 0.55 (SE 0.07) mmol/l) and glycerol (by 29.3 (SE 6.7) μmol/l) and prolonged exercise time to exhaustion by 22 (SE 7) min more than placebo ($P < 0.05$). Neither dose of caffeine had any significant effect on resting or exercising plasma lactate or catecholamines, or RER during exercise.

The present study confirms that a high dose (6 mg/kg) of caffeine prolongs exercise time and raises plasma NEFA and glycerol concentrations. The lower dose of caffeine had some effects on plasma NEFA and glycerol but did not affect exercise time. The effect of the higher dose of caffeine on finger tremor, in the absence of a change in catecholamines, suggests that a direct effect of caffeine on skeletal muscle contractile function might contribute to the increased exercise endurance time.

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The effect of creatine supplementation upon dynamic short-term maximal exercise. By EUGENE MORIARTY, BERNARD DONNE and FRED ANDREWS. *Department of Physiology, Trinity College, Dublin 2, Republic of Ireland*

Recently creatine supplementation has been proposed as one of the most effective naturally occuring ergogenic aids, enhancing performance in short maximal sprint type sporting events. It has been postulated that the reduction in force production during intense muscular contraction is related to the depletion of muscular phosphocreatine (PCR) stores (Greenhaff *et al.* 1993), with the resultant limitation of ADP rephosphorylation to ATP. This has been supported by evidence that links fatigue during intense muscle contraction to the rapid utilization of PCR stores in type II fibres (Soderlund *et al.* 1992). Previous studies have examined the effects of creatine supplementation on high intensity exercise, interspersed with varying degrees of rest periods (Greenhaff *et al.* 1993; Balsom *et al.* 1995).

We investigated the effects of creatine (CR) supplementation on short-duration dynamic maximal exercise (simulated 1 and 4 km time-trials) in eight male competitive road-racing cyclists. Subjects were administered 5 g CR + 1 g glucose as the test preparation and 6 g glucose as a placebo, four times daily, for 6 d in a subject-blinded crossover design study, with a 2-week washout period between treatments. Subjects were assessed on three occasions before supplementation, on day 4 (CR4) and day 6 (CR6) of supplementation and 2 d post-supplementation (CR8) for each treatment. Simulated time-trials were carried out on a standard road-racing bicycle mounted on a test rig (Kingcycle). Body mass (kg) was measured on each test day, Heart rate (HR) was continously monitored using a Polar Vantage heart rate monitor and blood lactate (LAC) was measured using a YSI 1500 Sports Lactate analyser. LAC was measured pre and post trial and also at 5 and 10 min post-trial. An additional LAC measurement was made at 2 km into the 4 km time-trial.

The results obtained were analysed using ANOVA for repeated measures, $P<0.05$ was considered significant. A significant increase in body mass over pre- supplementation values was recorded on day CR6 and CR8 of the supplementation phase (pre, 73.3 (SE 1.2); day 6, 74.2 (SE 1.2); day 8, 74.1 (SE 1.1)). No significant increase in body mass was seen on placebo treatment. Placebo supplementation had no effect on performance times for either 1 or 4 km simulated time-trials. CR supplementation resulted in an improvement in both 1 and 4 km time trial times, which was significant ($P<0.05$) for 1 km trials at CR4 and CR8 of supplementation (pre 66.3 (SE 0.6); day 4, 65.1 (SE 0.5); day 6, 65.6 (SE 0.6) and day 8, 65.1 (SE 0.6). No significant changes in HR or pre and post LAC were recorded for either treatment.

In conclusion, therefore, the results of this study confirm that CR supplementation in trained cyclists results in a significant performance improvement in maximal short-duration dynamic exercise (1 km time-trial). Further investigation appears warranted into longer duration near maximal efforts.

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Studies on a monoclonal antibody recognizing gluten-derived peptides implicated in coeliac disease. By R.D. HUSAIN, G.M. BRETT, E.N.C. MILLS, I.J. COLQUHOUN and M.R.A. MORGAN, *Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA*

Coeliac disease in humans is due to mucosal damage induced by prolamin (alcohol-soluble protein fractions of cereals), resulting in a malabsorption syndrome (Tatham *et al.* 1990). The molecular basis of this disease is unknown, and the advice given to patients is total dietary avoidance of cereals. Attention has been focused on identifying the protein components that activate the disease. α-Type gliadin proteins have been implicated in the activation of the disease in genetically predisposed individuals. In the present study we used a monoclonal antibody (Mab) which recognizes the peptide LGQQQPFPPQQPY (GB1), corresponding to A gliadin (major α gliadin subfraction) residues 31-43 and the sequence QPFPQQP (GB13) containing repetitive motif QPFPQ, which have proven *in vivo* coeliac toxicity (Marsh, 1992). The work was done with a view to following the uptake of α gliadin in gluten-challenged coeliacs.

Recognition of the synthesized peptides by the Mab has been studied using ELISA and fluorescent quenching experiments. The method used for the production of Mab IFRN 065 was essentially as described by Mills *et al.* (1990) with the following modification. A total glutenin fraction of wheat (cultivar Avalon) was used as the immunogen and for the selection of the Mab. Peptide-dependent quenching of the tryptophan fluorescence in the Mab IFRN 065 was used as an indicator of binding in the affinity determination. In a typical experiment 5-20 μl portions of a 40 μg/ml solution of the peptide were added to a 40 μg/ml antibody solution and the fluorescence was recorded. The titration was stopped when the fluorescence showed no further quenching with added peptide. The data were arranged in the form of a Scatchard plot with the intercept giving the binding constant as previously described (Scatchard, 1949). An affinity constant (K_a) of 3.55×10^9 $mole^{-1}$ was calculated for GB1 whilst for GB13 the K_a was 1.47×10^8 $mole^{-1}$.

For the inhibition (competitive) ELISA experiment, microtitration plates were coated with total glutenins at 1 μg/well as described by Plumb *et al.* (1995). A serial dilution of each of the two peptides was made providing solutions ranging from 0.01 μg to 100 μg/ml. Each sample or buffer alone was added in duplicate (0.1 ml/well) to the coated plate. Mab IFRN 065 (0.1 ml/well) was added to each well and plates incubated at 37^0. Plates were then developed as described for direct ELISA by Plumb *et al.* (1995). An inhibition curve was obtained for GB1 which had a detection limit of 0.1 μg/ml peptide. No curve was observed for GB13.

Results from fluorescent quenching studies suggest that both peptides are recognized by the Mab but with different affinities. Lower affinity for peptide GB13 results in no inhibition curve being observed. The above findings indicate that the Mab IFRN 065 has the required specificity and activity to use as a probe in coeliac-related studies.

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Breastfeeding and later introduction of gluten: their effect on the immune response to wheat protein. By V.E. FREEMAN[1], H.M.V. HOEY,[2] C. O'FARRELLY[3] and M.J. GIBNEY[1], [1]*Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Republic of Ireland,* [2]*Department of Paediatrics, Trinity College, Dublin 2, Republic of Ireland and* [3]*St Vincent's Hospital, Dublin 4, Republic of Ireland.*

Coeliac disease is thought to be caused by an abnormal immunological response to the ingestion of wheat protein. Raised serum levels of IgG antibodies to α gliadin, a fraction of the gluten peptide of wheat protein, have been used as a screening test for the disease. However, because of the high levels of false positives in children without coeliac disease, this test has been replaced by the more specific endomysial antibody assay. A high prevalence of coeliac disease has been reported in Ireland, but here, as elsewhere, the numbers presenting in childhood are falling (Stevens *et al.* 1987). These improvements appear to be the consequence of changes in infant feeding patterns, namely an increase in the uptake and duration of breastfeeding, modification of infant formulae, and later introduction of gluten-containing foods to the infant's diet. The aim of this study was to examine the effect of infant feeding practice on the immune response to wheat protein in normal children.

In a sample of normal healthy children who had been followed longitudinally from birth, a detailed feeding record was available including the duration of breastfeeding, the age of introduction of gluten and of solids. Illnesses were also documented. IgG α gliadin antibody (AGA) levels were measured in 70 children at age 2 years. If levels were raised, (based on an in-house standard), serum was tested for the presence of endomysial antibodies. Multiple regression analysis was used to explain AGA levels from other variables.

Values for IgG AGA ranged from 1-13 units/ml. Six children (9%), whose levels were raised, were subsequently tested for endoymsial antibodies, but all were found to be negative. All children were normal weight and height and showed no clinical evidence of disease. Multiple regression analysis showed that duration of breastfeeding (r 0.42, P=0.001) and the number of months when gluten-containing foods were included in the diet between the ages 2-5 months (r -0.29, P=0.05) were associated with AGA levels. If the age when solid foods were introduced was included in the regression, instead of gluten exposure, a similar pattern emerged. The later the solids were introduced, the higher the AGA levels. However, there was a significant positive correlation between the cessation of breastfeeding and introduction of solids (r 0.39, P=0.000).

In this study, more prolonged breastfeeding and later introduction of gluten resulted in moderately raised IgG AGA levels. In retrospective case-controlled (Greco *et al.* 1985 & 1988), and epidemiological (Ascher *et al.* 1993) studies, these same dietary variables have been found to be protective against coeliac disease. Breastfeeding in early infancy is known to enhance the immune response and provide protection against infection which remains effective even after breastfeeding has ceased (Wright *et al.* 1995). Raised IgG AGA levels may be an indicator of this enhanced response and may, at a certain level, protect from, rather than be an indicator of, coeliac disease. However, the duration of this protective effect may not extend into adulthood. For example, a screening programme in Italy resulted in 1 in 305 asymptomatic children, aged 11-15 years, being conclusively diagnosed with the disease (Catassi *et al.* 1994). Protection may also be associated with the presence of gluten in breast milk. Gradual exposure to wheat protein, through breastfeeding, may modulate, in susceptible individuals, the extreme immune response which might result from high intakes of cereal at weaning time. The present findings may explain the falling incidence of coeliac disease in young children, while the numbers presenting in adulthood are on the increase (Stevens *et al.* 1987).

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Cardiovascular and metabolic response to nasogastric and oral feeding in young healthy volunteers By T.A. STUBBS and I. A. MACDONALD, *Department of Physiology and Pharmacology, Nottingham University Medical School, Queen's Medical Centre, Nottingham NG7 2UH*

Oral ingestion of food evokes acute cardiovascular responses (Sidery *et al* 1991; Sidery & Macdonald, 1993). Feeding via a nasogastric tube is a common clinical practice but the cardiovascular and metabolic effects are not well established. The present study assessed the acute cardiovascular and metabolic responses to both oral and nasogastric (NG) feeding in healthy volunteers.

Six subjects (18-30 years, three male, 1.66 (SE 0.04) m, 64.9 (SE 2.2) kg) fasted for 6 h before randomized visits to receive either a drink of 400 ml Fresubin (Fresenius Ltd, GB, Runcorn, Ches.) on one occasion, or insertion of a NG tube and infusion of either water or Fresubin at 200 ml/h for 2 h on the other two occasions. Subjects gave written, informed consent, and the protocol was approved by the Medical School Research Ethics Committee. Baseline measurements of cardiac output (CO), superior mesenteric artery blood flow (SMABF), respiratory exchange ratio (RER) and metabolic rate (MR) were made before consuming the drink or starting the infusions. These measurements were then repeated at 20 min intervals for 140 min, the NG infusions were discontinued after 2 h and measurements continued for a further 20 min.

The oral ingestion of Fresubin (OF), was accompanied by an increase in CO, with a peak change of 1.15 (SE 0.22) litres/min, 40 min post-drink. NG Fresubin (NGF) administration was accompanied by a gradual increase in CO, with a maximum rise of 0.47 litres/min. Following NG water (NGW) there was no significant change in CO. SMABF increased most markedly after OF, with a peak response at 60 min post-drink of 71.2 (SE 4.0) ml/min, compared with increases of 32.8 (SE 5.5) after NGW and 31.2 (SE 9.4) ml/min after NGF. After NGW, RER remained at or below baseline values. After OF and NGF, RER rose gradually, with rises of 0.05 (SE 0.01) (OF) and 0.07 (SE 0.03) (NGF) at 80 min. Following OF, MR increased by 0.42 (SE 0.22) kJ/min at 40 min, and remained elevated throughout. By contrast, MR did not change significantly after NGW or NGF.

The mode of delivery of nutrients (oral *v.* NG) appears to affect the cardiovascular and metabolic rate responses, but does not substantially alter the RER response. Thus, acute NG feeding may produce smaller metabolic and cardiovascular disturbances than oral feeding.

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Benefit of magnesium supplementation (200 mg/d) in relief of the symptoms of the premenstrual syndrome. By ANN F. WALKER[1], MIRIAM COELHO DE SOUZA[1] AND MICHAEL F.VICKERS[2], [1]*Hugh Sinclair Unit of Human Nutrition, Department of Food Science and Technology, The University of Reading, Reading RG6 6AP,* [2]*formerly of Department of Clinical Biochemistry, Royal Berkshire Hospital, Reading RG1 5AN*

Mg deficiency has been suggested to be involved in the aetiology of the premenstrual syndrome (PMS). Low Mg status among women in the UK may be a reflection of low intake, as the mean intake of British women is 88 % (Gregory *et al.* 1990) of the reference nutrient intake (RNI; Department of Health, 1991) of 270 mg/d. Facchinetti *et al.* (1991) reported that a daily 370 mg Mg supplement administered from day 15 to the end of the menstrual cycle significantly reduced negative affect in PMS patients. Inspired by these findings, the present study was designed to investigate the effect of a daily supplement of 200 mg of Mg (as MgO) throughout the cycle. The study was a randomized, double-blind, crossover design, in which each treatment was administered for two menstrual cycles.

The twenty-four volunteers who successfully finished the study were female undergraduates and employees from The University of Reading and nineteen were in the age range 18-25 years. Recruitment screening was based on responses to a menstrual health questionnaire modified from Warner & Bancroft (1990). Volunteers were required to keep a daily record of their symptoms whilst taking supplements, using a 4-point scale in a menstrual diary comprising twenty-two items and to collect samples of urine at baseline (no tablet taking) and on day 15 or thereabouts during the second month of each treatment. For analysis, symptoms were grouped into six categories adapted from Abraham (1982): PMS-A (anxiety), PMS-C (craving), PMS-H (hydration), PMS-D (depression), PMS-O (other), and PMS-T (total overall symptoms).

There was no effect of Mg supplementation compared with placebo in any category in the first month of supplementation. In the second month (manifesting at about week 7 of supplementation) there was a significant effect ($P < 0.05$) of the Mg supplementation in the alleviation of PMS-H only. In the second month of Mg supplementation ANOVA showed that the mean of the estimated 24 h urinary output of Mg (100.8 mg) was significantly greater ($P < 0.02$) than the mean for placebo after the same time of administration (74.1 mg), using baseline data (mean 71.6 mg) as covariate in the analysis.

The finding of a significant effect of a daily supplement of 200 mg of Mg compared with placebo in the alleviation of PMS-H (weight gain, swelling of extremities, breast tenderness, abdominal bloating) but not PMS-A (nervous tension, mood swings, irritability, anxiety) is not in accord with the findings of Facchinetti *et al.* (1991). However, our study was carried out on young women among whom PMS-H symptoms were more prevalent than PMS-A. The beneficial effect of magnesium supplementation for the relief of symptoms of PMS found in the present study should encourage further research in this field, including studies on older women.

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A synergistic effect of magnesium and vitamin B_6 supplementation for the relief of symptoms of the premenstrual syndrome (PMS). By MIRIAM COELHO DE SOUZA[1], ANN F. WALKER[1], KIM M. BOLLAND[2] AND PAUL A. ROBINSON[3], [1]*Hugh Sinclair Unit of Human Nutrition, Department of Food Science and Technology, The University of Reading, Reading RG6 6AP,* [2]*MPS Research Unit, Department of Applied Statistics, The University of Reading, Reading RG6 6FN,* [3]*Department of Clinical Biochemistry, Royal Berkshire Hospital, Reading RG1 5AN*

Although no studies of combined Mg and vitamin B_6 supplementation have been carried out, both have been reported to reduce PMS symptoms when taken as single supplements. In the present study the administration of vitamin B_6 with Mg was investigated for synergy for the relief of symptoms of PMS and for enhancing the uptake of Mg (as suggested by Abraham *et al.* 1981) as estimated by urinary Mg output.

Volunteers were recruited from the Reading area by a feature article in a local newspaper, screened using a menstrual health questionnaire (MHQ) and requested to record their daily symptoms for one menstrual cycle (baseline) in a menstrual diary of thirty items using a five-point scale. By means of a double-blind, placebo-controlled, crossover design, about sixty-four women, average age 32 (range 18-52) years, were randomized to receive each of four treatments for one menstrual cycle each treatment. The treatments were: (a) 200 mg Mg/d, (b) 50 mg vitamin B_6/d, (c) 200 mg Mg/d + 50 mg vitamin B_6/d, and (d) placebo. Subjects scored their daily symptoms in a menstrual diary and provided a mid-cycle urine sample (time of day not specified) whilst taking each treatment.

Symptoms were classified using a modification of Abraham's (1982) classification: PMS-A (anxiety), PMS-C (craving), PMS-D (depression), PMS-H (hydration), PMS-O (other symptoms) and PMS-T (total symptoms). Of the forty-four subjects who successfully completed the study, the most prevalent form of PMS was PMS-A followed by PMS-H, with a non-significant trend for older women (>40 years) to suffer more PMS-A and younger women (<30 years) more PMS-H. Compared with baseline, placebo exerted a considerable benefit in reducing PMS symptoms (a decline in symptom scores ranging from 16-38% for the six PMS categories).

ANOVA revealed no overall significant treatment effect for any of the PMS scores. Further treatment comparisons were conducted using factorial contrasts and these showed a significant ($P<0.05$) interaction effect of 200 mg Mg/d + 50 mg vitamin B_6/d on both PMS-A (nervous tension, mood swings, irritability or anxiety) and PMS-C (headache, craving for sweets, increased appetite, palpitations, fatigue, dizziness or faintness). Although the mean symptoms scores for Mg treatment were reduced only in the presence of vitamin B_6, they were, nevertheless, close to placebo values. The estimated 24 h urinary Mg showed no overall treatment effect. However, the largest output of Mg was during the combined supplementation (mean value of 101.2 mg/d) compared with Mg alone (mean value of 94.9 mg/d), vitamin B_6 alone (mean value of 91.4 mg/d) and placebo (mean value of 85.6 mg/d). In conclusion, a synergistic effect for the relief of anxiety- and craving-related PMS symptoms of combined Mg and vitamin B_6 supplementation was demonstrated after about 20 d. Further studies are needed to confirm enhanced absorption of Mg by vitamin B_6 supplementation, with greater attention given to standardization of urine sampling procedures.

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Feeding and behavioural thermoregulation in obese mice. By PETRINA S. SYMES, SIOBHAN McBENNETT and J.F ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Republic of Ireland*

The genetically obese mouse develops its obesity through a combination of excess energy intake with increased energy efficiency (Thurlby & Trayhurn, 1979). The latter is due to a reduction in both thermoregulatory and diet-induced thermogenesis resulting from dysfunctional brown adipose tissue. While such physiological aspects of thermoregulation have been studied extensively, behavioural aspects have received less attention.

Lean (LN) and obese (OB) Aston mice were placed individually into a double-cage system where the mice were free to choose between a warm (30°) "nest" cage interconnected with a cooler (20°) "foraging" cage (containing food and water). Deep body temperature (DBT) was recorded continuously for 7 d using radiotelemetry in each of the following models: (1) warm foraging model; both nest and food cages held at 30°, food placed in hopper of food cage (WFH), (2) cold foraging models; food cage held at 30° while nest cage held at 20°, (i) food placed in hopper of food cage (CFH), (ii) food placed on floor of food cage (CFF), (iii) dilute food placed in hopper of food cage (CFDH). Standard laboratory chow (Red Mills, Co.Kilkenny) was used except in 2(iii) where pellets of reduced (to 85%) energy density were used. Food could only be eaten in the food cage in all models except 2(ii) where the food could be carried into the nest cage. Cold exposure could be monitored in the cold foraging model by distinguishing the source of the DBT signal.

		% Time foraging			
		Lean		Obese	
Foraging model	n	Mean	SD	Mean	SD
WFH	4	31.7*	12.00	24.5*	5.15
CFH	4	17.7	4.73	18.4	2.46
CFF	2	11.2	5.44	19.2	2.51
CFDH	2	22.7	8.84	19.2	2.51

* Significantly different from CFF, $P<0.05$.

Similar foraging patterns were seen for both LN and OB mice within the different models. When the food cage was held at 30° the mice spent over 25% of their time foraging. However this proportion decreased when the temperature was lowered to 20° and further decreased when food was placed on the floor of the food cage at this temperature; most mice preferred to hoard the food in their nest cage. This latter decrease in foraging time is not as great as might have been expected and may be attributed to a mouse's innate tendency to forage even when there is a plentiful supply of food. Finally, the proportion of time spent foraging increased again when dilute food was placed in the hopper as the mice were forced to spend more time in the cold food cage if they were to fulfil the same energy requirements.

There was no significant difference between the LN and OB foraging times in all foraging models, contrary to findings by Jakobson & Andrews (1992) where OB mice in the warm stayed in the food cage most or all of each day, while their LN counterparts chose to stay away from the food for most of each day. Such differences may be attributed to an experimental design where mice were housed in pairs, rather than singly. In the WFH model DBT of LN mice (37.00±0.24°) was significantly greater than of OB mice (35.69±0.59°), $P<0.05$). The cold foraging models did not significantly affect DBT.

In conclusion, changes in environmental conditions, food presentation and food density affect the behaviour of mice, however this experimental design was unable to show any distinction between LN and OB mice.

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The pattern of tissue mobilization and storage in the growing rat. By C.J.K. HENRY[1], P.R. PAYNE[2] and A. GHUSAIN-CHOUEIRI[1]. *[1]School of Biological and Molecular Sciences, Oxford Brookes University, Gipsy Lane , Oxford OX3 0BP and [2]Centre for Human Nutrition, Taviton Street, London WC1H 0BT.*

It has been proposed that the pattern of fat and lean tissue deposition or mobilization in an adult individual is defined by a fixed ratio 'P' (Payne & Dugdale, 1977). This ratio represents the energy that is mobilized or stored as protein, to the total energy stored or mobilized. These authors predicted that when measured in adults, the ratio during fasting (P_{fast}) would be found to be identical to that measured during subsequent re-feeding (P_{refed}). When computed in children, however, the values varied substantially with age (Dugdale & Payne, 1975). The present paper reports the results of measurements made on growing rats, of 'P' ratio during total fasting and on subsequent re-feeding.

Thirty weight-matched (105 SE 0.42 g) weanling male rats (30 d of age) were assigned to any one of three groups. Group 1 (eight rats) were killed at the beginning of the experiment. Group 2 (ten rats) were fasted for 3 d and then killed. Group 3 (eleven rats) were first fasted for 3 d and then had free access to stock diet (R&M 3 breeding diet, Lillico & Sons Wanham, Surrey) for a further 17 d before being killed. Carcass energy and N content were determined at death in all three groups. In addition, N loss during the 3 d fast, food intake and faecal losses during the re-feeding period were determined in group 3. Incremental changes in body composition with time were calculated by differences between groups. The changes in body N on starvation were calculated from urinary N loss (Henry *et al*, 1985). P_{fast} and P_{refed} were calculated as N (loss or gain) x 6.25 x 4 x 4.18 kJ ÷ energy (loss or gain).

During fasting, the 30 d old rats lost 32% of the initial body weight, whereas N loss was only 12% of initial body N. Body energy changes were found to be linearly related to body weight change (gross body energy (kJ) = 6.55 W - 77.30 (r 0.985, P<0.001)). This very close relationship was used to estimate the body energy content of each individual in group 3, at the end of the initial fasting period. Hence each individual in group 3, provided 'P' ratio during both fasting and re-feeding periods.

	Balance			
	Fasted (30-33 d)		Refed (33-50 d)	
Variable	Mean	SE	Mean	SE
Weight loss or gain (g)	33.3	0.843	154.5	3.66
Energy loss or gain (KJ) (ME)	209.7	5.56	1086.5	55.28
Urine energy loss (KJ)	32.8	1.204	-	-
Protein energy gain (KJ) (ME)	-	-	457.5	26.14
P ratio	0.156	0.003	0.421	0.0113

ME, metabolizable energy

The P_{fast} and P_{refed} values indicate that about 15% of total energy lost during fasting was due to protein catabolism, whereas during re-feeding, 42% of energy stored was as protein. The large difference between the P_{fast} and P_{refed} ratios, is probably due both to the fasting - refeeding change itself, and to the age-related changes expected during this intensive growth stage of development. Despite the fact that on average, each individual in group 3, increased its 'P' ratio by about 2.5 times during re-feeding, a close relationship between P_{fast} and P_{refed} was preserved in individual animals (r 0.668, P<0.05). This striking observation indicates that rats with a low 'P' ratio on fasting exhibit a low 'P' ratio on refeeding.

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A novel method using high-performance liquid chromatography to isolate lysine in amounts suitable for isotope-ratio mass-spectrometry. By R. BUNDY and A.A. JACKSON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

There has been recent interest in the physiological significance of urea-N salvaged from the human colon. It has been suggested that amino acids synthesized *de novo* through the actions of the colonic microflora, may be utilized by the body directly and this may be of importance when dietary protein is limiting (Jackson, 1995). Lysine is an essential amino acid whose C skeleton can neither be synthesized nor aminated by the body. Metabolic studies which attempt to follow the fate of urea-N in the body use ^{15}N as a tracer, and it follows that ^{15}N enrichment of lysine incorporated into body protein would have to derive from *de novo* lysine synthesis in the colon. To investigate this hypothesis, a means of isolating lysine from protein hydrolysates, in quantities that are sufficient for subsequent analysis of isotopic enrichment by isotope-ratio mass-spectrometry (IRMS), is required. To accomplish this we have developed a method employing HPLC as a preparative step.

Three separate amino acid mixtures of 50 mmol each were prepared. A 100 µl sample of each (in triplicate) was dried down under vacuum. A derivatization procedure similar to that employed by Bidlingmeyer *et al.* (1984) using phenylisothiocyanate was performed on each sample, which resulted in the formation of PTC-amino acid derivatives. Samples were dissolved in buffer and then run in triplicate on a C18 reverse-phase HPLC column using a graded bi-solvent system. Amino acid derivatives were identified with a UV detector which measured absorbance at 254 nm. A peak at 35.7 (SD 0.3) min was present in the twenty amino acid mixture and the lysine solution, but was absent from the nineteen amino acid mixture (lysine omitted).

To quantify the efficiency of the derivatization and separation procedure, [^{3}H]lysine was added to the twenty amino acid mixture and radioactivity measured initially, after derivatization, and after separation and subsequent collection. Results are shown in the Table.

	Radioactivity (dpm)		Percentage of initial sample	
	Mean	SD	Mean	SD
Initial sample	33645	888	100	3
After derivatisation	25313	6618	75	20
After separation	20481	3706	61	11

The results show that about 25% of the sample was lost during the process of derivatization and a further 14% lost through separation and collection. The amount of lysine recovered was calculated to be sufficient to measure the N enrichment with IRMS (>5 ugN).

Using a novel HPLC method, we have identified and separated lysine from a mixture of twenty amino acids in sufficient amounts to subsequently measure isotopic enrichment. The separation process is relatively rapid compared with conventional column chromatography, and is in the process of being automated.

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Transfer of ^{15}N from urea to the circulating dispensable and indispensable amino acid pool in the human infant. By N. GIBSON[1], E. AH-SING[1], A. BADALLOO[2], T. FORRESTER[2], A. JACKSON[3], and D.J. MILLWARD[1] . *[1]Nutrition and Food Safety Research Centre, School of Biological Sciences, University of Surrey, GU2 5XH, [2]Tropical Metabolism Research Unit, University of the West Indies, Jamaica and [3]Institute of Human Nutrition, University of Southampton, SO16 7PX.*

We have reported data which indicates that the extensive process of urea salvage in the human infant, which can account for more than half the urea produced, is of biological importance in terms of amino acid economy. Thus we showed that the salvaged N returns to the systemic amino N pool as indispensable amino acids as indicated by our demonstration that lysine isolated from the urinary amino acid pool of children given orally administered ^{15}N dilabelled urea was enriched with ^{15}N (Yeboah *et al* 1996). We interpreted this as indicating that the lysine was synthezised *de novo* by the colonic microflora and was able to cross from the colon into the systemic circulation. We report here further measurements of the enrichment of dispensable amino acids, namely alanine, glycine and histidine.

20 malnourished children (5-6 kg body weight) were studied on admission (after stabilization), during recovery and after recovery on diets which supplied either adequate or generous protein levels (0.53-0.69 and 2.97 g protein/kg stabilization phase, 3.05-3.1 and 4.59 g/kg catch-up phase and 0.52-0.62 and 3.04 g/kg recovered). Energy supplied was 414 kJlkg or 699 kJ/kg during catch-up. ^{15}N labelled urea was administered over 36 h in a primed multiple oral dose procedure. In order to avoid blood sampling, amino acids were isolated from the urinary pool which derives from the renal arterial blood. This was collected every 6h over 42 h. Glycine, alanine, lysine and histidine were isolated from urine by preparative ion-exchange with fraction collection of all column effluent and with peak identification by a fluorimetric, multiscan plate reader procedure for evaluating the distribution of sample peaks. The fractions containing alanine, glycine, histidine and lysine were desalted, lyophilized, taken up into buffer and analysed for ^{15}N enrichment in a Europa-Roboprep 20-20 combustion IRMS instrument. Confirmation of the identity and purification of the amino acid peaks was obtained by means of Waters Pico-tag HPLC analysis of a small sample of the final solution before ^{15}N analysis.

We observed significant enrichment in the majority of children. The degree of enrichment of lysine varied from zero up to 0.09 atom% excess. Furthermore when enrichment of lysine was compared with the degree of urea salvage calculated from the urinary urea enrichment, it was apparent that labelling was only observed in children where urea salvage was more than 40% of production with an obvious correlation between labelling and salvage as the latter process increased up to 75% of production. Glycine and alanine were generally more enriched than lysine. The relative enrichment ratios were glycine, 1.63 (range 0.18-3.15); alanine, 1.96 (range 0.7-3.73) whilst for histidine the labelling was similar to lysine, 0.9, (range 0.4-1.8). The higher mean enrichment in alanine and glycine is consistent with their N originating from both transamination reactions from labelled NH_3 derived from the colon and from *de novo synthesis* by colonic microflora. Overall the results support the concept that urea salvage is a nutritionally significant source of indispensable and dispensable amino acids.

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Postprandial protein utilization of wheat protein from a single meal in normal adults. By A. FEREDAY[1], N. GIBSON[1], M. COX[1], D. HALLIDAY[2], P.J. PACY[2] and D. J. MILLWARD[1], *[1]Centre for Nutrition and Food Safety , School of Biological Sciences, University of Surrey, Guildford GU2 5XH, [2] Unit of Metabolic Medicine, St Mary's Hospital, Praed Street, London W2 1PG*

We have reported studies of the extent and mechanisms of postprandial protein utilization (PPU) from wheat-based food, employing a [^{13}C]leucine balance protocol to measure utilization of wheat protein in normal adults given repeated small meals in order to maintain an isotopic and metabolic steady state (Fereday *et al.* 1994). Since meal feeding involves ingestion of bolus amounts of food, the utilisation may differ from that during small meal feeding. Following a large single meal the utilisation of protein can be measured from leucine oxidation and balance measured by continuous monitoring of $^{13}CO_2$ excretion and plasma α-keto-isocaproate (KIC) enrichment, assuming that plasma KIC represents the mean intracellular KIC enrichment and we have reported such a study with a milk protein meal (Fereday *et al.* 1995). We report here a similar study of the utilization of wheat gluten following a 50g protein-2092kJ meal.

We measured [^{13}C]leucine balance in five adults (four male one female), during a 9 h prime dose constant intravenous infusion of [1-^{13}C]leucine, commencing at 08.00 hours, 12 h after the last meal. CO_2 production was continuously monitored with a ventilated hood and blood and breath were sampled every 15 min between 120 and 180 min, every 10 min up to 300 min and every 20 min untill the end of the infusion at 540 min. Leucine oxidation was calculated from $^{13}CO_2$ excretion and plasma KIC enrichment assuming that bicarbonate recovery increased from 0.76 (postabsorptive) to 0.911(postprandial) following the same time course as the increase in CO_2 production. The meal, a dry fried pancake made of equal portions of wheat gluten and plain flour contained (per kg body weight): 0.5 g protein and 22.9 kJ, accompanied by a drink containing dissolved hydrolysed potato dextrose and paracetamol (1.5 g added as an absorption marker), and was consumed over 15 min at 180 min. The meal absorption was monitored with measurements of plasma glucose, insulin, leucine and paracetamol.

The meal-protein-related increase in leucine oxidation was calculated as postprandial oxidation minus postabsorptive oxidation of leucine measured between 120 and 180 min. PPU was then calculated as leucine intake minus cumulative excess leucine oxidation over the 6 h after the meal.

As judged by changes in plasma glucose, insulin and paracetamol, the meal absorption was essentially complete by 4 h although the leucine concentration did not return to baseline untill 6 h after the meal. After the meal the plasma leucine ^{13}C mol % excess (MPE) increased, reaching a peak after about 1 h, then falling to a minimum at about 3 h and then increasing to a slightly elevated enrichment at the end of the study. Although the changes were not so marked, the pattern of response of the KIC MPE values was similar. CO_2 enrichment increased following the meal reaching a maximum at 300 min after the meal and remained elevated for the duration of the studies. The pattern of increase in leucine oxidation was a delayed increase reaching a maximum at 4 h after the meal and remaining elevated throughout the infusion.

PPU, calculated as intake minus the cumulative oxidation expressed as a fraction of the leucine meal content was 0.711 (SD 0.064, CV 9.0%) This is similar to the value previously reported for milk protein measured in this way, 0.766 (SD 0.013, CV 1.7%).

In conclusion in healthy adults, wheat protein given in a single high protein meal is utilized for postprandial protein deposition as efficiently as milk protein over the 6 h following the meal.

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The effects of creatine and carbohydrate ingestion on whole-body creatine retention in vegetarians. By A. L. GREEN, I. A. MACDONALD and P. L. GREENHAFF, *Department of Physiology and Pharmacology, Nottingham University, Nottingham NG7 2UH*

Although it is capable of being synthesized *de novo*, creatine (CR) is also found in the diet, mainly in the form of meat (Walker, 1979). Dietary CR supplementation can increase muscle CR concentration, which is further augmented if CR is ingested with carbohydrate (CHO; Green *et al.* 1995). Dietary intake of CR is limited in vegetarians and normal reference values for serum CR are lower in these individuals (Delanghe *et al.* 1989). The aim of the present study was, therefore, to investigate the effects of CR supplementation, and any additive effect of CHO ingestion, on whole-body CR retention in vegetarians.

On day 1, twenty-four fasted men gave a blood sample and then consumed 250 ml of a sugar free orange drink (SFO). Arterialized-venous blood samples were then obtained at 20 min intervals for the next 260 min, while subjects remained in a supine position. Subjects undertook a 24 h urine collection, beginning in the morning of day 1. On day 8, subjects were randomly divided into four groups (A-D, *n* 6), with groups A and B consisting of lacto-vegetarians. A resting blood sample was obtained and subjects then consumed: 5 g CR in 250 ml SFO (groups A and C); 5 g CR in 250 ml SFO + 500 ml Lucozade (groups B and D). This dose of CHO has previously been shown to augment muscle Cr uptake in non-vegetarians (Green *et al.* 1995). The subjects repeated the same procedures as on day 1 and consumed three more CR preparations throughout day 8. Plasma and urinary CR were measured using HPLC, serum insulin using a diagnostic radio immunoassay kit and whole-blood glucose using a Yellow Springs glucose analyser. Statistical analysis was performed using ANOVA and a post-hoc Student's unpaired *t* test or Fisher *t* test, where appropriate.

	Vegetarian A (CR)		Vegetarian B (CR + CHO)		Non-vegetarian C (CR)		Non-vegetarian D (CR + CHO)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
AUC (μmol/l per min)	120489	7013	76739***	3521	155310	1727	98045†††	2240
Urinary CR(g)	5.72	0.45	4.75	0.48	9.13	0.17	5.09†††	0.59
Peak insulin (mIU/l)	5.8	1.0	59.6***	1.7	7.3	1.2	148.7†††	14.7
Peak glucose (mmol/l)	4.6	0.2	8.4***	0.3	4.5	0.1	8.5†††	0.3

*** Mean values in group B were significantly different from those in group A, $P<0.001$. ††† Mean values in group D were significantly different from those in group C, $P<0.001$.

On day 1, plasma CR, serum insulin and blood glucose concentrations remained constant throughout the experiment and urinary CR excretion was negligible in all four treatment groups. The area under plasma CR/time curve (AUC), urinary CR, peak insulin and peak glucose on day 8 are shown in the table. On day 8, AUC and urinary CR were lower in group A than in group C ($P<0.001$), suggesting that whole body CR retention was greater in vegetarians than in non-vegetarians following CR supplementation alone. In non-vegetarians, AUC and urinary CR were lower in group D than in group C, suggesting that CHO ingestion augmented CR retention, which is in agreement with our previous findings (Green *et al.* 1995). In contrast, although AUC was lower in group B than in group A, no difference was found when comparing their 24 h urinary CR excretion. Thus, over the course of four doses of CR, CHO ingestion had no further effect on tissue CR accumulation in the vegetarians. No difference was found when comparing urinary CR excretion in groups A, B and D indicating that tissue CR accumulation was similar in these three treatment groups.

Ethical approval was granted for this study which was supported by the Defence Research Agency.

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The effect of dietary glycerol on the rate of postprandial glycogen synthesis in cachectic tumour-bearing rats. By A. A-W. ALHAMDAN, O. A. OBEID and P.W. EMERY, *Department of Nutrition and Dietetics, King's College London, Campden Hill Road, London W8 7AH*

We found previously that the rate of postprandial glycogen synthesis in tumour-bearing rats was twice that of control rats (Emery *et al.* 1993). However, the test meal included Intralipid, which contains a significant quantity of glycerol, a known precursor for glycogen synthesis. The present experiment was designed to investigate whether increased glycogen synthesis in tumour bearing rats is dependent on the presence of glycerol in the meal. Fourteen male Fischer 344 rats bearing a transplantable Leydig cell tumour (TB) and fourteen healthy controls (AD) were fed *ad libitum* on a semi-purified diet supplying 20% energy as protein, 23% as fat (maize oil) and 57% as carbohydrate, then fasted overnight. Half the rats from each group were given a 16 kJ liquid control meal based on the previous diet (C), while the rest were given the same meal in which 50 mg carbohydrate was replaced by glycerol (G). The rats were immediately injected intraperitoneally with 259 MBq ^3H_2O, killed 1 h later and blood and livers were taken for analysis. The rate of glycogen synthesis was calculated as μmol ^3H_2O incorporated into glycogen-glucose/h per g liver and the percentage of glycogen derived via pyruvate was calculated from the ratio of ^3H labelling at C6 and C2 in the glycogen-glucose (Kuwajima *et al.* 1986)

	Control		Glycerol			Analysis of variance		
	AD	TB	AD	TB	Pooled SE	TB	G	TBxG
Plasma glucose (mmol/l)	8.5	7.3	8.3	8.8	0.51	NS	NS	NS
Plasma glycerol (μmol/l)	366	359	393	319	22.5	NS	NS	NS
Glycogen synthesis	47.4	74.2	65.6	74.2	8.47	*	NS	NS
Glycogen via pyruvate (%)	44.1	56.5	38.2	48.8	4.77	*	NS	NS

* $P<0.05$.

Increased glycerol intake did not affect plasma glucose or glycerol concentration 1 h after the meal, which suggests that glycerol was cleared from the circulation very rapidly. The rate of synthesis of glycogen in tumour-bearing rats was higher than that in controls, in line with our previous observations, but the addition of glycerol to the meal did not affect glycogen synthesis in either group of rats. The percentage of glycogen derived via pyruvate (indirect pathway) was higher in tumour-bearing rats than in controls, and this was not affected by the glycerol content of the meal. Thus it appears that increased postprandial glycogen synthesis in tumour bearing rats occurs by the indirect pathway via pyruvate, and may involve recycling of lactate and/or amino acids. However it is not affected by the glycerol content of the meal.

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Emery, P.W., Carpenter, T.T.A. & Obeid, O.A. (1993). *Nutrition and Cancer* **20**, 231-240.
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The effect of meal size on the rates of postprandial glycogen and lipid synthesis of tumour-bearing rats. By O. A. OBEID, J. A. KHAYATT and P. W. EMERY, *Department of Nutrition and Dietetics, King's College London, Campden Hill Road, London W8 7AH*

We found previously that the rate of postprandial glycogenesis was greater in cachectic tumour-bearing rats than in normal controls, while rates of lipogenesis changed in the opposite direction (Emery *et al.* 1993). The partitioning of ingested carbohydrate between glycogen and fat deposition is known to be affected by meal size and frequency. We have therefore investigated whether the changes observed in tumour-bearing rats can be reversed by increasing the size of the meal. Fourteen male Fischer 344 rats bearing a transplantable Leydig cell tumour (TB) and fourteen *ad libitum*-fed controls (AD) were fed on a semipurified diet supplying 20% energy as protein, 23% as fat and 57% as sucrose-starch (1:1, w:w). After an overnight fast half the rats from each group were tube-fed with a 4 ml liquid meal containing 12 kJ of their normal diet, while the rest were tube-fed with a meal of similar volume but containing 24 kJ of the same diet. The rats were immediately injected intraperitoneally with 259 MBq ^3H_2O, killed 1 h later and blood, livers and epididymal fat pads were taken for analysis. Rates of synthesis of glycogen and lipid were determined as described previously (Emery *et al.* 1993) and the percentage of glycogen derived via pyruvate was calculated from the ratio of ^3H labelling at C6 to that at C2 in the glycogen-glucose (Kuwajima *et al.* 1986).

	12 kJ Meal		24 kJ Meal			Analysis of variance		
	AD	TB	AD	TB	Pooled SE	TB	M	TBxM
Plasma glucose (mmol/l)	9.5	9.0	10.2	10.1	0.17	*	***	NS
Glycogen synthesis†	28.8	43.6	33.8	53.1	6.79	**	NS	NS
Glycogen via pyruvate (%)	40.4	45.5	33.1	38.6	3.05	NS	*	NS
Liver lipogenesis‡	10.8	8.0	16.7	9.3	1.32	***	*	NS
Epididymal fat pad lipogenesis‡	5.73	5.74	7.28	8.50	0.94	NS	*	NS

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

† μmol ^3H_2O incorporated into glycogen-glucose/g liver per h.

‡ μmol ^3H_2O incorporated into saponifiable lipid/g tissue per h.

Plasma glucose concentration showed a greater increase in response to the larger meal, but was slightly lower in tumour bearing rats than in the corresponding controls. Liver glycogen synthesis rate was greater in the tumour bearing rats than in controls, in line with our previous observations, but was not significantly affected by the size of the meal. The percentage of glycogen synthesized via pyruvate was not significantly affected by tumour growth, but increasing meal size caused a significant decrease, indicating greater activity of the direct pathway of glycogen synthesis from glucose. The rate of fatty acid synthesis in the liver was lower in tumour-bearing rats than in controls. Increasing meal size caused an increase in hepatic fatty acid synthesis, although the magnitude of this effect appeared to be somewhat smaller in the tumour-bearing rats than in the controls. Thus although the larger meal tended to counteract some of the metabolic abnormalities associated with tumour growth the extent of this improvement was rather limited.

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Plasma metabolite, hormone and lipoprotein lipase activity responses to foods enriched with *n*-3 polyunsaturated fatty acids. By C.N.BROOKS[1], J.A.LOVEGROVE[2], M.C.MURPHY[1], B.J. GOULD[1] and C M WILLIAMS[2], [1]*Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH,* [2]*Hugh Sinclair Unit of Human Nutrition, University of Reading, Whiteknights, Reading RG6 6AP*

The purpose of this dietary intervention study was to increase the intakes of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from current levels of intake to 1.8 g/d using a range of normal foods which had been enriched with these fatty acids using a fish-oil product (ROPUFA, Hoffman La Roche) during manufacture.

Nine normolipidaemic male subjects, mean age 50 (SD 7.2) years, BMI 25.7 (SD 2.6) kg/m^2 were recruited to participate in a 2 x 21 d randomized single-blind cross over trial using *n*-3 polyunsaturated fatty acid (PUFA)-enriched and control foods. The volunteers were encouraged to consume 1.8 g EPA+DHA/d by substituting the test foods for similar foods in their usual diet. On day 22 the subjects received a standard test meal breakfast after a 12 h overnight fast. Postprandial blood collections were made for the following 8 h. A bolus dose of heparin was then given followed by 5 and 15 min blood collections. Habitual nutrient intakes and intakes whilst on the two 21 d interventions were assessed by means of 3 d weighed records. Nutrient analysis was carried out using the FOODBASE programme (Version 1.2, Institute of Brain Chemistry and Human Nutrition).

A mean intake of 1.36 (SD 0.34) g/d EPA+DHA on the *n*-3 enriched diet was achieved and this was significantly higher ($P<0.001$) than the intakes on the control 0.36 (SD 0.22) g/d and habitual 0.12 (SD 0.06) g/d diets. However we did not manage to achieve our target enrichment of 1.8 g EPA+DHA/d. Mean energy intakes on the *n*-3 enriched and control diets 12.01 (SD 1.29) MJ/d and 11.98 (SD 1.94) MJ/d respectively, were both significantly higher ($P<0.001$) than the habitual diet 9.23 (SD 1.14) MJ/d. Of the extra energy consumed, 60% came from carbohydrate and 40% came from fat.

Results of fasting plasma triacylglycerol (TAG), total and HDL-cholesterol and area under the time concentration curves (AUC) for plasma TAG, non-esterified fatty acids (NEFA), insulin and glucose are presented in the Table:

	n-3 Enriched diet		Control diet	
	Mean	SD	Mean	SD
TAG (mmol/l)	1.49	0.37	1.53	0.63
Total cholesterol (mmol/l)	5.52	0.83	5.27	0.54
HDL-cholesterol (mmol/l)	1.04*	0.33	0.87	0.29
AUC TAG (min.mmol/l)	1149.2	401.7	1061.6	308.1
AUC NEFA (min.mmol/l)	160.3	56.9	171.9	38.3
AUC glucose (min.mmol/l)	2864.7	515.1	2629.1	166.7
AUC insulin (min.pmol/l)	184807	124035	197369	172125
Postheparin LPL (15 min) (mU)Ψ	153.93	26.45	160.74	39.54

*Mean value was significantly different from control, $P<0.02$

Ψ1mU = amount required to release 1 nmol oleate/ml per min at 37°.

Apart from a significantly raised HDL-cholesterol concentration on the *n*-3 enriched diet, no other significant differences were found between the biochemical variables analysed.

We conclude that it is feasible to significantly increase daily EPA+DHA levels using *n*-3 enriched normal foods. At 1.3 g EPA+DHA/d no significant hypotriacylglycerolaemic effects were noticed which is contrary to supplement trials using a similar dose (Harris, 1989). This may be due to the negative effects of the concurrent increase in energy or due to differences in efficacy of EPA+DHA from foods as opposed to capsule supplements.

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Apolipoprotein B-48 measurements as a marker for chylomicron metabolism and the effect of chronic *n*-3 PUFA intake. By J.A. LOVEGROVE[1], C.N. BROOKS[2], M.C. MURPHY[2], B.J. GOULD[2] and C.M. WILLIAMS[1], [1]*Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading RG6 6AP,* [2]*Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

To date no definitive marker has been identified for the investigation of dietary-derived lipids, chylomicrons (CM) and their remnants (CM-r). Triacylglycerol (TAG) concentration and retinyl ester (RE) enrichment in plasma and TAG-rich lipoproteins (TRL) fractions have been used as markers of CM, but this approach has been criticized (Krasinski *et al.* 1990). The aim of the present study was to investigate the feasibility of using apolipoprotein B-48 (apo B-48), the apolipoprotein uniquely associated with CM in humans, as a marker for dietary-derived fat. The effect of chronic ingestion of *n*-3 PUFA on their postprandial concentrations was also investigated.

A randomized, controlled, single-blind crossover study was performed on nine normotriacylglycerolaemic men, mean age 50 (SD 7.2) years, BMI 25.7 (SD 2.6) kg/m^2. Each subject followed two periods (21 d) of dietary intervention separated by 5 months washout period. On the enriched diet subjects were asked to substitute manufactured foods, enriched with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (ROPUFA, Hoffman La Roche), for normal foods in their diet. The control diet consisted of similar foods which were not enriched. A mixed test meal containing 82 g fat, 6.4 MJ energy, 163 g carbohydrate, 43 g protein and 340,000 IU of retinyl palmitate was given after a 12 h overnight fast, the day after both of the two intervention periods. Hourly blood samples were collected from the subjects for an 8 h period. TRL fractions were prepared by overlaying the plasma with an equal volume of saline (1.006 g/ml), followed by ultracentrifugation 5.0 x 10^6 g_{max}.min. The TRL fraction was aliquoted, with 5% preservative, and frozen at -20° until required for analysis. The RE, TAG and apo B-48 concentrations were determined in the TRL fractions (Lovegrove *et al.* 1996).

	Incremental area under curve (IAUC)				Time of peak (minutes)			
	Enriched		Control		Enriched		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TAG (mmol/l.min)	271	175	245	251	220	90	202	96
Apo B-48 (μg/ml.min)	68	78	53	79	227	107	267	91
RE (μg/ml.min)	1153	1354	1857	2909	413	87	373	140

A mean daily intake of 1.36 (SD 0.34) g EPA + DHA was achieved on the enriched diet. This was significantly higher ($P<0.001$) than the daily intakes from the control 0.36 (SD 0.22) g and from subjects habitual 0.12 (SD 0.06) g diets, but did not reach the target intake of 1.80 g/d. The postprandial TAG and apo B-48 responses to the test meals were similar on the enriched and the control diets. Earlier postprandial peak responses were observed for apo B-48 and TAG, compared with the RE for both diets. The individual variability was large for all three parameteres measured.

A daily intake of 1.36 g of EPA + DHA in the form of enriched foods did not achieve significant reduction in either fasting or postprandial TAG concentrations. Preliminary data suggest that apo B-48 is a valuable marker of CM numbers since the postprandial TRL TAG and apo B-48 responses were similar. However lack of concordance with the RE responses lends further evidence of the limitations of using RE measurements as a marker for CM and CM-r.

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Detection of orally administered [1-^{13}C]palmitic acid in the triacylglycerol component of chylomicrons by gas chromatography-isotope ratio mass spectrometry. By M. STOLINSKI [1], M.A. HUMAYUN[1], A. HOUNSLOW[1], A.E. JONES[1], J.L. MURPHY[1], S.A. WOOTTON[1] and R. PRATLEY[2]
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The gastrointestinal handling and metabolic disposal of dietary lipid has been examined by measuring the enrichment of ^{13}C on breath and in stool following oral administration of [1-^{13}C]palmitic acid using isotope ratio mass spectrometry (IRMS) and gas chromatography-isotope ratio mass spectrometry (GC-IRMS) (Murphy *et al.* 1995; Stolinski *et al.* 1996). A more complete description of the partitioning and metabolism of absorbed lipid would be gained by measurements of enrichment in blood lipid fractions during the postprandial period. Our present understanding has been restricted by the analytical difficulties associated with the low levels of isotopic enrichment in triacylglycerols (TAG) isolated from blood. This presentation describes the use of GC-IRMS to determine the appearance of tracer in TAG isolated from plasma chylomicrons following the oral administration of [1-^{13}C]palmitic acid.

Following an overnight fast, four male subjects aged 34-50 years with a BMI of 28.7-42.8 kg/m^2 consumed a standardized test breakfast (35% of measured 24 h energy expenditure: 40% energy as lipid, 40% carbohydrate, 20% protein) containing [1-^{13}C]palmitic acid (20 mg/100 kJ). Venous blood samples were obtained before the meal and at 2 h intervals over the 8 h postprandial period for the determination of plasma total TAG and the isolation of chylomicron fraction (CM) by gradient ultra centrifugation (<0.95 SvU). Lipids were extracted and TAG isolated by TLC. The enrichment of ^{13}C label in fatty acid methyl esters (FAME) and fatty acid composition was determined by GC-IRMS (Stolinski *et al.* 1996) using an Orchid GC-IRMS (Europa Scientific Ltd, Crewe). The results are shown in the Table (median values with minimum and maximum values in parentheses; *ND*, not detected).

	Time (h)				
	0	2	4	6	8
Plasma total TAG (mmol/l)	0.59 (0.46,0.87)	0.72 (0.58,0.97)	1.12 (0.72,1.63)	0.69 (0.54,1.71)	0.60 (0.31,1.28)
CM TAG [^{13}C]16:0 (‰^{13}C)	-18 (-20,-13)	131 (49,147)	325 (130,587)	219 (138,606)	192 (167,328)
TAG total 16:0 (μg/ml CM)	68 (61,91)	86 (80,96)	108 (82,209)	64 (49,269)	94 (50,128)
TAG [^{13}C]16:0 as % total 16:0	*ND*	4 (2,4)	8 (4,14)	5 (3,14)	5 (3,8)

High levels of enrichment observed in the chylomicron TAG suggested that less label could be administered without compromising analytical precision. The initial appearance of the labelled tracer in chylomicron TAG followed a similar pattern to that of plasma total TAG, but remained elevated at 8 h even though plasma TAG had returned to pre-meal values. Labelled palmitic acid accounted for approximately 5% of the total palmitic acid present in chylomicron TAG over the postprandial period. Small increases in enrichment (usually <10 ‰^{13}C) were also observed in other fatty acids including 16:1, 18:0 and 18:1 in the 6 h and 8 h samples. These observations further demonstrate the potential application of GC-IRMS in the routine determination of ^{13}C enrichment of FAME in blood lipid fractions to trace the fate of exogenous fatty acids in the study of lipid metabolism.

The support of MAFF, National Institutes of Health and Scientific Hospital Supplies Ltd is gratefully acknowledged.

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Gastrointestinal handling and metabolic disposal of emulsified [^{13}C]-tripalmitin in healthy children, healthy women and patients with cystic fibrosis. By J.L. MURPHY, K. LAIHO, A. HOUNSLOW and S.A. WOOTTON, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

The factors that determine the digestion, absorption and metabolic disposal of dietary lipid are not well understood under normal circumstances or when additional demands may be imposed due to growth during childhood or in ill health such as the maldigestion and malabsorption observed in cystic fibrosis. The present study utilized stable-isotope tracer methodology to examine the gastrointestinal handling and metabolic disposal of [1,1,1-^{13}C]-tripalmitin (10 mg/kg body weight) presented in a novel form as a glucose-sucrose-casein emulsion in a group of six healthy women (aged 23-29 years), nine healthy children (six males and three females; aged 5-8 years) and seven patients with cystic fibrosis (CF;three males and four females; aged 7-23 years) using pancreatic enzyme replacement therapy (PERT).

Following an overnight fast, the subjects ingested the ^{13}C-label with a standardized test meal (11g lipid; 1660 kJ) of low natural ^{13}C abundance. Breath samples were collected before and during a postprandial period (fasting) at hourly intervals for at least 6 h and then after 10 h and 24 h. Whole-body breath CO_2 excretion was measured by indirect calorimetry (GEM, Europa Scientific Ltd) before and at hourly intervals for at least 6 h after label administration. A baseline stool sample and all stools passed were collected for at least a 3 d period. Enrichment of $^{13}CO_2$ on breath and^{13} C in stool samples was analysed by mass spectrometry (ABCA and ANCA systems, Europa Scientific Ltd). The results shown are for ^{13}C excretion in stool and ^{13}C excretion on breath $^{13}CO_2$ as a percentage of the administered label (% admin dose).

	Stool ^{13}C (% admin dose)		Breath $^{13}CO_2$ (% admin dose)	
	Median	Range	Median	Range
Women	1.8	0 - 6.4	22.8	15.9 - 29.1
Children	5.7*	1 - 12.7	31.4	14.2 - 42.9
CF	27.9††	0 - 78.8	16.4†	0 - 31.3

* Median values were significantly different from those for healthy women, $P<0.05$. Median values were significantly different from those for healthy women and children: †$P<0.05$, ††$P<0.01$ (Mann-Whitney U test).

Emulsified tripalmitin appeared to be well digested and absorbed in both healthy children and women. More of the label was excreted in stool in CF patients (despite PERT) than in healthy children and women ($P<0.01$). The excretion of the label on breath as $^{13}CO_2$ peaked between 1 and 6 h after the test meal returning to baseline in most subjects by 24 h. The proportion of administered ^{13}C-label excreted on breath was reduced in CF patients compared with healthy women and children ($P<0.05$). Whilst there were only modest inverse relationships between the excretion of ^{13}C-label in stool and breath in healthy women (r -0.76) and CF patients (r -0.49), no association was observed in healthy children (r -0.27). These results suggest that the emulsified form of ^{13}C-labelled tripalmitin may have the potential to provide a clinical tool to assess gastrointestinal function in the form of a 'breath test'. Further studies need to address both the specificity and sensitivity of the test and the assumptions underlying these observations by characterizing the nature of ^{13}C-label in stool.

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The influence of a high-oleic-acid test meal compared with butter fat on postprandial lipaemia and factor VII coagulant activity in young men. By FRANCESCA OAKLEY[1], T.A.B. SANDERS[1] and G.J. MILLER[2], *[1]Nutrition, Food & Health Research Centre, King's College, University of London, Campden Hill Road, London W8 7AH and [2]MRC Epidemiology and Medical Care Unit, Wolfson Institute of Preventive Medicine, Medical College of St Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ*

We reported that factor VII coagulant (FVIIc) activity is increased following a high fat-test meal in which the fat was olive oil (Yahia & Sanders, 1995) compared with a low-fat test meal. We also observed less postprandial lipaemia in subjects who had received a diet for 3 weeks in which most of the fat was provided by butter fat compared with a diet in which most of the fat was provided by olive oil (Oakley *et al.*1994). The present study compared a low-fat test meal containing 5.4 MJ, 41 g protein, 249 g carbohydrate, 18 g medium-chain triacylglycerol (MCT) with three high-fat test meals (5.4 MJ, 41 g protein, 72 g carbohydrate, 95 g fat) enriched with either butter fat (90 g butter oil, 5 g safflower oil) or a high-oleic sunflower oil (95 g) or an admixture of a high-oleic sunflower oil and MCT (75 g high oleic sunflower oil, 2 g safflower oil, 18 g MCT). In designing the test meals, care was taken to ensure that the level of linoleic acid and stearic acid were similar in all three test meals. Twelve healthy male subjects received the four diets in random order 1 week apart according to an orthogonal Latin-square design. The subjects received a low-fat diet on the day preceding the test meal and a venous blood sample was obtained between 16.00 and 17.00 hours. After an overnight fast from 22.00 hours, venous blood samples were collected between 08.30 and 09.00 hours and the subjects then consumed the test meal. Further venous blood samples were obtained at 3 and 7 h after the test meal and capillary blood samples were obtained at 1, 2, 4 and 5 h after the test meal. Plasma triacylglycerol concentrations were determined on all blood samples and FVIIc was determined on the venous blood samples. The degree of postprandial lipaemia was determined by the measuring the area under the curve (AUC) for plasma triacylglycerol (TAG) concentrations following the test meal.. The results are shown in the Table.

	Low fat		High oleic		Butter		Oleic + MCT	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
TAG (AUC)	1.6[a]	0.41	11.1[b]	1.81	7.1[b]	1.09	8.6[b]	1.38
FVIIc %								
Low fat	109	7.30	111	7.97	115	5.95	111	7.57
Fasting	118	7.95	113	7.66	123	9.27	124	8.46
3 h	116	7.71	131	8.78	132	6.76	135	8.36
7 h	103[a]	6.46	123[b]	7.22	130[b]	6.63	127[b]	7.64

[a,b] Values with different superscripts in the same row were significantly different, $P<0.05$.

The postprandial lipaemia as measured was most marked with the high-oleic-acid diet and lowest with the low-fat diet. The lipaemia was lower on the butter diet than on the high-oleic diet ($P=0.076$). Both the high-oleic and oleic + MCT diets led to a significant increase in FVIIc at 3 h compared with the fasting value, whereas FVIIc declined from the fasting level to the 7 h value on the low-fat diet ($P< 0.005$). The 7 h values for FVIIc were greater on all the high-fat diets compared with the low-fat diet, and a significant diet x time interaction ($P<0.05$) for the three high-fat diets, was observed (ANOVA). The results of this study suggest that a low-fat meal decreases FVIIc and that fats high in long-chain fatty acids increase FVIIc.

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Postprandial coagulation factor VII activity in northern and southern Europeans. By HELEN M. ROCHE[1], ANTONIS ZAMPELAS[2], MARIA KAPSOKEFALOU[3], ANTONY KAFATOS[3], CHRISTINE M. WILLIAMS[4] and MICHAEL J. GIBNEY[1], *[1]Deptartment of Clinical Medicine, Trinity Health Sciences Centre, St. James's Hospital, Dublin 8. [2]School of Biological Sciences, University of Surrey, Guildford, GU2 5XH, [3]University of Crete, Iraklion, Crete, Greece. [4]Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading.*

Coagulation factor VII activity (FVIIc) is positively related to the incidence of cardiovascular disease (CVD). The activity of factor VII increases during postprandial triacylglycerolaemia. The present study investigated whether northern and southern European subjects exhibit differences in postprandial coagulation FVIIc following the ingestion of a fat-rich test meal.

The postprandial response was investigated in thirty northern European (UK and Irish) males and in thirty southern European (Cretan and Athenian) males who were matched for age and plasma lipid concentrations. Subjects consumed a test meal containing 40 g fat, providing different levels of monounsaturated fatty acids (MUFA) (12% and 24% energy). Fasting and postprandial samples were drawn every 2 h and analysed for plasma triacylglycerol (TAG) and apolipoprotein B48 (apo B48) concentrations and FVIIc. Statistical analysis was completed using repeated measures ANOVA. There were no significant differences following the low- and high-MUFA meals, therefore all data reported represent the mean of the values following both meals.

Postprandial FVIIc activity is presented below. Repeated measures ANOVA demonstrated a significant centre x repeat interaction ($P = 0.03$) for FVIIc. The southern European population had lower fasting FVIIc which increased sharply and to a greater extent during the early postprandial phase and returned to fasting levels, compared with the northern European population who showed greater fasting and postprandial FVIIc.

Time (hours)		0	2	4	6	8
N. European	mean	67.054	70.195	70.910*	71.267*	70.624*
(% reference plasma)	SE	3.548	3.933	3.570	3.627	3.523
S. European	mean	61.254=	63.462=	72.195*	65.938=*	62.429=
(% reference plasma)	SE	4.950	4.949	5.473	5.605	5.249

= Significantly ($P = 0.03$) different between groups; * Significantly ($P = 0.03$) different from fasting values.

Repeated measures ANOVA of plasma TAG and apo B48 concentrations demonstrated a highly significant centre x repeat interaction ($P \leq 0.0001$). The southern Europeans showed a greater rise in plasma TAG and apo B48 concentrations during early postprandial lipaemia, while the northern Europeans showed a more prolonged rise of TAG and apo B48. Multivariate regression analysis was completed to determine the factors which affected the magnitude of the postprandial FVIIc response, and it was shown that the time to peak TAG concentration was the single most important factor affecting FVIIc, the earlier the peak in plasma TAG concentrations the greater the increase in postprandial FVIIc. The different postprandial responses between the two groups may be ascribed to dietary and / or genetic differences between populations.

The role of growth hormone in regulation of lipolysis in normal subjects after an overnight fast.

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Lipolysis is the process by which stored body fat is hydrolysed to provide metabolic fuel in the form of non-esterified fatty acids (NEFA) and glycerol. This process is modulated by a number of hormones. The possible role of hormones other than catecholamines in stimulating lipolysis has come into prominence because microdialysis work has shown that, in normal subjects at rest after an overnight fast, the net adrenergic effect on lipolysis is inhibitory (Arner *et al.* 1990). Growth hormone (GH) is a potential stimulator of lipolysis in normal subjects.

We have investigated the role of the nocturnal rise in plasma GH on the following day's regulation of lipolysis by measurement of arterio-venous differences across a subcutaneous adipose tissue depot. Arterialized blood was obtained from a vein draining a hand warmed in a box at 65 °, and venous blood from a superficial inferior epigastric vein draining the adipose tissue of the anterior abdominal wall. Eight healthy subjects, median age 25 (range 19-37) years, median BMI 21.2 (range 18.4-25.5) kg/m^2, were studied on two occasions after a standard evening meal. During the first night either octreotide was given (50 μg at 23.00 and 07.00 hours) to suppress GH release, or a control study was carried out. The veno-arterial (v-a) differences were measured for triacylglycerol (TAG), NEFA and glycerol across the adipose tissue bed, and adipose tissue blood flow was measured by the clearance of ^{133}Xe (Larsen *et al.* 1966) at hourly intervals from 08.00 to 14.00 hours the next day.

	Control		Octreotide	
	Mean	SE	Mean	SE
v-a difference				
NEFA (μmol/l)	821	124	576*	83
TAG (μmol/l)	45	9	45	7
Glycerol (μmol/l)	171	16	126*	15
Blood flow (ml/min per 100 g)	4.21	0.55	3.96	0.35
GH (mU/l)	6.26	1.33	2.47*	0.50

Mean values were significantly different from control, * $P < 0.05$.

The Table shows mean values for plasma GH concentration from 23.00 to 14.00 hours, and mean values for v-a differences and blood flow from 08.00 to 14.00 hours. Plasma GH concentrations were suppressed with octreotide. The mean v-a differences for NEFA (indicative of intracellular lipolysis) were significantly higher in the control study than after the GH suppression, but the plasma TAG differences (indicative of intravascular lipolysis) were not significantly different. There was no significant difference in adipose tissue blood flow. These results suggest that in normal subjects the nocturnal surge in GH secretion leads to increased release of NEFA by the adipose tissue in the postabsorptive state on the following day.

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Suppression of endogenous lipolysis during exogenous triacylglycerol infusion. By JASWINDER S. SAMRA, SARAH L. GILES, CATE L. RAVELL, MO L. CLARK, SANDY M. HUMPHREYS and KEITH N. FRAYN, *Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE*

Changes in energy balance are reflected in changes in the body fat stores. There are close correlations between fat balance and energy balance even over periods as short as 24 h (Abbott *et al.* 1988). Carbohydrate intake may regulate fat stores via changes in insulin secretion, but it is less clear whether or how pure fat intake would have an effect on the body's triacylglycerol store. In the present study we have looked at the rate of endogenous lipolysis in normal subjects fasted overnight who then received an intravenous infusion of a triacylglycerol emulsion.

Six normal males (aged 21 - 37 years; BMI 23.0 - 25.9 kg/m^2) were studied. Cannulas were placed in a vein draining the subcutaneous adipose tissue, and a vein draining a hand which was warmed to provide arterialized blood. Basal blood samples were taken before beginning an intravenous infusion of Intralipid (100 g/l) at a rate of 1.85 ml/kg body weight per h. Samples were taken at intervals during a 4 h infusion and then for a further 90 min after stopping the infusion. Total efflux of non-esterified fatty acids (NEFA) from the subcutaneous adipose tissue was calculated as the veno-arterial difference multiplied by plasma flow, which was measured by the clearance of ^{133}Xe. Clinical and analytical methods were as described previously (Frayn *et al.* 1994).

NEFA efflux decreased during Intralipid infusion, and then further decreased after the infusion was stopped (Table). This occurred despite unchanged insulin concentrations (Table). Interestingly, the arterial NEFA concentration rose during Intralipid infusion (Table), implying a dissociation of the relationship usually seen between arterial NEFA concentration and release from adipose tissue.

	Before infusion		During infusion		After infusion	
	Mean	SE	Mean	SE	Mean	SE
NEFA Efflux (μmol/100 g per min)	2.59	1.07	1.66*	0.74	0.94*	0.55
Arterial NEFA (μmol/l)	654	80	1012*	44	853*	68
Arterial insulin (mU/l)	4.7	0.6	5.2	0.7	5.1	0.7

*Significantly different from column before, $P<0.05$ (2-tail Wilcoxon Signed-Rank).

During Intralipid infusion fatty acids are generated both through intracellular lipolysis and by the action of lipoprotein lipase (*EC* 3.1.1.34) on the triacylglycerol emulsion. Total NEFA efflux from adipose tissue will therefore overestimate intracellular lipolysis. Intracellular lipolysis therefore decreased markedly both during and after Intralipid infusion. There is no obvious mechanism by which this might have been brought about, since insulin concentrations did not change. We speculate that some message, possibly humoral, has responded to the influx of fat and down-regulated adipose tissue lipolysis, although we are unable to identify this message. In addition we are unable to explain the increased systemic NEFA concentration during Intralipid infusion unless lipolysis of the Intralipid emulsion is occurring in other adipose depots, in other tissues or simply in the vascular compartment.

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Adverse effect of 18:1*trans* v. 18:1*cis* on plasma HDL- cholesterol subfractions in men. By T.A.B. SANDERS[1], FRANCESCA OAKLEY[1], G.J. MILLER[2] and D. CROOK[3], *[1]Nutrition, Food & Health Research Centre, King's College London, Campden Hill Road, London W8 7AH, [2]MRC Epidemiology and Medical Care Unit, Wolfson Institute of Preventive Medicine, Medical College of St Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ and [3]Wynn Institute for Metabolic Research, Wellington Road, London NW8*

The plasma concentration of HDL-cholesterol, particularly that in the HDL_2 fraction, is a powerful predictor of risk of coronary heart disease. *Trans* monounsaturated fatty acids lead to a reduction in HDL-cholesterol and an increase in LDL-cholesterol (British Nutrition Foundation, 1995) when substituted for *cis*-monounsaturated fatty acids in the diet. It is uncertain whether the HDL cholesterol-lowering effect of *trans* fatty acids is similar to that of diets high in carbohydrate, nor is it clear how the distribution of cholesterol in the HDL subfractions is affected. We report the influence of diets containing *trans* fatty acids on HDL subfractions in healthy men. Subjects were fed three isoenergetic diets for consecutive 2-week periods in random order using an orthogonal Latin-square design. The three diets compared were a high-carbohydrate diet (30% energy from fat), a high-oleic-acid diet (40% energy from fat) and a high-*trans* diet (40% energy from fat). All three diets provided 10% energy as saturated fatty acids and were identical except for the level and type of fat. In the high-carbohydrate diet, 10% of the monounsaturated fat was replaced with carbohydrate. The high-oleic and high-*trans* diets contained an additional 10% energy as either 18:1 *cis* or 18:1 *trans* respectively, provided as margarine specially formulated by Unilever Research Laboratories, Vlaadingham. Fasting venous blood samples were collected on day 13 and day 14 of each period and HDL subfractions were separated (Gidez *et al.*1982) and their cholesterol content determined using enzymic assays. The results are shown in the Table below for eighteen subjects who completed all three dietary periods (age range 18-38, mean BMI: 24kg/m^2).

Diet...	High carbohydrate		High oleic		High *trans*	
	Mean	SE	Mean	SE	Mean	SE
HDL cholesterol mmol/l	1.20^a	0.065	1.28^b	0.057	1.16^a	0.063
HDL_2 cholesterol mmol/l	0.18^a	0.042	0.18^a	0.042	0.14^b	0.019
HDL_3 cholesterol mmol/l	1.03^a	0.045	1.10^b	0.045	1.02^a	0.047

^{a,b} Values with different superscripts in the same row were significantly different, $P<0.01$.

Compared with the high-oleic diet the high-*trans* diet lowered HDL cholesterol significantly. This change was due mainly to a decrease in the proportion of cholesterol in the HDL_2 fraction on the high-*trans* diet and an increase in the proportion in the HDL_3 fraction on the high-oleic-acid diet. The high-carbohydrate diet lowered HDL-cholesterol compared with the high-oleic-acid diet but did not affect the HDL_2 fraction. The results of this study suggest that *trans* fatty acids have adverse effects on HDL subfractions with regard to risk of coronary heart disease.

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Effects of meals of varying monounsaturated fatty acid content on postprandial hormones and lipaemia in young and middle-aged men. By J.M.E. KNAPPER[1], D.H. WEBB[1], A. ZAMPELAS[1], K. JACKSON[1], J.A. TREDGER[1], L.M. MORGAN[1], J. WRIGHT[1] and C.M. WILLIAMS[2], [1] *Centre for Nutrition and Food Safety, University of Surrey, Guildford GU2 5XH and* [2]*Hugh Sinclair Unit of Human Nutrition, University of Reading, Whiteknights, Reading RG6 6AP*

The magnitude and duration of the postprandial lipaemic response have been implicated in the development of coronary heart disease. The influence of age on postprandial lipaemia is largely accounted for by differences in fasting triacylglycerol (TAG) levels. In our current study we recruited fifteen healthy young men (YM) (aged 18-28 years, BMI 20.2-26.6 kg/m^2) and fifteen healthy middle-aged men (MAM) (aged 40-53 years, BMI 22.6-29.2 kg/m^2). Subjects were matched for fasting TAG and lifestyle factors such as participation in sport. All subjects were non-smokers. On two separate occasions subjects attended the Clinical Investigation Unit at a local hospital after an overnight fast. On each visit they consumed a mixed meal of 4.18 MJ comprising 150 g carbohydrate, 24 g protein and 43 g fat. The fatty acid composition of the two test meals provided either 12 or 24% energy as MUFA at the expense of saturated fatty acids. Meals were consumed in a random order. Two fasting blood samples were collected and the meal consumed over a 20 min period. Blood was sampled every 30 min for the first 2 h and then hourly until 9 h after the meal. Plasma glucose and TAG were measured by automated enzymic methods. Insulin, glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1(7-36)amide) were measured by specific radioimmunoassay.

There were no significant effects of the MUFA concentration of the test meal on the postprandial responses of the two groups and for this reason the total areas under the time response curves (TAUC) for the 12% MUFA meal only are shown in the Table.

	Plasma glucose TAUC (mmol/l.min)		Plasma TAG TAUC (mmol/l.min)		Plasma insulin TAUC (nmol/l.min)		Plasma GIP TAUC (nmol/l.min)		Plasma GLP-1 TAUC (nmol/l.min)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12% MUFA YM	2725	233	778.9	199.9	149.4	60.9	75.9	16.3	10.9	1.0
12% MUFA MAM	3162**	261	1015.1	394.5	137.5	70.0	91.1	30.7	11.4	4.6

** Significantly different from YM by one-way ANOVA, $P<0.01$.

Fasting levels for TAG, insulin, GIP and GLP-1(7-36)amide were similar at the start of the two test meals for both age groups. There were significant differences in fasting glucose levels between the two age groups (YM 4.8 (SD 0.4), MAM 5.5 (SD 0.5) $P<0.01$) and this accounts for the significant difference in TAUC. There were significant differences ($P<0.01$) in the patterns of the response curves between the two age groups for glucose, TAG and insulin with the MAM achieving higher levels of glucose and TAG at all time points monitored. The incremental areas under the curves (IAUC) for TAG were significantly greater for the MAM ($P<0.01$). The rise in plasma insulin was slower in the MAM compared with the YM.

Within a mixed meal physiological amounts of MUFA as a substitute for saturated fatty acids had no modifying effect on plasma glucose, TAG, insulin, GIP or GLP-1(7-36)amide in healthy individuals. Differences are seen in the time course of postprandial TAG and insulin responses between healthy YM and MAM which are not accounted for by differences in fasting levels. However differences in IAUC for TAG do suggest an effect of age on secretion and/or TAG clearance rates.

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Postheparin lipase activities and postprandial triacylglycerol responses to meals of varying monounsaturated fatty acid content in young men from the UK and Greece. By K.G. JACKSON[1], A. ZAMPELAS[1], J. M. E. KNAPPER[1], J. WILSON[1], A. KAFATOS[2], M. KAPSOKEFALOU[2], C.M. WILLIAMS[3] and B. J. GOULD[1], [1]*Centre for Nutrition and Food Safety, University of Surrey, Guildford GU2 5XH,* [2]*Department of Social Medicine and Nutrition, University of Crete, Iraklion, Greece and* [3]*Hugh Sinclair Unit of Human Nutrition, University of Reading, RG6 6AP*

The magnitude and duration of postprandial lipaemia have been correlated with the risk of development of coronary artery disease (CAD) (Patsch *et al.* 1992). Recent studies have suggested that the fatty acid composition of triacylglycerol-rich lipoproteins is an important determinant of their removal (Sethi *et al.* 1993). At present there is a lack of knowledge of the effect of *n*-9 monounsaturated fatty acids (MUFA), especially oleic acid, on postprandial lipid responses.

The present study was carried out to investigate the effect of three test meals of varying MUFA content (12%, 17% and 24% of dietary energy) on postprandial plasma triacylglycerol (TAG) responses and postheparin lipoprotein lipase (LPL, *EC* 3.1.1.34) and hepatic lipase (HL, *EC* 3.1.1.3) activities in subjects who habitually consume varying amounts of MUFA in their background diet. Twenty four subjects (aged 18-30 years, BMI 20-25 kg/m^2) were recruited, eight from the UK (low MUFA in background diet) and sixteen from Greece (GC) (high MUFA in background diet). Following a 12 h fast, subjects were given a test meal which contained 150 g carbohydrate, 24 g protein and 42 g of fat. Two fasting blood samples were taken before the meal and then hourly samples were collected for 9 h. At the end of the test period, 7500 IU of heparin were injected and two blood samples were collected, 5 and 15 min following the injection, for the analysis of lipase activites. Plasma TAG was measured by an automated, enzymic, colorimetric method and postheparin lipases by the measurement of free fatty acids released from a labelled triolein substrate emulsion (Nilsson-Ehle & Schotz, 1976). Lipase activities and area under the time response curve (AUC) for the plasma TAG responses are shown in the Table.

	Plasma TAG-AUC (mmol/l.min)		LPL activity (mU/ml)†		HL activity (mU/ml)	
	Mean	SD	Mean	SD	Mean	SD
12% MUFA - UK	773.70	251.21	344.45	78.06	70.96	33.06
GC	738.20	260.65	271.87	61.30	121.13*	46.50
17% MUFA - UK	697.19	203.12	331.95	72.28	64.89	21.89
GC	790.46	253.73	300.88	85.63	120.85**	40.03
24% MUFA - UK	832.10	335.08	359.34	84.93	71.45	21.00
GC	777.96	240.73	299.97	89.51	123.64***	44.77

Significant differences between UK and GC for same MUFA test meals. * $P < 0.02$, ** $P < 0.001$ and *** $P < 0.005$. † LPL and HL activities are expressed as mU/ml where 1 mU represents 1 nmol of fatty acids released/ml per min at 37°.

Fasting levels for TAG were similar for both populations and not significantly different among study days for any of the test meals. No significant differences were observed in the TAG-AUC, although an earlier peak plasma TAG concentration and a faster return to baseline levels were observed in the plasma TAG response curves in the GC compared with the UK subjects. LPL activities were not significantly different between the two populations. We therefore conclude that the significantly higher HL activities in subjects habituated to a diet high in MUFA may partially explain their faster removal rates of remnants, and hence may have implications in the development of CAD.

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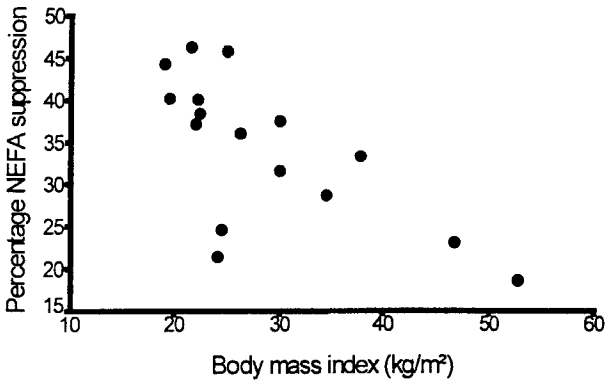
The effect of body mass index on postprandial non-esterified fatty acid suppression. By LUCINDA K.M. SUMMERS, BARBARA A. FIELDING, VERA ILIC and KEITH N. FRAYN, *Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE*

Increased BMI is associated with the insulin resistance syndrome. Insulin is an important regulator of non-esterified fatty acid (NEFA) concentrations after a mixed meal (Coppack *et al.* 1989). Decreased NEFA suppression and features of the insulin resistance syndrome occur in some individuals with normal glucose tolerance (Byrne *et al.* 1995). We investigated the relationship between BMI, insulin concentration and postprandial NEFA suppression.

Sixteen healthy subjects (four male) median age 44 (range 19 - 68) years and median BMI 25 (range 19 - 53) kg/m^2, were studied twice after an overnight fast. Subjects were given a meal containing 60 g fat, 85 g carbohydrate and 13 g protein. Arterialized blood was obtained from a hand vein warmed in a hot-box at 65° at -20, 0, 30, 60, 90, 120, 180, 240, 300 and 360 min. Plasma NEFA and insulin concentrations were measured.

'Basal' NEFA concentration was the mean of the -20 and 0 values for both experiments. The 'minimum' NEFA concentration was the mean of the minimum NEFA concentrations for the two experiments. The maximal fall in arterial NEFA concentration was the difference between these two concentrations. Percentage NEFA suppression was calculated as the maximal fall divided by the basal value multiplied by 100 (Byrne *et al.* 1995). Percentage NEFA suppression varied considerably between subjects (median 73.4%, range 37.4 - 92.9%). The Spearman rank correlation test showed that percentage NEFA suppression was strongly negatively correlated with BMI (r_S -0.70, $P < 0.005$) (Fig.). However, there was no correlation between basal insulin or postprandial rise in insulin concentration and suppression of NEFA concentration, although basal insulin levels were strongly correlated with BMI (r_S 0.70, $P < 0.005$).

BMI *v.* percentage NEFA suppression (*n* 16)



The decreased NEFA suppression seen in obesity may be a further feature of the insulin resistance syndrome. Basal insulin concentrations and postprandial rise in insulin concentration were not related to percentage NEFA suppression, but neither of these values accurately reflects insulin sensitivity.

This research was funded by a grant from the Ministry of Agriculture, Fisheries and Food.

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Very early changes in gastrointestinal morphology in response to riboflavin depletion. By CATHERINE A. YATES, GARETH S. EVANS and HILARY J. POWERS, *University Department of Paediatrics, Sheffield Children's Hospital, Sheffield S10 2TH*

Factors which disturb events during the neonatal period of gastrointestinal development may have long-term effects on gastrointestinal function. We have demonstrated that the induction of riboflavin deficiency at weaning results in morphological and kinetic changes in the gastrointestinal tract which are not readily reversed by repletion (Ayree *et al.* 1996; Williams *et al.* 1996). Of particular significance is the observed failure to develop the normal number of villi in the neonatal period, an effect that is evident after only 7 d of feeding a riboflavin-deficient diet. We have explored further the sensitivity of gastrointestinal development to riboflavin depletion very early in the neonatal period.

Thirty-two weanling female Wistar rats were weight matched and allocated to one of two groups to receive a riboflavin-deficient diet or a control diet , *ad libitum*. After 45 h or 69 h eight animals from each group were killed for the study of duodenal morphology and cytokinetics. At 90 min before kill, each animal received an intraperitoneal injection of the metaphase arrest agent, vincristine. At kill, intestines were washed, and 10 mm segments cut at defined positions from the proximal end. One segment was immediately treated with Weiser's solution in order to prepare intact crypts, which were then studied for the frequency of bifurcation using an inverted light microsope. Villus counts and crypt:villus ratio were measured in whole mounts, using Feulgen staining and light microscopy. Crypt dissection and squash of similarly stained segments permitted calculation of the number of metaphase-arrested cells per 10 crypts, giving a mitotic index (MI). Transverse sections were cut and stained appropriately for the qualitative assessment of mucins, and the expression of alkaline phosphatase (*EC* 3.1.3.1). Haematoxylin-and-eosin-stained sections were studied by light microscopy, using a crossed scale eyepiece graticule to permit the measurement of crypt dimensions.

	Riboflavin deficient				Control			
	45 h (*n* 8)		69 h (*n* 8)		45 h (*n* 8)		69 h (*n* 8)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Crypt bifurcation (%)	9.8	3.45	9.0	3.20	10.0	4.28	9.8	1.98
Villus number †	1703	558	1714	260	1611	108	1624	362
Crypt:villus ratio	15.2	1.74	15.0	1.19	13.6	2.20	15.8	1.59
Mitotic index ‡	7.6	0.7	9.3	0.4	7.2	0.9	8.5	0.9
Crypt depth (μm)	139	15.9	162*	8.7	134	7.5	137	3.5
Crypt width (μm)	39	5.3	32	2.7	36	2.2	33	1.7

* Significantly different from control group and value at 45 h ($P<0.05$).
† Number of villi per 10 mm length of duodenum.
‡ Average number of metaphase-arrested cells per 10 crypts.

There was no effect of either the dietary group or the period of feeding, on the frequency of crypt bifurcation, villus number per 10 mm length of duodenum, crypt:villus ratio or MI. There was no evidence of a difference in the expression of brush-border alkaline phosphatase, or in the degree of sulphation of mucins between the two dietary groups at either time point. However, a 2-way ANOVA revealed an effect of both diet ($P=0.000$) and time ($P=0.001$) on crypt depth, and an interaction between these factors ($P=0.008$). Post hoc analysis (Mann-Whitney U) showed relative crypt hypertrophy after only 69 h of feeding a deficient diet ($P<0.05$). Disturbances to gastrointestinal development occur remarkably fast in response to feeding a riboflavin-deficient diet. Further studies will clarify the mechanisms involved.

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An investigation of the role of dietary copper and α-tocopherol on the oxidative stability of poultry muscle. By L.M. O'NEILL[1], K. GALVIN[1], P.A. MORRISSEY[1] and D.J. BUCKLEY[2], *Departments of [1]Nutrition and [2]Food Technology, University College Cork, Republic of Ireland*

Transition metals such as Fe and Cu are important catalysts of lipid oxidation, resulting in deterioration in the organoleptic and nutritional quality of muscle foods. Animal feeds usually contain Fe and Cu supplements resulting in total feed levels in excess of dietary requirements. Evidence suggests that withdrawal of supplemental Fe shortly before slaughter improves the storage stability of muscle foods (Kanner *et al.* 1990). However, little is known about the effects of dietary Cu on oxidative stability. The purpose of the present study was to examine the effects of dietary α-tocopheryl acetate supplementation and the withdrawal of dietary Cu supplementation, for 1 or 2 weeks before slaughter, on the oxidative stability of chicken muscle.

Cobb 500 broilers (1 d old) were divided into six groups and fed on diets containing either a basal (30 mg/kg) or supplemental (200 mg/kg) level of α-tocopheryl acetate. Supplemental Cu (8 mg/kg feed) was withdrawn for 1 or 2 weeks before slaughter. Control groups received supplemental Cu for the entire feeding period. Birds were slaughtered after 6 weeks. Thigh muscle was ground, cooked and stored at 4° for up to 12 d. The extent of lipid oxidation was determined by measuring thiobarbituric acid-reacting substances (TBARS) by the method of Ke *et al.* (1977).

Storage time at 4° (d) . . .		0		3		6		9		12	
α-Tocopheryl acetate (mg/kg diet)	Cu withdrawal before slaughter-(Weeks)	TBARS (mg malonaldehyde/kg muscle)									
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
30	Control	1.03	0.20	6.52	0.45	7.44	0.41	7.80	0.42	7.90	0.39
	1	0.78	0.70	5.15*	0.31	6.63	0.29	6.84	0.46	7.54	0.80
	2	0.81	0.60	5.07*	0.29	7.15	0.44	6.79	0.38	6.86	0.47
200	Control	0.00	0.00	4.55	0.21	4.68	0.36	5.75	0.45	6.24	0.32
	1	0.00	0.00	2.89*	0.29	5.33	0.30	5.07	0.41	6.47	0.46
	2	0.00	0.00	2.29*	0.31	3.41*	0.41	4.50*	0.47	4.37*	0.42

* Significantly different from corresponding control group (ANOVA one-way; LSD test): ($P<0.01$).

Supplementation with α-tocopheryl acetate resulted in lower TBARS throughout the storage period in all groups. In basal and supplemented groups, withdrawal of Cu supplementation resulted in lower TBARS after 3 d. However, Cu withdrawal did not affect the oxidative stability of muscle from basal groups after this point. In supplemented groups, after day 3, TBARS were significantly reduced ($P<0.01$) when Cu was withdrawn for 2 weeks before slaughter. The results indicate that removal of supplemental Cu from chicks diets for 2 weeks before slaughter improved the oxidative stability of chicken muscle. Inclusion of supplemental α-tocopheryl acetate enhanced this effect.

This project was supported under the EU AIR programme.

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Effects of vitamin A on inflammatory responses of Caco2 colonic epithelial cells. By S.M. FILTEAU[1,2], D. HOUSE[2] and J.G. RAYNES[2], *[1]Centre for International Child Health, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, and [2]Department of Clinical Sciences, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 4T8*

Prophylactic vitamin A supplements decrease mortality and severe morbidity among young children in less developed countries with virtually all benefit due to improved resistance to gastrointestinal disease. However, therapeutic vitamin A for established diarrhoea appears to have no benefit. Proposed mechanisms generally explain neither the tissue specificity nor the difference between prophylaxis and therapy. We showed (Filteau *et al.* 1995) that prophylactic vitamin A supplements to Ghanaian children increased their inflammatory responses, assessed as acute phase protein (APP) production, in association with gastrointestinal, but not other, symptoms. Inflammation is an early response to infection and may benefit from vitamin A before diarrhoea is established. Vitamin A could either directly increase APP production or could do so through increasing inflammatory cytokine production. To investigate the former, we have studied vitamin A and gut inflammatory responses *in vitro*.

Caco2 cells, a colonic epithelial line which differentiates *in vitro* to a small intestine-like phenotype, were cultured under standard conditions in twenty-four-well plates and used at various times post confluence. Cells were stimulated for 24 hours with interleukin-1 (IL1) in the presence or absence of retinol or retinoic acid. ELISA assays were used to measure APP and interleukin-8 (IL8, a positive control) in the medium. Analyses of variance with IL1 and vitamin A metabolites as main effects and day of assay as a covariate were performed.

Caco2 cells produced similar amounts of α_1-antichymotrypsin (ACT) and about one fifth as much haptoglobin (HAP) and α_1-acid glycoprotein (AGP) as HuH-7 hepatoma cells, used as a control since hepatocytes are the major source of APP. IL1 (1 ng/ml) increased production by Caco2 cells of HAP by 49% (F 3.97, P 0.056, n 19) and ACT 7-fold (F 16.9, P 0.0003, n 18), but did not affect AGP (4% decrease, F 0.06, P 0.8, n 20). Retinoic acid (10^{-7} M) did not affect APP production by IL1-stimulated Caco2 cells. IL1 induced a 24-fold increase (F 121, P 0.0001, n 18) in production of the neutrophil chemoattractant, IL8. Retinoic acid decreased release of IL8 in stimulated cultures by 20% but this was not significant (F 1.82, P 0.19, n 18). Similar results were seen using 10^{-6} M retinol. These effects were observed in Caco2 cells at all stages of differentiation post confluence, and were not due to increased cell death as measured by release of lactate dehydrogenase into the medium.

The results indicate that vitamin A does not directly affect APP production in gut epithelial cells. Further work should address the effects of vitamin A on inflammatory cytokine production by gut cells. In addition, the possible involvement of decreased IL8 production in decreasing inflammatory diarrhea after vitamin A supplementation should be investigated further *in vivo*.

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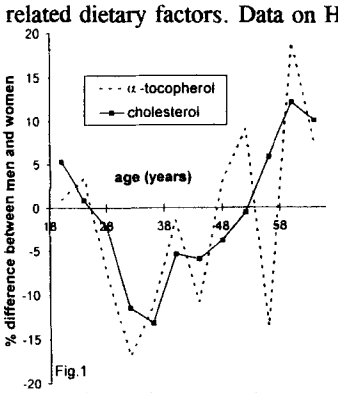
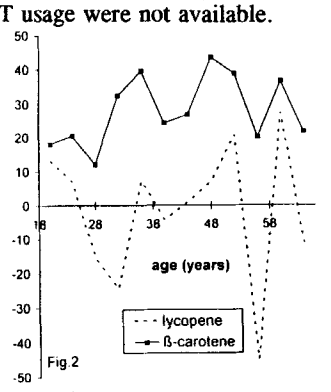
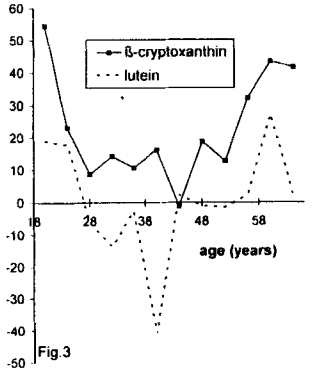
The effect of menopause on lipoprotein-associated biomarkers. By L.M. EDMOND and D.I. THURNHAM, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

The pre-menopausal risk of coronary heart disease (CHD) in women is significantly lower than that in men of the same age. The risk of CHD for the sexes approaches unity over several years post-menopause (Robbins *et al.* 1994). Hormone replacement therapy (HRT) can partly reduce this increased risk suggesting that oestrogen is involved in protecting females from CHD (Robbins *et al.* 1994). Since oestrogen increases lipoprotein turnover (Demacker *et al.* 1991), biomarkers associated with lipoproteins, such as vitamin E, cholesterol and the carotenoids are expected to show changes associated with menopause.

As part of the Adult study in 1986 (Gregory *et al.* 1990), the blood from 1800 randomly chosen people across Great Britain, in the age range 18-65 years, was analysed for cholesterol, carotenoids and vitamin E. For each biomarker, the concentration values were separated into discrete age ranges (4 years) for each sex and the median value for each group calculated. The median male values were subtracted from the median female values. These differences were expressed as percentages (%D) of the median value for the entire data set and plotted against age (Fig.1-3). This enables changes associated with menopause to be seen. By calculating %D, males serve as controls and correct for age-related dietary factors. Data on HRT usage were not available.







Female cholesterol values rose above those of males at about age 52 years (Fig.1)(NB higher mortality of males with high plasma cholesterol was not corrected for). Similar changes were seen for vitamin E (Fig.1). ß-Carotene (Fig.2) showed no significant changes with age (regression) and the significance of the sex difference could be largely eliminated by correcting for differences in body weight (17%), as intakes for each sex were similar. Lycopene, like ß-Carotene showed no change with age though also no difference between sexes (Fig.2). ß-Cryptoxanthin (Fig.3) rose sharply at about 52 years and was very similar to the lutein (*n* 900) graph (Fig.3). Higher turnover of lipoproteins (between puberty and menopause in women) increases the turnover of the more hydrophilic carotenoids such as lutein relative to the more hydrophobic carotenoids such as ß-Carotene (Edmond *et al.* 1995).

In conclusion, the data confirm the expected changes which would result from a decrease in lipoprotein turnover in women after menopause.

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Effect of oral supplementation with vitamin E on carotene levels in different lipoprotein fractions in humans. By M. CHOPRA, U. M^cLOONE, M. O'NEILL and D.I. THURNHAM, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

In the present study we have investigated the effect of vitamin E supplementation on the levels of micronutrients in lipoproteins from healthy volunteers. 65 normolipidemic, healthy, non-smoker volunteers were supplemented with 100 mg vitamin E/day for one month and levels of antioxidant micronutrients were measured by reverse phase HPLC at the start (week 0) and end (week 4) of supplementation.

After supplementation there was a significant increase in plasma α-tocopherol levels. However levels of γ-tocopherol and carotenes were significantly decreased. When tocopherol and carotene levels in different lipoprotein fractions were measured, α-tocopherol levels were found to increase and γ-tocopherol decreased in all fractions. Changes in carotenes varied between the different lipoprotein fractions.

		Micronutrients[a]										Cholesterol[b]	
		α-Tocopherol		γ-Tocopherol		ß-Carotene		Lutein		Lycopene			
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma	W0	27.0	1.15	1.53	0.13	0.46	0.03	0.29	0.01	0.85	0.06	4.49	0.96
(*n* 65)	W4	33.9***	1.59	0.57***	0.06	0.40**	0.03	0.25*	0.01	0.61***	0.05	4.32	1.03
VLDL	W0	10.40	0.80	1.0	0.06	0.065	0.01	0.074	0.01	0.135	0.01	2.11	1.37
(*n* 47)	W4	13.76***0.70		0.51***	0.00	0.063	0.01	0.058*	0.00	0.120	0.01	2.01	1.92
LDL	W0	4.08	0.75	0.166	0.01	0.14	0.02	0.038	0.003	0.232	0.02	2.83	1.20
(*n* 63)	W4	4.75*	0.42	0.079***0.01		0.07**	0.01	0.037	0.003	0.157***0.01		3.04	1.37
HDL	W0	7.92	0.89	0.444	0.06	0.098	0.01	0.112	0.01	0.148	0.02	1.53	0.13
(*n* 61)	W4	9.51	0.84	0.170***0.03		0.117	0.02	0.093	0.01	0.111	0.03	1.39	0.09

*Mean values were significantly different from those for week 0: *P<0.05; **P<0.01; ***P<0.001.*
[a]*Concentration of micronutrients in plasma is expressed in μmol/l and in VLDL, LDL and HDL is expressed as μmol/mmol cholesterol.*
[b]*Concentration of cholesterol is expressed as mmol/l.*

The table shows that vitamin E supplementation had no effect on cholesterol levels of any of the lipoprotein fractions. However it had a significant effect on the carotene levels of LDL and VLDL. Hydrocarbon carotenes were lowered significantly in LDL fractions while xanthophyll carotene lutein levels were significantly lowered in VLDL fraction only. The reduction in LDL carotene levels may be due to an increased transfer of carotenes to storage tissues. Change in carotenes in the HDL fractions was not significant, suggesting that vitamin E supplementation does not alter the carotene composition of HDL.

The results of this study suggest that vitamin E effects carotene metabolism. It is possible that vitamin E supplementation increases the transport of carotenes to tissues, or reduces the release of carotenes into lipoproteins from storage depots e.g. liver.

MC, MO, UML are supported by AAIR2-CT93-0888.

Quantitation of vitamin E and a carotenoid in human lens nuclei, and a preliminary study of micronutrient supplementation. By ANDREW MACDONALD[1], SU-JING CHEN[1], CHRISTOPHER BATES[1] and ROGER HOLDEN[2], [1]*MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ and* [2]*Derbyshire Royal Infirmary, London Road, Derby DE1 2QY*

In view of recent evidence that diet and vitamin supplements may affect the development of senile cataract (Seddon *et al.* 1994; Mares-Perlman *et al.* 1995), an intervention trial with ß-carotene, vitamin C and vitamin E is being undertaken (Chylack *et al.* 1995). The present study addressed associated questions, namely: which fat-soluble antioxidant nutrients are detectable in human lens nuclei obtained during cataract operation, and are their concentrations altered by dietary supplements? An assay procedure, suitable for blood plasma and lens nuclei, was developed. Following lipid extraction with heptane, ethanol, sodium dodecyl sulphate and butylated hydroxytoluene, using tocopheryl acetate as internal standard, samples were evaporated, redissolved in mobile phase (acetonitrile, methanol, dichloromethane) and injected on to a Waters HPLC system with a 5 µ Spherisorb ODS-2 (Alltech) column and a Waters 490E 4-channel optical density detector, to quantitate vitamin E at 292 nm and carotenoids at 450 nm. Analysis of nine lens nuclei from unsupplemented subjects with senile cataract indicated the presence of α-tocopherol: 4.7 (SE 0.4) nmol/g wet lens; g-tocopherol: 1.5 (SE 0.3) nmol/g and lutein/zeaxanthin: 0.028 (SE 0.009) nmol/g. ß-Carotene and other blood carotenoids were not detectable, the detection limit being 0.01 nmol/g. The Table shows comparisons between blood plasma and lens nuclei from subjects who were either not supplemented, or supplemented (vitamin C, 750 mg; α-tocopherol, 600 mg and ß-carotene 18 mg/d, in three divided doses, for 3 months before cataract operation).

	Not supplemented (*n* 9)				Supplemented (*n* 3)			
	Plasma (µM)		Lens Nucleus (µM)		Plasma (µM)		Lens Nucleus (µM)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
α-tocopherol	32.9	1.2	4.3	0.4	100.0	1.5	3.7	0.2
g-tocopherol	2.9	0.6	1.1	0.1	1.1	0.1	0.5	0.1
ß-carotene	0.17	0.03	<0.01	-	2.04	0.55	<0.01	-

Despite a 3-fold increase in plasma α-tocopherol and a 12-fold increase in plasma ß-carotene in the supplemented group, lens nucleus α-tocopherol was not increased, and lens nucleus ß-carotene remained undetectable. In aqueous humour, vitamin C concentrations increased from 56 µM in the unsupplemented, to 1145 µM in the supplemented, subjects. Vitamin E and carotenoids were not detected in aqueous humour.

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Absorption of carotenoids. By M.E. O' NEILL and D.I. THURNHAM, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA.*

Much evidence to date suggests that dietary carotenoids may be protective against both heart disease and cancers (Gey, 1993). Most studies have traditionally been carried out on ß-carotene, however there is growing interest in the non pro-vitamin A carotenoids since work has shown that these may have better antioxidant properties than ß-carotene (Chopra et al. 1993).

Twelve normolipidaemic non-smoking healthy volunteers (six females, six males) were given 40 mg of specific encapsulated carotenoids, ß-carotene, lutein or lycopene, with a meal containing 42 g of fat, 96 g of carbohydrate, 52 g of protein, 4.0 MJ and devoid of carotenoids and Vitamin A, although retinol palmitate (50,000IU) was added in order to quantify triacylglycerol-rich-lipoprotein (TRL) clearance when lutein and lycopene were tested. Fasting and then hourly postprandial blood samples were collected for 8 h. Carotenoids in plasma and the TRL fraction (density < 1.006 kg/l) were measured by HPLC (Thurnham et al. 1988). Postprandial response curves of the carotenoids and retinyl esters in the TRL fraction were used to measure absorption of carotenoids in males and females and to act as a baseline for future work on "modifiers"of carotenoid absorption.

Positive responses for all carotenoids were observed in the TRL fraction with little change occurring in plasma. Peak postprandial absorption for the different carotenoids ranges between 2 h for lutein in men, to 5 h for lycopene in both males and females. In both sexes the majority of ß-carotene is represented as retinol in the first 3 h of absorption, with ß-carotene being absorbed intact after this time.

Table. *Area under curve after 40 mg supplementation of specific carotenoids (nmol/l.h)*

	Lutein			*Lycopene*			*ß-Carotene + Retinol*		
	n	Median	Range	*n*	Median	Range	*n*	Median	Range
Males	3	136	16-257	3	113	75-267	6	254	200-650
Females	3	227	44-257	3	255	146-287	6	174	136-510

Large variations between individuals in their absorptive capacity of the carotenoids are shown in the Table. For the two non-nutritional carotenoids, although peak absorption for lutein occured earlier in males (2 h) than females (4 h), there was no significant difference in the total amount absorbed. However, in the case of lycopene, peak absorption times were similar (4-5 h). These results suggest that no significant differences exist between males and females in the absorption of any of the carotenoids studied.

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We thank Hoffman-La-Roche for supply of Carotenoid supplements.

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Relationships between dietary intakes and plasma concentrations of carotenoids. By T.K. HA, L. MAHER, A.S. ANDERSON and M.E.J. LEAN, *University of Glasgow, Department of Human Nutrition, Glasgow Royal Infirmary, Glasgow G31 2ER*

Plasma concentrations of retinol and α-tocopherol have previously been found to correlate poorly with preformed vitamin A and vitamin E intake (Gregory *et al.* 1990). We have evaluated fasting plasma carotenoid concentrations, measured using HPLC, as potential biomarkers in ninety adults who completed a 7 d weighed diet inventory, analysed using COMPEAT (Nutrition Systems, London). Daily intakes of preformed vitamin A ranged from 67 to 348 μg/4184 KJ, carotene equivalents from 95 to 2430 μg/4184 KJ, α-carotene from 0 to 1032 μg/4184 KJ and β-carotene from 0 to 3832 μg/4184 KJ.

Our results confirmed a lack of relationship between plasma retinol and intake of preformed vitamin A, or between plasma α-tocopherol and dietary intake. Since food values of carotenoids are incomplete, plasma concentrations were related to total carotene equivalent intake. There were significant relationships with lutein (R 0.50, $P<0.001$), α-carotene (R 0.41, $P<0.001$) and β-carotene (R 0.41, $P<0.003$). It is postulated that the weaker association with α- and β-carotene might reflect their conversion to retinol. Thus lutein appears to be the best of a relatively weak set of dietary markers.

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Comparison of the antioxidant activities of lycopene, lutein and crocetin at atmospheric and low oxygen partial pressures in an *in vitro* model system. By A.E. DUGGAN, E.P. PRENDERGAST and N.M. O'BRIEN. *Department of Nutrition, University College Cork, Republic of Ireland*

Many studies suggests an inverse relationship between chronic disease risk and the consumption of fresh fruit and vegetables. Carotenoids are one component of fruit and vegetables that have evoked considerable research interest. Carotenoids are thought to inhibit mutagenesis, to be photoprotective, to scavenge free radicals and to act as antioxidants (Krinsky, 1993) . We have previously shown that the antioxidant properties of β-carotene can vary under different partial pressures of O_2 (Lawlor and O' Brien, 1994). In the present study the ability of lutein, lycopene and crocetin to protect against paraquat-induced oxidative stress at normal and reduced partial pressures of O_2 in a cell culture model was assessed.

Primary cultures of chicken embryo fibroblasts (CEF) were cultured in HAM's F10 medium at 37° in a humidified atmosphere at 150 torr (atmospheric O_2 partial pressures) or 7.5 torr (low O_2 partial pressure). The medium was supplemented with the carotenoids at concentrations from 0-1000 nM. Oxidative stress was induced by exposing the cells to 0.25 mM paraquat for an 18 h period. Lipid peroxidation, as indicated by thiobarbituric acid-reactive substances (TBARS) and the activities of the antioxidant enzymes catalase (CAT, *EC* 1.11.1.6) and superoxide dismutase (SOD, *EC* 1.15.1.1), were measured as indices of oxidative stress.

		150 torr					7.5 torr			
		CAT(U/mg protein)		TBARS(nmol MDA/ mg protein)			CAT(U/mg protein)		TBARS(nmolMDA/ mgprotein)	
PQ (mM)	Lycopene (nM)	Mean	SE	Mean	SE	Lycopene (nM)	Mean	SE	Mean	SE
0	Control†	3.53*	0.10	1.90*	0.44	Control†	3.07*	0.15	1.37*	0.12
0.25	0	7.38	0.31	9.33	0.57	0	6.76	0.20	6.14	0.35
0.25	0.1	7.31	0.10	7.07*	0.91	0.1	6.60	0.23	4.75*	0.49
0.25	1.0	7.35	0.17	5.27*	0.10	1.0	4.37*	0.09	4.36*	0.22
0.25	10	4.01*	0.08	3.60*	0.23	10	3.62*	0.09	1.57*	0.18
0.25	100	3.81*	0.15	4.23*	0.70	100	2.81*	0.12	1.72*	0.14
0.25	1000	2.55*	0.10	3.94*	0.17	1000	2.42*	0.06	1.51*	0.09
	LSD (P<0.05)	0.72		0.34		LSD (P<0.05)	0.55		0.59	

MDA, malondialdehyde;LSD, least significant difference.

*Mean values were significantly different from PQ-treated cells not supplemented with lycopene (P<0.05, one-way ANOVA followed by LSD; n 6 for all groups). † Control cells not exposed to PQ and not supplemented with the carotenoids

Incorporation of added lycopene (10 nM at 150 torr and 1.0 nM at 7.5 torr) into PQ-treated cells returned CAT levels to those seen in non-PQ-treated cells. Incorporation of added lycopene (10 nM at 150 and at 7.5 torr) returned SOD levels to those seen in non-PQ-treated cells (results not shown). TBARS levels increased on exposure of CEF to PQ under both normal and low partial pressures of O_2. Lycopene (10-1000 nM) was slightly more effective in protecting against increased TBARS levels under low O_2 pressures (7.5 torr) than at atmospheric O_2 (150 torr). Therefore, the ability of lycopene to modulate the effects of PQ-induced oxidative stress in CEF was influenced slightly by partial pressure of O_2. In contrast, the ability of lutein and crocetin to modulate the effects of PQ-induced oxidative stress in this model did not appear to vary with different partial pressures of O_2 (results not shown).

This work was supported by the Department of Agriculture, Food and Forestry, Dublin.

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Supplementation of the diet with β-carotene or lycopene: comparison of effects on DNA damage in primary T-lymphocytes as assessed using the 'Comet' assay. By SIÂN B. ASTLEY, DAVID A. HUGHES, ANTHONY J.A. WRIGHT, ABIGAEL C.J. PEERLESS and SUSAN SOUTHON, *Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA*

Diets rich in vegetables and fruit have been found to be associated with a reduced risk of chronic diseases including cancer. Cancer aetiology is a complex multi-step process arising essentially from damage and subsequent misrepair of DNA. The present study investigated whether increased β-carotene or lycopene intake, at levels achievable from the diet, protected DNA from oxidative damage.

Single strand breaks, a measure of DNA integrity, were detected using the 'Comet' assay. After staining with a fluorescent DNA-dye, undamaged DNA was observed as a bright core while damaged DNA moved away from this core, in the direction of the anode, forming an image described as a comet (McKelvey-Martin *et. al.*, 1993; Fairbairn *et. al.*, 1995). Lymphocytes, isolated from twenty-five volunteers were analysed using the Comet assay, before and after exposure to H_2O_2 *ex vivo* (500 μmol/ l, 5 min). In the presence of metal ions, H_2O_2 induces DNA single-strand breaks. Comets were digitised with a low-light-level video camera and quantitative analysis performed by image processing.

Volunteers were allocated to one of two groups and individuals given the placebo or β-carotene (15 mg) in a double-blind, crossover study for 4 weeks. Measures of DNA single-strand breaks in T-lymphocytes and plasma carotenoids concentrations were performed at baseline and then repeated at 4 and 8 weeks, respectively. This procedure was repeated, 12 month later, using a lycopene (15 mg) supplement. Data from groups 1 and 2 were not combined because plasma β-carotene concentrations did not return to baseline, even after 4 weeks of placebo treatment. Supplementation with β-carotene significantly ($F \geq 25$, $P \leq 0.0001$) raised the circulating plasma concentration, which was associated with a significant decrease in single-strand DNA breaks in lymphocytes subjected to H_2O_2 *ex vivo* ($F \geq 5.6$, $P \leq 0.03$). However, there was an increased incidence of single-strand breaks observed in control cells (i.e. those not exposed to H_2O_2 *ex vivo*) compared to control cells at baseline ($F \geq 28.6$, $P \leq 0.01$) following supplementation with β-carotene. Increased intake of lycopene had no significant effect on the incidence of single-strand DNA breaks in control lymphocytes or following exposure to H_2O_2.

		Baseline			Week 4			Week 8		
Variable	Group	Mean	SEM	Range	Mean	SEM	Range	Mean	SEM	Range
β-carotene										
baseline	1*	0.294[a]	0.03	0.181-0.557	0.368[ab]	0.01	0.258-0.438	0.420[b]	0.03	0.174-0.605
	2*	0.237[a]	0.02	0.143-0.340	0.419[b]	0.03	0.208-0.558	0.452[b]	0.05	0.202-0.696
+ H_2O_2	1	0.613[a]	0.04	0.391-0.822	0.642[a]	0.07	0.371-1.000	0.461[b]	0.03	0.293-0.628
	2	0.519[a]	0.05	0.285-0.713	0.470[a]	0.03	0.322-0.602	0.455[a]	0.03	0.255-0.588
difference	1	0.319[a]	0.04	0.000-0.501	0.327[a]	0.10	0.000-1.000	0.042[b]	0.03	0.000-0.135
	2	0.209[a]	0.07	0.000-0.423	0.051[b]	0.02	0.000-0.203	0.003[b]	0.05	0.000-0.353
lycopene										
baseline	1	0.115[a]	0.01	0.068-0.181	0.199[b]	0.03	0.068-0.369	0.131[ab]	0.01	0.086-0.199
	2	0.148[a]	0.01	0.125-0.176	0.223[b]	0.03	0.096-0.344	0.143[a]	0.01	0.095-0.211
+ H_2O_2	1	0.311[a]	0.04	0.092-0.458	0.256[a]	0.02	0.160-0.398	0.255[a]	0.01	0.180-0.324
	2	0.423[a]	0.05	0.194-0.680	0.408[a]	0.04	0.216-0.616	0.294[b]	0.02	0.217-0.391
difference	1	0.196[a]	0.03	0.007-0.297	0.058[b]	0.02	-0.068-0.156	0.128[a]	0.01	0.069-0.194
	2	0.275[a]	0.05	0.026-0.538	0.184[a]	0.06	-0.075-0.444	0.150[a]	0.03	0.006-0.268

[a,b] mean values within a row, not sharing a common superscript letter, were significantly different ($P \leq 0.05$; one-way ANOVA followed by two-tailed paired *t* test). *Group 1 received placebo first and then the supplement of β-carotene or lycopene (15 mg/ d for 4 weeks). Group 2 received the supplement of β-carotene or lycopene (15 mg/ d for 4 weeks) and then the placebo. McKelvey-Martin, V.J., Green, M.H., Schemzer, P., Pool-Zobel, B.L., De Meo, M.P. & Collins, A. (1993). *Mutation Research* **288**, 47-63. Fairbairn, D.W., Olive, P.L., & O'Neill, K.L. (1995). *Mutation Research* **339**, 37-59.

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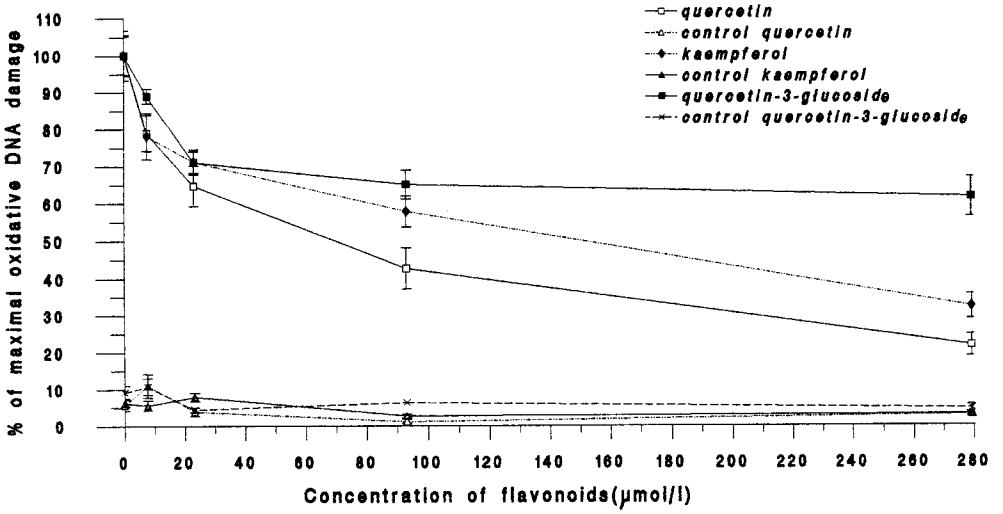
Protection from various flavonoids against oxygen-radical-generated DNA damage in the single-cell gel electrophoresis assay. By M. NOROOZI[1], W.J. ANGERSON[2], A. COLLINS[3] and M.E.J. LEAN[1], *Departments of [1]Human Nutrition and [2]Surgery, Glasgow University, Royal Infirmary, Glasgow G31 2ER, and [3]Rowett Research Institute, Aberdeen AB2 9SB*

Flavonoids are polyphenolic compounds the main dietary sources of which are fruit and vegetables (Hertog *et al.* 1992). We have examined the antioxidant effects of quercetin, kaempferol and quercetin-3-glucoside using a single-cell gel electrophoresis assay (SCGE, "comet assay") a sensitive technique for measuring DNA breakage in mammalian cells (McKelvey-Martin *et al.* 1993) and sensitive enough for detecting oxidative DNA strand breaks (Collins *et al.* 1995).

We isolated fresh peripheral human lymphocytes with histopaque 1077 and incubated them with different concentrations (0 - 279 μmol/l) of quercetin, kaempferol and quercetin-3-glucoside. After treatment with flavonoids (30 min, 37°C) cells were washed with phosphate-buffered saline and were treated with H_2O_2 (100 μmol/l, 5 min on ice), suspended in low-melting-point agarose, set on a microscope slide and lysed with lysis solution containing 2.5 M NaCl and 1% Triton x 100 for 1 h. Gel electrophoresis was then used to measure DNA strand breaks by estimating tail DNA content of 600-1200 lysed nuclei (comets) for each flavonoid concentration, using an Imaging Research BRS2 Image Analyser, with the fluorescence dye ethidium bromide.

All three agents produced a dose-dependent reduction in oxidative DNA damage. The protective effect of quercetin was significantly greater than that of both kaempferol and quercetin-3-glucoside at doses of 93 μM (kaempferol, p=0.05, quercetin-3-glucoside p=0.01) and 279 μM (kaempferol p=0.02, quercetin-3-glucoside p<=0.001). kaempferol was significantly more protective than quercetin-3-glucoside at 279 μM (p=0.001). (ANOVA and Student's t test). The concentrations of the three flavonoids that produced a 50% reduction in DNA damage, as estimated by linear regression analaysis of percent of maximal damage versus log-concentration, were: quercetin 53 μM, kaempferol 104 μM, quercetin-3-glucoside 949 mM.



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A longitudinal study of vitamin intake and plasma levels in the premature infant. By K.M. SILVERS[1]*, A.T. GIBSON[2] and H.J. POWERS[1]. [1]*University Department of Paediatrics, Children's Hospital, Sheffield S10 2TH* and [2]*Neonatal Intensive Care Unit, The Jessop Hospital for Women, Sheffield S3 7RE*

Premature infants have low circulating levels of vitamins A and E at birth. Conversely, levels of plasma vitamin C at birth can be five times higher than those found in adults (Silvers *et al.* 1994). There is evidence to suggest that the vitamin intakes of premature infants are inadequate to maintain status and that the mode of vitamin delivery may reduce intakes further (Inder *et al.* 1995). The aim of the present study was to observe the association between plasma levels and intakes of vitamins A, C and E over the first month of life in non-survivors, well survivors and survivors with bronchopulmonary dysplasia (BPD).

One hundred and forty-four babies requiring intensive care were recruited by parental consent. Blood was collected from an *in situ* arterial line within 2 h of birth and thereafter with routine clinical sampling over the first month of life. Samples were processed immediately and aliquots of plasma were stored at -70°. Plasma was stored with 10% metaphosphoric acid for the analysis of vitamin C. Levels of retinol and α-tocopherol were determined by HPLC analysis (Thurnham *et al.* 1988). Vitamin C was measured by a fluorometric assay (Vuillemier & Keck, 1989) using a centrifugal analyser with a fluorescence attachment. Daily intakes of vitamins A, C and E per kg body weight were calculated for each baby using the manufacturers data for intravenous nutrition and proprietary feed information and the DHSS report (1977) for mature human breast milk.

The mean gestational age in well survivors was 30.6 (SD 2.4) weeks which was significantly longer than the gestational age in either other group ($P<0.05$). Survivors who went on to develop BPD had a mean gestational age of 27.7 (SD 1.9) weeks which was significantly longer than the mean of 26.2 (SD 2.1) weeks in non-survivors ($P<0.05$).

Plasma retinol levels were significantly associated with vitamin A intake over the first month of life in well survivors (r 0.286, n 598, $P<0.001$) and survivors with BPD (r 0.417, n 322, $P<0.001$), but not in non-survivors (r 0.122, n 208, $P>0.05$). The plasma α-tocopherol:cholesterol ratio was significantly associated with vitamin E intake over the first month of life in non-survivors (r 0.274, n 200, $P<0.001$). Plasma α-tocopherol:cholesterol ratios were only weakly associated with vitamin E intakes over the first month of life in well survivors (r 0.187, n 568, $P<0.001$) and not in survivors with BPD (r 0.118, n 312, $P<0.05$). The distribution of vitamin E intakes and hence plasma α-tocopherol:cholesterol ratios in well survivors and survivors with BPD was bimodal, reflecting vitamin E supplementation with the introduction of enteral feeds. Plasma vitamin C levels were significantly associated with vitamin C intake over the first month of life in well survivors (r 0.359, n 531, $P<0.001$) and survivors with BPD (r 0.469, n 307, $P<0.001$). In contrast, there was a significant negative association in non-survivors (r 0.257, n 154, $P<0.001$).

Plasma retinol levels failed to respond to intake in non-survivors. This may have been due to adherence of retinol to the tubes of the delivery sets, or an inability of these infants to absorb retinol in the gastrointestinal tract, or increased requirements. The weak response of plasma levels of α tocopherol to intake in those babies who went on to develop BPD suggests that α-tocopherol intakes were adequate. Pharmacological doses of vitamin E to prevent the development of BPD seem not to be justified. The fall in the plasma vitamin C despite increasing intakes in non-survivors suggests a difference in the handling of vitamin C in this group.

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Neonatal neutrophil vitamin C concentration, effect on function and viability. By A. LOBAN[1], N. REBUCK[1], S.C. YONG[2], A.T. GIBSON[2], A. FINN[1] and H.J. POWERS[1], *[1]University Department of Paediatrics, The University of Sheffield, S10 2TH and [2] The Jessop Hospital for Women, Sheffield S3 7RE*

It has been observed that high plasma concentrations of vitamin C at birth in premature babies are deleterious (Silvers *et al.* 1994). This is believed to be at least partly due to the inhibitory effect of vitamin C on caeruloplasmin (Powers *et al.* 1995).

Vitamin C is believed to have an important role in neutrophil function. Vitamin C is found in millimolar amounts in neutrophils and is believed to quench reactive oxygen species, such as superoxide, which are generated during the respiratory burst in order to preserve the immune cell integrity and protect surrounding tissues (Stankova *et al.* 1975). High plasma vitamin C concentrations in premature babies at birth could reflect high concentrations in neutrophils or an impaired ability to transport vitamin C into tissues. An abnormal neutrophil vitamin C concentration could adversely affect neutrophil function. A low vitamin C concentration could fail to protect the neutrophils so they self-destruct leading to an impaired host defence, and a high vitamin C concentration could protect the neutrophils such that they continue to produce superoxide causing tissue damage.

We measured the ability of neutrophils isolated from adult (n 7) whole blood and neonatal (n 8) cord blood to take up ascorbic acid. The neutrophil respiratory burst, after stimulation with phorbol myristate acetate (PMA), was assessed using a flow cytometric method which measures the oxidation of 2'7'-dichlorofluorescein (DCFH) by superoxide (Himmelfarb *et al.* 1992). Cell viability after stimulation was assessed using flow cytometric analysis after staining with propidium iodide (Darzynkiewicz *et al.* 1995).

Plasma vitamin C concentrations were significantly higher in the neonates than the adults (87.9 (SD 39.08)μM (n 28) *v.* 54.5 (SD 11.53)μM (n 17), $P<0.001$); neutrophil vitamin C concentrations were also significantly higher in the neonates compared with the adults (0.9 (SD 0.57)mM *v.* 0.6 (SD 0.21)mM, $P<0.05$). The rate of uptake of ascorbic acid over a range of concentrations was the same in neonatal cord neutrophils as adult neutrophils. Incubating neutrophils with vitamin C did not influence the generation of superoxide by neonatal or adult neutrophils. However, incubation of adult neutrophils with 250 μM ascorbic acid resulted in a significantly higher viability after stimulation with 125 nM-PMA than without incubation with ascorbic acid ($P<0.05$), but did not protect neonatal cells.

These results suggest that neutrophils of neonates are as efficient at accumulating vitamin C as adult neutrophils and that the high plasma concentrations of vitamin C observed in these babies at birth are not due to impaired transport into tissues. Increasing vitamin C concentration in adult neutrophils is protective against the damaging effects of the respiratory burst but does not seem to have the same protective function in neonatal neutrophils.

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The radioprotective effect of dietary antioxidants on murine haematopoietic cells. By E.S. GALLIGAN, S.F. SWEETMAN, S.R. McKEOWN and J.J. STRAIN, *School of Biomedical Sciences, University of Ulster, Jordanstown, BT37 OQB.*

Vitamin C supplementation in the diet can modify the radioresponsiveness of isolated human lymphocytes to ionizing radiation (Green *et al.* 1994). It is possible therefore that human tissue radiosensitivity may be modified by dietary changes.

To examine this hypothesis further we have studied the effects of antioxidants on X-ray-induced DNA damage on P388 murine lymphoblastic cells and isolated murine lymphocytes (0 -5 Gray (Gy)). Before irradiation P388 cells were previously exposed for 24 h to a range of antioxidants: β-carotene (2 μM), ascorbate (60 μM), α-tocopherol (90 μM). *In vivo* experiments were carried out using male CBA mice were pretreated with daily (5 d) intra-peritoneal injections of each antioxidant: ascorbate (300 mg/kg body weight), α-tocopherol (400 mg/kg body weight) and β-carotene (100 mg/kg body weight). On day 5 lymphocytes were isolated and irradiated *in vitro* (0-5 Gray (Gy)). The Comet assay (McKelvey-Martin *et al.* 1993) was used to measured DNA damage immediately following exposure to the X-irradiation: cells in which the DNA contained single and double strand breaks showed streaming of the DNA from the nucleus on electrophoresis under alkaline conditions. (Cells were visualized using ethidium bromide staining and analysed using image analysis. Mutagenicity experiments (Cole *et al.* 1988) were also performed and cells evaluated for percentage survival.

Mann-Whitney U test showed that all of the antioxidants significantly increased protection of P388 cells at the lower radiation doses (1, 2 and 3 Gy). In addition β-carotene and vitamin E were observed to exert a protective of the cells at the higher radiation dose of 5 Gy in contrast to ascorbate. All antioxidants showed similar and significant protection ($P<0.05$) of the lymphocytes irradiated *in vitro* at these clinically relevant doses. Antioxidant-supplemented P388 cells exhibited a significantly lower ($P<0.05$) mutant frequency and increased percentage survival than non-supplemented cells. This shows that supplementation of cells *in vitro* and *in vivo* with dietary antioxidants can decrease the extent of radiation- induced DNA damage at low doses of X-radiation.

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Influence of antioxidant vitamin supplementation on cellular DNA damage and repair in Raji human lymphoblastoid cells. By SANDRA F. SWEETMAN, VALERIE J. McKELVEY-MARTIN and J. J. STRAIN, *School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

Oxidative damage in biological systems may occur in the presence of high levels of reactive oxygen species produced as by-products of normal metabolism and during inflammation. The targets for these reactive species include the DNA, protein and lipids of living cells. Oxidative damage is thought to play a role in ageing and degenerative diseases including cancer and cardiovascular disease. Antioxidants play a major role in controlling levels of oxidative stress and so may contribute to the protection of the integrity of cellular DNA (Binkova *et al.* 1990; Dyke *et al.* 1994). For example Green *et al.* (1994) have shown that ascorbic acid may alter human lymphocyte radiosensitivity by reducing oxidative damage.

In the present study the possible protective effects of ascorbic acid and α-tocopherol (singly and in combination) on Raji human lymphoblastoid cells exposed to various treatments of either X-rays (1,3 and 5 Gy) or H_2O_2 (5, 20 and 50 μM) were examined. DNA strand breaks and alkali-labile sites were measured using the alkaline comet assay (McKelvey-Martin *et al.* 1993); percentage survival and hypoxanthine guanine phosphoribosyl transferase (HPRT) mutant frequency were measured using the colony forming assay (Cole *et al.* 1988). Ascorbic acid (60 μM) and α-tocopherol (30 μM) were added either singly or together to cell culture medium 24 h before treatment and were present during treatment.

After the 24 h supplementation period with ascorbic acid alone, α-tocopherol alone and ascorbic acid and α-tocopherol together, the level of endogenous DNA damage was significantly decreased ($P<0.05$, Mann Whitney-U test.) relative to the non-supplemented culture as assessed by the comet assay (using the parameter of tail moment). No significant differences were observed with percentage survival, however the endogenous level of mutant frequency was significantly decreased ($P<0.05$ Mann Whitney-U test.) in the presence of ascorbic acid relative to the non-supplemented culture.

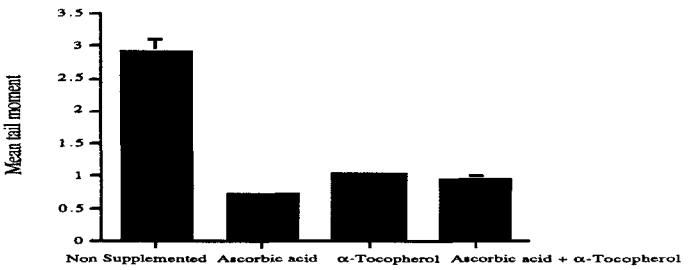

Fig. 1 Mean tail moment in supplemented and non-supplemented untreated Raji cells. Each value represents the mean with its standard error of three independent experiments.

The findings obtained with vitamin supplements and X-ray and H_2O_2 treatments, although not consistent for each dose of each agent nor at each time point, are, nevertheless generally consistent with the concept that ascorbic acid alone, α-tocopherol alone, and ascorbic acid and α-tocopherol together can, under certain conditions, provide protection against oxidatively-induced DNA damage. The most convincing evidence for an antioxidant protective effect against X-ray and H_2O_2 induced damage in the Raji cell line, under the conditions used was obtained with ascorbic acid.

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Ferric reducing ability of plasma (FRAP) as a novel method of measuring 'total antioxidant power'. By IRIS F.F. BENZIE[1] and J.J. STRAIN[2]. *[1]Department of Health Sciences, The Hong Kong Polytechnic University, Hong Kong and [2]Human Nutrition Research Group, University of Ulster at Coleraine, BT52 1SA, Northern Ireland*

Measurement of 'total antioxidant power' may be useful in studies of pro-oxidant : antioxidant balance. Such tests, however, are often time consuming and require sophisticated expensive equipment. The ferric reducing ability of plasma, measured by the FRAP assay, is a novel test of 'total antioxidant power' which is quick, simple and inexpensive. In the FRAP assay antioxidant-induced reduction of Fe^{3+}, present in stoichiometric excess and complexed to tripyridyltriazine (TPTZ) at low pH, causes an immediate change in absorbance at 593 nm. The reaction is non-specific, in that any half-reaction with a lower redox potential, under the test conditions, will drive the Fe^{3+} reaction. Test conditions favour reduction of the Fe^{3+}/TPTZ complex, provided that a reducing co-reactant is present.

The absorbance at 593nm of the FRAP reagent, containing 25 ml of 300 mmol/l acetate buffer, pH 3.6, 2.5 ml of 10 mmol/l TPTZ and 2.5 ml of 20 mmol/l ferric chloride, is measured immediately before and 4 min after sample addition. The ΔA593nm over the 0-4 min reaction time is related to that given by reaction of a known concentration of Fe^{2+} (ferrous sulphate) and a FRAP value (µmol/l) calculated.

Using a Cobas Fara centrifugal analyser the FRAP assay can be performed on 10µl of sample and results are available within 5 minutes of sample + reagent mixing. In run and between run CV are <1% and <3% respectively, the test is linear to at least 2000 µmol/l, relative to Fe^{2+}, and no sample pre-treatment is required. Ascorbic acid, α-tocopherol and uric acid each display stoichiometric factors of 2.0, bilirubin 4.0 and albumin 0.1. Glucose does not react, and there was no interaction between the various antioxidants tested. Stoichiometric factors are constant in the FRAP assay test system, and excellent agreement was obtained between measured and calculated (from known molar concentration and measured stoichiometric factors) FRAP values.

Fresh plasma from healthy adults gave FRAP values of 612-1634 values µmol/l (mean 1017, median 1000, *n*, 141). There was a significant correlation between FRAP and uric acid (*r*, 0.914, $P<0.001$) and between FRAP and ascorbic acid (*r*, 0.233, $P<0.05$).

The FRAP assay offers a simple, speedy and straightforward test of 'antioxidant power' and should be valuable in nutritional antioxidant studies of biological fluids and of food and drug extracts.

High-performance liquid chromatographic (HPLC) determination of aldehydes in low-density lipoprotein. By ANGELA L. BAILEY, G. WORTLEY and SUSAN SOUTHON, *Institute of Food Research, Norwich Research Park, Colney Lane, Norwich NR4 7UA*

Low density lipoprotein (LDL) lipid peroxidation and aldehyde formation are believed to be major contributors to atherosclerosis (Esterbauer,1993). An improved HPLC method for the quantification of n-alkanals, hydroxyalkenals and furfural, in small samples (100 µl) of plasma LDL has been developed by modification of the method for determination of plasma aldehydes by Holley *et al*. (1993).

Plasma LDL was isolated by density gradient ultracentrifugation and aldehydes derivatized with 1,3-cyclohexanedione (CHD), separated by reversed-phase HPLC with gradient elution, detected fluorometrically and expressed as concentrations per mg of LDL protein.The aldehydes measured are listed in the Table. The sensitivity was increased, and the sample preparation miniaturized, to reduce sample volumes required. Analysis time was reduced by removal of a solid phase extraction step and the poor recoveries experienced by Holley *et al.* (1993). greatly improved by use of a standard addition technique to quantify aldehyde concentrations. Use of solvents during sample preparation was minimized to avoid high concentrations of aldehydes in reagent blanks, due to contamination of solvents with aldehydes. The range of aldehydes measured has been extended to include furfural and formaldehyde. It is demonstrated that this method has capacity to determine concentrations of the biologically important, trans-2-alkenals (Esterbauer *et al.* 1990) whose CHD derivatives are clearly resolved from the n-alkanals and hydroxyalkenals.

	Mean aldehyde concentration (per mg protein)	%CV (*n* 11)	Recovery of added aldehyde %	(range)	*n*	Minimum detection level (dl)
HNE (hydroxynonenal, µmol)	<dl	-	116.0	(104.4-140.0)	8	8 nM
HHE (hydroxyhexenal, µmol)	4.7	30.6%	119.6	(104.6-138.4)	8	5 nM
HPE (hydroxypentenal, µmol)	12.6	46.9%	103.01	(90.1-117.9)	8	13 nM
C1 (formaldehyde, mmol)	2.6	25.6%	94.7	(69.4-123.1)	6	0.1 µM
C2 (acetaldehyde, µmol)	341	4.2%	123.8	(111.8-140.5)	8	4 nM
C3 (n-propanal, µmol)	<dl	-	109.8	(98.2-127.6)	8	5 nM
C4 (n-butanal, µmol)	4.1	25.8%	102.1	(93.3-114.5)	8	5 nM
C5 (n-pentanal, µmol)	1.4	64.9%	104.0	(96.1-121.1)	8	4 nM
C6 (n-hexanal, µmol)	1.0	113.4%	102.1	(91.2-122.1)	8	4 nM
C7 (n-heptanal, µmol)	<dl	-	98.7	(78.9-121.0)	8	12 nM
C8 (n-octanal, µmol)	<dl	-	99.6	(81.1-135.3)	8	26 nM
C9 (n-nonanal, µmol)	<dl	-	90.5	(76.3-121.6)	8	80 nM
C10 (n-decanal, µmol)	<dl	-	110.7	(80.9-127.7)	8	250 nM
2-furfural, (mmol)	368.5	110%	108.5	(101.6 -119.7)	6	0.5 µM

<dl = below detection limit

The table shows aldehyde concentrations found in a small sample (100 µl) of typical human LDL, intra-assay coefficients of variation (% CV), recoveries of aldehydes added to LDL (mean and range), and minimum detection levels calculated as peaks detectable above three times baseline noise.

The method has potential for investigation of the degree of LDL lipid peroxidation in fresh human blood plasma and hence the protection afforded against oxidation by antioxidant nutrients. Measurement of many individual aldehydes offers the advantage of providing a much more detailed picture than the non-specific determination of thiobarbituric acid reactive substances (TBARS) or measurement of malondialdehyde or HNE alone. The method has capacity for application to the measurement of a greater range of aldehydes in LDL and other biological samples.

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The effect of cholesterol oxidation products on the cholesterol content of intracellular membranes of primary cultures and finite cell lines. By A. M. WILSON, R. M. SISK and N. M. O'BRIEN, *Department of Nutrition, University College, Cork, Republic of Ireland*

Cholesterol (cholest-5-en-3β-ol) is an unsaturated lipid which is ubiquitous in mammalian tissues. The unsaturated nature of the cholesterol molecule readily allows it to undergo autoxidation by diverse oxygen species, yielding cholesterol oxidation products (COP). COP have been reported as having a wide variety of biological activities including cytotoxicity. The mechanisms of cytotoxic effects of COP may be due to their effects on cell membranes (Peng *et al.* 1985). COP can alter membrane function, fluidity, permeability and stability (Smith & Johnson, 1989).

The aim of the present study was to determine the cholesterol content of intracellular membranes within cells preincubated with various COP. Two different cell types were investigated: a primary cell culture, chicken embryo fibroblasts (CEF) and a finite cell-line, Chinese hamster ovary (CHO) cells. The cells were incubated for a 24h period in the presence or absence of 20 μM-cholesterol, cholestan,3β,5α,6β,triol, 7-ketocholesterol, 25-hydroxycholesterol, α-epoxide or β-epoxide. Four enriched fractions were isolated from the cells by differential centrifugation. The three enriched pellets, nuclear (P1), mitochondrial (P2) and microsomal (P3), were obtained by centrifugation at 600 ***g***, 15,000 ***g*** and 100, 000 ***g*** respectively. The cytosolic fraction present in the supernatant fraction (S3) was also retained for analysis following centrifugation at 100, 000 ***g***. Capillary GC was used to detect the amount of cholesterol and COP in each fraction.

	Cholesterol content (μg/mg protein) of intracellular fraction of CEF cells							
	P1		P2		P3		S3	
Incubation	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control †	1.114	0.515	5.025	0.389	0.714	0.108	3.533	0.009
Cholesterol	0.895	0.049	17.095*	5.180	1.929*	0.054	11.765*	3.373
Cholestan-triol	0.430*	0.128	0.580*	0.104	0.122*	0.007	1.799*	0.239
7-ketocholesterol	0.877	0.344	0.720*	0.133	0.188*	0.019	3.195	0.525
25-hydroxy ‡	0.188*	0.096	0.266*	0.035	0.226*	0.032	1.442*	0.189
α-epoxide	0.162*	0.021	0.674*	0.036	0.147*	0.055	1.920	0.033
β-epoxide	1.017	0.273	3.158	0.652	0.394*	0.040	1.386*	0.388
LSD (P<0.05)	0.400		2.744		0.240		1.723	

*P<0.05 significantly different from control.
†Control cells cultured with growth medium alone; ‡25-hydroxy - 25-hydroxycholesterol.
ANOVA one-way followed by least significant difference (LSD), n 4 for all treatments.

Following incubation with the various COP, each COP was subsequently identified in the different intracellular locations of both cell types; these levels of COP were significantly higher than in the control or cholesterol supplemented cells (results not shown). The addition of COP to the growth media of the CEF cells led, in nearly all cases, to a reduced cholesterol content of each of the enriched fractions (Table). However, when the CEF cells were cultured with 20 μM-cholesterol-supplemented growth media, an increase in the cholesterol content of the P2, P3 and S3 fractions was observed relative to that of the control. Similarly with the CHO cells, the addition of COP resulted in a reduction of the cholesterol content of the enriched fractions (results not shown). The depletion of membrane cholesterol could be due to the potent inhibitory effects of COP on hydroxymethylglutaryl Co A reductase (*EC* 1.1.1.88). Alternatively, the direct insertion of COP into the cellular membranes may affect the cholesterol content.

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A comparison of the cytotoxicities and the extent of lipid peroxidation of cholesterol oxidation products in *in vitro* model systems. By R.M. SISK, A.M. WILSON, S.A. AHERNE and N.M. O'BRIEN, *Department of Nutrition, University College, Cork, Republic of Ireland*

Cholesterol is susceptible to autoxidation by diverse oxygen species to yield cholesterol oxidation products (COP). The production of COP is dependent on conditions such as temperature, time, pH, and form of the substrate (Kim & Nawar, 1993). COP have been reported to have a wide variety of biological activities including altering membrane function, DNA synthesis, cell growth, proliferation and cytotoxicity (Peng *et al.* 1992).

The aim of the present study was to investigate the extent of COP-induced toxicity and lipid peroxidation in finite and primary cultures. The finite cell-line, Chinese hamster ovary (CHO) cells and primary culture, chicken embryo fibroblasts (CEF) were selected for investigation. The CHO and CEF were cultured in the presence or absence of cholesterol, 25-hydroxycholesterol, 7-ketocholesterol or cholestan,3β,5α,6β-triol. Cytotoxicity was measured by two separate assays namely the neutral-red uptake assay (Hunter *et al.* 1987) and lactate dehydrogenase (LDH) release assay (Vassault, 1983). The extent of lipid peroxidation, as indicated by thiobarbituric acid reactive substances (TBARS) was also measured.

	% CELL VIABILITY					
	CEF cells			CHO cells		
	100%	75%	50%	100%	75%	50%
Cholestan,3β,5α,6β,triol	5 μM	10 μM	15 μM	10 μM	15 μM	20 μM
7-Ketocholesterol	5 μM	15 μM	30 μM	5 μM	15 μM	25 μM
25-Hydroxycholesterol	5 μM	15 μM	40 μM	10 μM	25 μM	50 μM

The neutral red uptake assay was used to measure the cytotoxicity of cholesterol or COP. Cholesterol was found to be non-toxic in either of the cell types (results not shown). The order of cytotoxicity of COP in both cell types was cholestan,3β,5α,6β-triol being the most cytotoxic followed by 7-ketocholesterol and then 25-hydroxycholesterol. Similar findings were observed with LDH release assay (results not shown). As the concentration of COP in the incubation media increased so the extent of lipid peroxidation also increased in both cell types. However, at the concentrations of COP which induced 50% cytotoxicity, the CEF had a significantly higher extent of lipid peroxidation ($P<0.01$) with an average value of 3.59 (SEM 0.280) nmol MDA/mg protein compared to 2.607 (SEM 0.145) nmol MDA/mg protein in CHO cells These findings demonstrate that COP are cytotoxic and induce lipid peroxidation in both primary cultures and finite cells. In addition, primary cultures are more susceptible to COP-induced toxicity than finite cells.

This work was supported by the Department of Agriculture, Food and Forestry, Dublin.

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Lipid peroxidation and caeruloplasmin activity in cord blood. By M.E. KIELY and P.A. MORRISSEY, *Department of Nutrition, University College, Cork, Ireland*

Human plasma contains molecules which reduce ferric ions to the more reactive ferrous state, leading to the formation of toxic oxygen radicals. Caeruloplasmin protects the extracellular environment by catalytically oxidizing ferrous ions. Transferrin binds ferric ions, thus ensuring that plasma is well protected from Fe-stimulated free-radical reactions (Gutteridge, 1991). Concentrations of caeruloplasmin are low in neonates in comparison with adults (Lindeman *et al.* 1992), suggesting that newborn infants may have a greater susceptibility to lipid peroxidation.

Caeruloplasmin activity was measured in forty-eight maternal and umbilical cord plasma samples by the method of Schosinsky *et al.* (1974). In a subset of thirteen, lipid peroxidation of plasma was stimulated using Cu ions. The thiobarbituric acid-malondialdehyde (TBA-MDA) complex was quantified on the basis of its first derivative absorption spectrum, where zero-crossing at 532 nm indicates its absorption maximum, as described in Espinosa-Mansilla *et al.* (1993). Peak-trough distance (D) was calculated and the results for each sample were expressed as area under the curve (AUC), with the x and y axes represented by incubation time in minutes and D respectively.

The caeruloplasmin activity of cord plasma (0.052 (SD 0.04) U/ml) was found to be substantially lower than that of mothers (0.377 (SD 0.17) U/ml). There was a significant correlation (r 0.39; P <0.01) between maternal and cord values. In an earlier study, we observed that the ability of cord plasma to prevent autoxidation in brain homogenate was weaker than that of maternal plasma. The caeruloplasmin results presented here substantiate that finding, and are in accordance with other published data.

Lipid peroxidation, assessed by the formation of the MDA-TBA complex, was found to be greater in maternal (284.82 (SD 35.16)) than in cord (35.24 (SD 54.52)) samples, which was an unexpected finding. In the case of the cord samples, caeruloplasmin activity was found to increase with increasing lipid peroxidation (r 0.7; P <0.02). One infant, in particular, was observed to have a caeruloplasmin activity of 0.112 U/ml and a TBA-MDA level of 205.16.

These results show that maternal plasma is considerably more susceptible to Cu-induced lipid peroxidation than cord plasma. However, this finding is not in agreement with several other researchers who maintain that antioxidant potential at birth is compromised. On the other hand, authors who have compared the free-radical trapping ability (TRAP) of cord plasma with maternal plasma have reported that both measured and calculated TRAP is higher in neonates than adults (van Zoeren-Grobben *et al.* 1994). The increased oxidative stability of cord plasma observed in the present study may be due to higher levels of certain plasma antioxidants, and/or more efficient interactions between plasma components with antioxidant activity.

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New vitamin D values for meat and their implication for vitamin D intake in British adults. By SIGRID A.GIBSON [1] and MARGARET ASHWELL [2], *[1]21 Orchard Rd, Burpham, Guildford GU4 7JH, and [2]Ashwell Associates, Ashwell St, Ashwell SG7 5PZ*

New analytical data for the composition of meat (Chan *et al.* 1995) now include the contribution from the metabolite 25-hydroxycholecalciferol ($25(OH)D_3$), not previously analysed. This metabolite is assumed to have a biological activity five times that of cholecalciferol (Chan *et al.* 1995) and is present in significant quantities in meat and liver. The contribution of meat to vitamin D intakes may therefore be much greater than the value (4%) calculated in *The Dietary and Nutritional Survey of British Adults* (Gregory *et al.* 1990). Preliminary calculations using new analytical data for beef, veal, pork and lamb suggested that vitamin D intake might be 20%, or more, above that currently estimated (Lee *et al.* 1995). We have used representative values from Chan *et al.* (1995), together with data from Gregory *et al.* (1990) on individuals' consumption of the eleven food codes containing meat, to recalculate vitamin D intakes and the contribution from meat and meat products (the meat group). Since values for $25(OH)D_3$ in meat products and liver were not available, vitamin D in liver was taken as 8 μg/kg and values for meat products were calculated from meat content, plus other ingredients. These calculations generated a new estimate of vitamin D intake, 3.58 (SE 0.05) μg/d, an increase of 21% on the previous value of 2.96 μg/d. On average, the meat group contributed 0.74 μg/d, i.e. approximately 21% of the total intake from food sources. In fact, the meat group is now the richest natural source of vitamin D, since the fat spreads group (providing 25% of intake) contains fortified products.

	Men (*n* 1087)					Women (*n* 1110)				
	Old values		New values			Old values		New values		
	mean	SE	mean	SE	new : old	mean	SE	mean	SE	new : old
Vitamin D from the Meat Group (μg/d)	0.13	0.01	0.90	0.01	6.9	0.10	0.01	0.58	0.01	5.8
Total vitamin D from food (μg/d)	3.43	0.08	4.20	0.08	1.22	2.51	0.05	2.99	0.05	1.19
Low meat intake (<25 pctile)	3.67	0.17	4.03	0.17	1.10	2.75	0.11	2.94	0.11	1.07
High meat intake (>75 pctile)	3.29	0.14	4.54	0.14	1.38	2.31	0.09	3.10	0.09	1.34

Vitamin D intakes were significantly higher in high consumers of the meat group (*n* 272 men and 277 women above the 75th percentile), than in low meat group consumers (*n* 270 men and 277 women below the 25th percentile). This is a reversal of the trend that would be shown using the old values. The effect was significant for men and women (Mann-Whitney U-test: P=0.0002 for men, P=0.0142 for women). The conclusion that meat makes a significant contribution to vitamin D intakes has most relevance to those groups (e.g. Asian women and the elderly) for whom vitamin D synthesis from exposure to sunlight is low, and for whom dietary sources are therefore particularly important.

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Food frequency questionnaire (FFQ) interpretation and analysis: issues from the UK Women's Cohort Study. By CLAIRE CALVERT[1], JANET CADE[1], AMANDA WOODHOUSE[1], JENNIFER BARRETT[2] and UKWCS STEERING GROUP*, *[1]Division of Public Health Medicine, Nuffield Institute for Health, 71-75 Clarendon Road, University of Leeds, Leeds, LS2 9PL; [2]Centre for Cancer Research, University of Leeds,Cookridge Hospital, LS16 6QB.*

The UK Women's Cohort Study is a national study of approximately 26 000 women aged 35-69 years. The study will investigate the relationship between diet,lifestyle and disease incidence over the next 10 years. Baseline data are being collected by post over a 12 month period and 5 months after the start of the study, 6254 questionnaires have been analysed. Dietary assessment is made from a detailed FFQ which includes questions on cooking and eating practices which act as a cross-check to the food frequency data.

The effect on nutrient intake of different methods of FFQ analysis was explored. This is presented for fruit only, which represents approximately 10% of the foods listed on the FFQ. Methods of analysis included; (1) comparing different portion weights (using an average of portion weights derived from Crawley (1988), women's portion sizes MAFF (1994) and pilot data from 7 day weighed records on vegetarian women *v* Crawley(1988)). (2) nutrient composition data for a mix of fresh,stewed,canned and frozen fruit *v* fresh fruit only (Paul & Southgate 1991). (3) adjusting frequency of fruit consumption by a weighting factor calculated as follows:

$$\frac{\text{Number of servings of fruit per week from the cross-check question}\ ✍}{\text{Number of servings of fruit per week from all fruits on the FFQ}}$$

This weighting was applied to each fruit item. The results for selected nutrients are summarized below.

Analysis method		Energy (kj) Mean	Energy (kj) Rank correlation	Fat (g) Mean	Fat (g) Rank correlation	Cho(g) Mean	Cho(g) Rank correlation	NSP (g) Mean	NSP (g) Rank correlation	Vitamin C Mean	Vitamin C Rank correlation
'Standard		9902	1.00	85	1.00	318	1.00	26	1.00	190	1.00
New portion	(1)	9911	0.99	85	1.00	320	0.99	26	0.99	194	0.99
New foods	(2)	9843	0.99	85	1.00	315	0.99	27	0.99	198	0.99
Adjusted	(3)	9380	0.98	85	0.99	289	0.96	23	0.95	147	0.91

Using these different methods of analysis for a single food group such as fruit has little effect on the ranking of subjects according to nutrient intake. Overall mean nutrient intakes are not greatly changed by varying fruit portion sizes or food types. Many women (63%) reported eating more than twice as many fruits on the frequency data as they recorded on the cross-check question. These results suggest that cross-check questions can be used to reduce the effect of over-reporting on the FFQ.

Using the standard analysis method, 15% of women had energy intakes less than 1.2 x basal metabolic rate (BMR). In terms of number of missing frequency responses, only 2% of the sample left more than 10% of food items unanswered. There was no evident relationship between nutrient intakes and number of missing frequencies.

During the 10 years of the study a sub-sample of the cohort will be followed up with a food diary and biomarkers. These results can then be compared with the FFQ analyses to see which method appears to be most reliable.

*Rhys Williams, University of Leeds; Barrie Margetts, University of Southampton; Margaret Thorogood, London School of Hygiene and Tropical Medicine.

✍Cross check question "How many servings of fruit or fruit-containing dishes do you usually eat each week?

Crawley, H. (1988) *Food Portion Sizes*. London:H. M. Stationery Office.
MAFF(1994). *The Dietary and Nutritional Survey of British Adults-Further Analysis*. London: H.M. Stationery Office.
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This work is funded by the World Cancer Research Fund.

Assessment of dietary nutrient consumption in alcoholic patients using a food assessment questionnaire. By, CLAIRE FORD[1] CHRISTOPHER J. SEAL[1], HELEN MILBURN[2], CHRISTOPHER P. DAY[2] MICHAEL NELSON[3] and JOHN C. MATHERS[1], *[1]Human Nutrition Research Centre, Department of Biological and Nutritional Sciences and [2]Department of Medicine, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU and [3]Department of Human Nutrition and Dietetics, Kings College London, Campden Hill Road, London W8 7AH*

Dietary assessment in alcoholics is difficult, especially in individuals still consuming alcohol. As part of a project to evaluate antioxidant status during the progression of liver disease from fatty liver to cirrhosis in alcoholic patients, we have attempted to use a food-frequency questionnaire to assess nutrient intake. Male alcoholic patients with cirrhosis of the liver (*n* 9, C group) and age-matched patients with fatty liver disease without cirrhosis (*n* 6, FL group) were recruited during routine visits to the outpatient clinic. Normal control subjects (*n* 9, N group) were similarly matched to the C group; the overall average age of the subjects was 49.2 (SE 1.68) years. Where possible, alcoholics were also matched for reported alcohol intake. Dietary assessment aimed to quantify habitual diet during the preceding 12 months and was by a food assessment questionnaire which combines food frequency questions with portion size food photographs currently under development by the Kings College London Group. Nutrient intakes were calculated from these data using an integrated dietary analysis programme. Results were analysed by ANOVA.

					Overall	*P* for specific contrasts	
	Normal	C group	FL group	Pooled SE	*P*	N *v.* (C+FL)	C *v.* FL
BMI (kg/m^2)	24.8	27.5	30.0	0.86	0.510	0.024	0.220
Calculated BMR (MJ/d)	7.4	7.6	7.9	0.14	0.414	0.220	0.523
Reported energy intake (MJ/d)	13.3	18.6	21.5	1.45	0.067	0.025	0.407
Physical activity ratio	1.80	2.47	2.74	0.193	0.126	0.047	0.566
% Daily energy from:							
Protein	13.3	14.3	15.1	0.56	0.452	0.232	0.609
Carbohydrate	44.5	34.1	33.2	1.58	0.001	0.000	0.765
Fat	37.1	35.3	40.7	1.09	0.151	0.667	0.056
Alcohol	3.0	9.6	6.4	1.07	0.023	0.019	0.211

On average, alcoholic patients had higher BMI values than the control group (P=0.024). Reported energy intake was significantly higher for C and FL groups (P=0.025) and was overestimated by all groups when compared with calculated energy requirements. There was considerable variability in reported intake especially in alcoholic subjects (range: 1.35-2.23 times BMR for control, 1.0-4.3 for C group and 1.9-5.1 for FL group). However, percentages of energy intake derived from macronutrients were much less variable and, with the exception of alcohol this variability was similar across all groups. The results suggest that the overestimation of intake is due to an exaggeration of the whole diet, not of specific dietary components and that this is greater in alcoholic subjects. This food assessment questionnaire may be useful in determining the overall pattern of macronutrient supply in the diet but not absolute nutrient intakes in this group of subjects.

Patterns of reported energy intakes and weight loss between repeated measurement periods during a slimming study in overweight subjects with ischaemic heart disease. By C.R. HANKEY and M.E.J. LEAN, *University of Glasgow Department of Human Nutrition, Glasgow Royal Infirmary, Glasgow G31 2ER*

Measurements of dietary intake are used in dietary intervention studies both to examine the effect of the specific intervention on reported dietary intake, and also to encourage motivation and compliance with a diet prescription (Bingham, 1987). Overweight, elderly volunteers with angina pectoris were recruited for this 12 week study. A weight-reducing dietary prescription was given, based on measured resting metabolic rate, with an activity factor of 1.3, and a daily energy deficit of 2510 kJ. A baseline record of food intake (7 d weighed diary) was made before dietary intervention to assess usual intake, and three other 7 d recordings were made at weeks 2, 7 and 11. Fifty four subjects were recruited and forty-nine, (twenty-seven males, twenty-two females) mean BMI 29.3 (SD 4.1) kg/m^2 completed the study. Baseline dietary intakes were compared in turn with each of the three reported food intakes for the principal macro-nutrients and micro-nutrients. The dietary recordings made after the intervention period all showed reductions in reported dietary intake in comparison with the baseline period. Significant reductions in body weight were seen during the intervention period, although the rate of weight loss decreased as the study proceeded. The reduction in rate of weight loss was accompanied by increases in reported dietary intakes (Fig. 1 and 2).

Reported energy intake (EI) was within 20% of the estimated average requirement (DOH, 1991) in a high proportion of subjects with angina. Reported EI rose in weeks 7 and 11 as weight loss plateaued, and angina frequency fell with weight loss over the 12 weeks from 3.2 (SD 4.5) to 1.4 (SD 2.5) incidents/week (P=0.009).

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Fig. 1 REI and body weight changes for females

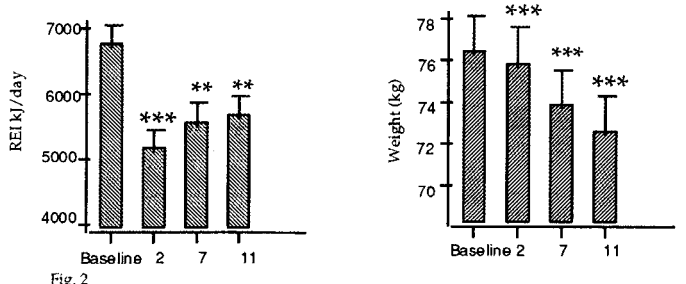
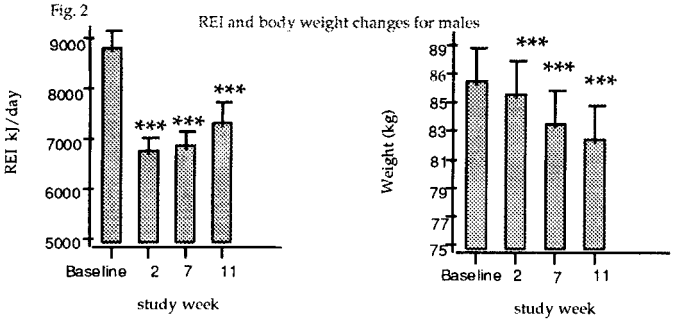


Fig. 2 REI and body weight changes for males



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Validation of a 7 d food and drink diary: a tool for the assessment of nutrient intake.

By MOIRA A. GEEKIE, MONIQUE M. RAATS, PAUL SPARKS and RICHARD SHEPHERD, *Consumer Sciences Department, Institute of Food Research, Reading Laboratory, Reading RG6 6BZ*

It is suggested that nutrient values assessed from an open-ended food diary are closely associated with those values obtained from a weighed food record (Bingham *et al.* 1994). In the interest of investigating the promotion of dietary change, a 7 d food and drink diary has been developed by means of which free-living individuals estimate and record their habitual food intake. In the present study the 7 d estimated food and drink diary has been validated by comparison with a 7 d weighed food inventory.

The diary, in A4 format, includes instructions for completion and contains two fold-out flaps listing eighty commonly eaten foods in 'medium portions', described in household measures. There are five pages of colour photographs, showing 'medium portions' of fifty-three of the listed foods.

Subjects record all food and drink they consume, describing their food either as portions, relative to the 'medium portions' in the photographs and lists provided, or in household measures, or from weights taken from food packaging.

Sixty-three subjects from the academic and administrative staff at the University of Reading (F 46, M 17, mean age 46 years) completed the 7 d estimated diary and a 7 d weighed food inventory, with an interval of no less than 6 weeks between the two.

	Estimated diary		Weighed diary			
	Mean	SEM	Mean	SEM	*t* value	Spearman corr. coeff between the two methods
Total energy (MJ)	8.6	0.3	8.6	0.3	-0.39	0.76***
Total protein (g)	75.0	2.2	74.5	2.2	0.29	0.71***
Total fat (g)	80.6	3.8	82.2	3.3	-0.53	0.64***
Saturated fat (g)	22.5	1.1	23.9	1.1	-1.64	0.71***
Total carbohydrate (g)	272.5	10.2	274.9	10.7	-0.33	0.79***
Starch (g)	142.9	6.6	142.6	5.8	0.08	0.78***
Sugars (g)	126.2	5.7	129.8	6.3	-0.97	0.78***
Alcohol (g)	11.2	1.6	10.1	1.3	0.99	0.77***
Fibre (g)	18.8	0.8	18.2	0.7	0.95	0.63***
Energy from protein (%)	15.3	0.4	15.0	0.4	1.09	0.75***
Energy from fat (%)	34.5	0.8	35.1	0.7	-1.05	0.64***
Energy from saturated fat (%)	9.7	0.3	10.3	0.3	-2.20*	0.63***
Energy from carbohydrate (%)	50.3	0.7	49.9	0.7	0.65	0.67***
Energy from starch (%)	26.0	0.6	25.9	0.5	0.32	0.79***
Energy from sugar (%)	23.6	0.8	23.6	0.7	0.13	0.74***
Vitamin A (μg)	969.0	110.2	965.1	89.0	0.03	0.18
Vitamin C (mg)	113.9	6.3	109.7	6.3	0.73	0.48***
Calcium (mg)	911.1	36.3	999.3	35.6	-3.25**	0.71***
Iron (mg)	15.6	0.9	15.9	0.9	-0.05	0.64***

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

For the estimated diary, the mean ratio of reported energy intake (EI) to estimated basal metabolic rate (BMR est.) was 1.44: for the weighed diary, mean EI:BMR est. was 1.46.

The Table shows that for all dietary components except Ca and the percentage energy from saturated fat, there was no significant difference in intake assessed by the two diaries. For all nutrients except vitamin A, correlations between the two methods were highly significant.

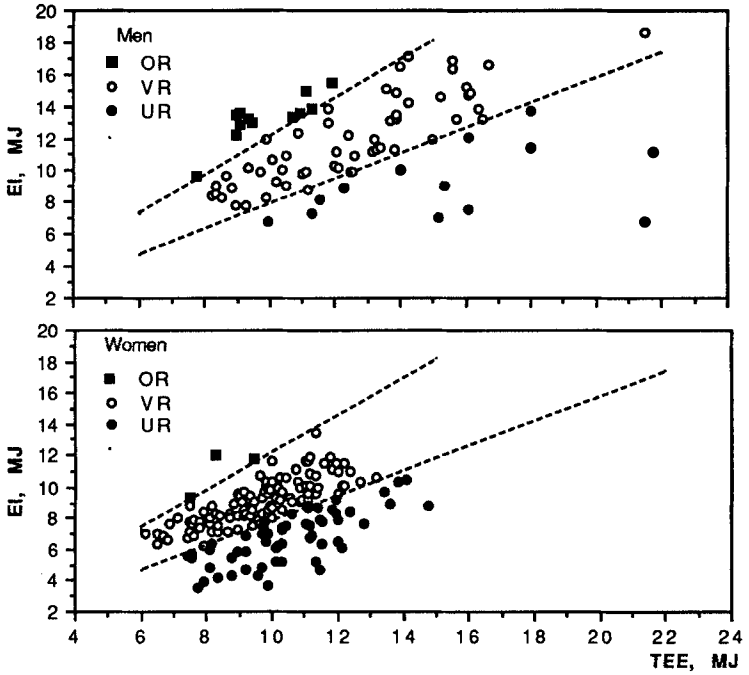
The estimated diary is commended as a suitable tool for the assessment of usual food intake in prospective studies. It is simple to administer, and diaries can be sent by post involving minimal personal contact. As food is not weighed, there is less intrusion on the subject's lifestyle, thus a greater chance of the record reflecting normal eating patterns and dietary composition.

This work is being funded by the Ministry of Agriculture, Fisheries and Foods in the UK.

Bingham, S.A., Gill, C., Welch, A., Day, K., Cassidy, A., Khaw, K.T., Sneyd, M.J., Key, T.J.A., Roe, L.& Day, N.E. (1994). *British Journal of Nutrition* **72**, 619-643.

Under-reporting of energy intake at all levels of energy expenditure: evidence from doubly labelled water studies. By ALISON E. BLACK, *MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH*

Under-reporting of food intake is a problem of dietary surveys (Black *et al.*. 1991), but the pattern of reporting across subjects is not known. Data from doubly-labelled water (DLW) studies can be used to explore this. Seventeen studies of 248 (81M, 167F) free-living subjects aged 18-80 years with measurements of energy expenditure (EE) by DLW, BMR and energy intake (EI) were combined. They were a subset of 574 values from a meta-analysis of EE in free-living subjects (Black *et al.* 1996). Under- (UR), valid- (VR), and over- (OR) reporters were defined as EI:EE <0.79, 0.79-1.21-and >1.21 respectively. The Fig. shows EI *v* EE for UR, VR, and OR (separated by lines) by sex.



For men and women respectively, mean EI:EE values were 0.96 (SD 0.23) and 0.85 (SD 0.19); and the proportions of VR, UR and OR were 69%, 16%, 15% and 63%, 35%, 2%. In men UR were balanced by OR and mean EI was unbiased. In women there was bias to UR.

Subjects were predominantly volunteers and from white collar occupations. The results may not be applicable to other studies. However, they demonstrate under-reporting at all levels of energy intake and expenditure. Studies that have examined the characterisitics of under-reporters as identified by comparison with a low physical activity of 1.55 x BMR (Price *et al.*, 1993; Pryer *et al.* 1994; Rutishauser *et al.* 1994) have been able to study only part of the pattern. There is a need to identify the characteristics of under-reporters across all levels of energy expenditure.

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Black, A. E., Coward, W. A., Cole, T. J. & Prentice, A. M. (1996). *European journal of Clinical Nutrition* **50**, 72-92.

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Pryer, J., Vrijheid, M., Nichols, R. & Ellitt, P. (1994). *Proceedings of the Nutrition Society* **53**, 235A.

Rutishauser, I. H. E., Wheeler, C. E., Conn, J. A. & O'Dea, K. (1994). *Australian Journal of Nutrition and Dietetics* **51**, 157-166.

Energy intake:basal metabolic rate (EI:BMR) for the evaluation of reported energy intake of individuals. By ALISON E. BLACK, GUNNAR JOHANSSON, AILSA WELCH and SHEILA A. BINGHAM. *MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH.*

For any diet study, mean reported energy intake (EI) may be expressed as a multiple of the mean BMR and the EI:BMR ratio can be compared with physical activity levels (PAL), where energy expenditure (EE) is expressed as a multiple of BMR, to detect the presence of under-reporting. Goldberg *et al.* (1991) have derived equations for calculating the value of EI:BMR below which it is unlikely that it represents either habitual intake or chance low intake. This formula calculates the lower 95% confidence limit of the agreement between mean EI:BMR and mean PAL taking into account daily variation in energy intake, precision of estimating or measuring BMR, variation in energy requirements expressed as PAL, number of days of dietary assessment and number of subjects studied. If this value is less than reported mean EI:BMR, then the study is biased to under-reporting. The equation will identify under-reporters (UR) at the individual level if the number of subjects is taken as $n = 1$. The present paper compares UR identified by the Goldberg equation for $n = 1$ with UR identified by direct comparison of EI and EE measured by doubly labelled water (DLW) to determine the variables that should be substituted in the equation for this purpose.

The subjects were eighteen women aged 50-65years and twenty-six men aged 45-87 years with 16 d of wieghed diet records over 1 year, and eleven post-obese (11F, 1M) subjects with 21 d diet records (Black *et al.* 1995). UR were defined as EI:EE <0.79, being the lower 95% confidence limit of agreement between EI and EE; the Table shows 7 women, 8 men and 6 post-obese to be UR by this definition. The Table also shows UR identified by the Goldberg *et al.* (1991) equation using as the energy requirement for comparison: first, a low PAL of 1.55; second, the mean PAL measured in each study (women 1.66, men 1.86, post-obese 1.59), and third, each individuals' own measured PAL. The calculations were done using both subjects' estimated (BMRe) from equations and measured BMRm by calorimtery.

If a low PAL of 1.55 was used for comparison, only 2/7 women and 3/8 men were identified as UR; using a measured rather than estimated BMR removed 1 man. Using the higher study specific PALs increased the number identified to 3/7 women and 4/8 men, but using estimated BMR also included 1 woman and 2 men not defined as UR by EI:EE. Using a subject specific PAL with the measured BMR, effectively a comparison of individual EI and EE with increased errors due to the inclusion of BMR, identified the 6/7 women and 5/8 men who under-reported to the greatest extent. Using an estimated BMR included 2 extra men not defined as UR by EI:EE. In the post-obese, the same 6 subjects were identified by all variations of the calculation. It can be noted however that the mean energy expenditure was only 1.59 x BMR and all these subjects under-reported by more than 30%; they were thus readily identified.

			Number of "under-reporters" identified by the Goldberg equation according to various criteria					
			PAL 1.55 x BMR		Study specific PAL		Subject specific PAL	
Study	Total *n*	EI:EE <0.79	EI:BMRe	EI:BMRm	EI:BMRe	EI:BMRm	EI:BMRe	EI:BMRm
Present study, women,	18	7	2	2	3+1	3	4	6
Present study, men	27	8	3	2	5+2	4	5+2	5
Black *et al.* 1995, post-obese	11	6	6	6	6	6	6	6

Use of the Goldberg equation assuming a mean energy expenditure for an inactive population can identify only a proportion of UR. Adequate identification of UR across the full range of energy expenditures requires some knowledge of individuals' physical activity. It is therefore desirable for dietary studies to include estimates of activity and information about occupation as routine, and for small scale studies also to include measurements of BMR.

Black, A. E., Jebb, S. A., Bingham, S. A., Runswick, S. & Poppitt, S. (1995). *Journal of Human Nutrition and Dietetics* **8**, 51-64.

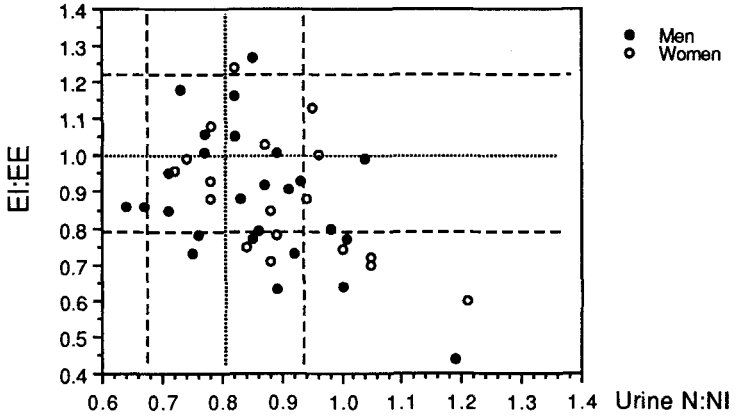
Goldberg, G. R., Black, A. E., Jebb, S. A., Cole, T. J., Murgatroyd, P. R., Coward, W. A. & Prentice, A. M. (1991). *European Journal of Clinical Nutrition* **45**, 569-581.

Double validation of dietary intakes against 24 h urinary nitrogen and energy expenditure by doubly-labelled water in men and women. By ALISON E. BLACK, AILSA WELCH, GUNNAR JOHANSSON and SHEILA A. BINGHAM, *MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH.*

External biomarkers for the validation of dietary studies have significantly enhanced understanding of the limitations of dietary intake data. Two techniques have been particularly used, the comparison of N intake (NI) with 24 h urinary N excretion (UrN) and of energy intake (EI) with energy expenditure (EE) measured by doubly labelled water (DLW), on the assumption that all measure habitual intake, excretion or expenditure and can therefore be compared. In separate studies, both techniques have identified individual subjects who have under-reported dietary intake (UR) (Black *et al.*, 1993; Bingham *et al.*, 1995). The present report combines data from two studies that have used both techniques and examines the agreement between them.

Eighteen women aged 50-65 years and 27 men aged 45-87 years kept 16 d weighed diet records for estimation of EI and NI and eight 24 h urine collections for measurement of UrN spread over 1 year.. EE was measured by DLW after the end of the main study: mean time elapsed for women 0.49 (SD 2.46) weeks, for men 19 (SD 13) weeks.

For all subjects, the mean ratio UrN:NI was 0.87 (SD 0.13) compared with an expected mean ratio of 0.81 (Bingham & Cummings, 1985), indicating a bias to under-reporting of about 7% for N. The mean ratio EI:EE was 0.89 (SD 0.18) compared with an expected ratio of 1.00, indicating a bias to under-reporting of about 11% for energy. The difference in under-reporting was significant ($p<0.01$).



The Fig. shows the relationship between UrN:NI and EI:EE. The dotted lines show the 95% confidence limits of each ratio taking into account the limits on the precision of measurement of each component. The combined error on EI:EE was ±21% and on UrN:NI was ±22%. Thus under-reporting by individuals was defined as UrN:NI>0.94 and EI:EE<0.79. Seven subjects were UR by both validations; five by UrN:NI only and eight by EI:EE only. The correlation between the two ratios was -0.45, $p<0.01$.

There was not absolute agreement between the two techniques in identifying UR. Possible explanations include lack of coincidence in time of EI and EE measurements, differential reporting of energy and N and underestimation of the errors of the measurements. The first is not thought to be a problem since EI and EE are not balanced on the short term. There is some evidence in the literature for differential reporting of energy and N.

Bingham, S. A., Cassidy, A., Cole, T. J., Welch, A., Runswick, S., Black, A. E., Thurnham, D., Bates, C. J., Khaw, K. T., Key, T. J. A. & Day, N. E. (1995). Validation of weighed records and other methods of dietary assessment using the 24h urine nitrogen technique and other biological markers. *British Journal of Nutrition* **73**, 531-550.

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The feasibility of measuring energy expenditure by heart rate monitoring in a population-based study of adults. By NICHOLAS J. WAREHAM[1], SUSIE H.J. HENNINGS[1], NICHOLAS E. DAY[1] and ANDREW M. PRENTICE[2], *[1]Department of Community Medicine, University of Cambridge, Cambridge CB2 2SR and [2]MRC Dunn Clinical Nutrition Unit, Cambridge CB2 2DH*

The assessment of habitual energy expenditure by questionnaires is beset by measurement error, and a more precise method for estimating both the pattern and total level of energy expenditure is required for use in population-based studies. Although the heart rate monitoring method has been shown to be valid by comparison with doubly-labelled water (Livingstone *et al.* 1990) and indirect calorimetry (Spurr *et al.* 1988), its feasibility has not previously been tested in a population-based study.

Volunteers (*n* 167) aged 30-40 years were randomly selected from a general practice age-sex register. Subjects attended the hospital in a fasted state. Resting energy expenditure, the slope and intercept of the regression line between energy expenditure and heart rate, and FLEX heart rate were assessed using the method described by Spurr *et al.* (1988). Subjects wore a heart rate monitor (SportsTester, Polar, Kempele, Finland) for four consecutive days; Three subjects were unable to complete the protocol. One had a learning disability and two were physically handicapped. BMR was estimated using standard equations (James & Schofield, 1990) and physical activity levels (PAL) were calculated as total energy expenditure divided by BMR.

Mean PAL in men was 1.89 (SD 0.40) and 1.76 (SD 0.31) in women. There was no relationship between PAL and weight, BMI or percentage body fat as measured by impedance.

			% of time with HR ≤ FLEX Heart Rate		Predicted VO_2max (ml O_2/kg per min)	
	PAL quartile	*n*	Mean	SD	Mean	SD
Men	<1.615	18	55.7	19.1	32.9	5.6
	1.615-1.825	18	43.2	12.0	34.9	8.0
	1.825-2.030	19	25.8	14.8	38.2	6.7
	>2.030	19	15.4	14.7	42.9	7.9
Women	<1.518	22	56.0	14.1	25.1	5.4
	1.518-1.761	22	44.4	14.1	29.7	6.7
	1.761-1.958	23	28.1	13.0	31.6	5.3
	>1.958	23	18.8	13.4	32.3	6.2

The Table shows the strong relationship between PAL and the percentage of time spent at or below FLEX heart rate and also physical fitness as assessed by VO_2max (tests for linear trend $P<0.0001$). The estimates for mean PAL in this population are comparable with those in a recent meta-analysis of results from doubly-labelled water measurements (Black *et al.* 1996). This study has therefore demonstrated the feasibility of using the heart rate monitoring method to assess energy expenditure in an epidemiological setting.

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A new multicompartment body composition model to estimate fat, skeletal muscle and visceral cell mass from measurements of total body water and extracellular fluid. By TOM PRESTON, *Isotope Biochemistry Laboratory, SURRC, East Kilbride G74 0QF*

Two-compartment body composition models that require measurement of total body water (TBW), potassium (TBK) or density to calculate lean body mass (LBM) and fat, assume that LBM has constant composition, which is clearly inaccurate. Moore & Boyden (1965) argued that the concept that LBM is of constant quality "never obtains in disease". More complex multicompartment models are needed for accurate assessment of body composition in studies of growth, nutritional status and disease. These can be established when two or more components are measured together (Anderson, 1963).

In vivo neutron activation analysis (NAA) can be used to determine the elemental status of the body with good accuracy. These data can be interpreted to yield the quantity of the major tissues (Wang *et al.* 1992). Furthermore, compartmental analysis of body N and TBK can be used to derive skeletal muscle and non-muscle mass (Burkinshaw *et al.* 1978). Although it is unlikely that NAA will be available in many centres worldwide, such data can be used to develop new body-composition models and to investigate less sophisticated approaches that may be used routinely. In the current study, a comprehensive NAA data set that is also supported by independent analyses, has been used to develop a new multicompartmental body composition model. Body cell mass (BCM), derived from measurements of TBK or intracellular fluid (ICF = TBW- ECF), is divided into muscle (MCM) and visceral cell mass (VCM), by predicting VCM:

$$\text{weight} = \text{fat} + (\text{MCM} + \text{VCM} + \text{ECF} + \text{ECP} + \text{ECM} + \text{ECB})$$
$$(\text{- - - - - lean body mass - - - - -})$$

Component	Abbreviation	Derivation	Mass (kg)*	Error (kg)
Fat	Fat	= weight - LBM	14.81	0.84‡
Muscle cell mass	MCM	= BCM - VCM	11.47	0.61‡
Visceral cell mass	VCM	= (10.70 x Ht^2) - 14.79; Ht in m	13.76	2.86†
Extracellular fluid	ECF	= ECF; (or, TBW - ICF)	14.19	0.73†
Extracellular protein	ECP	= VCM x 0.296	4.07	0.84‡
Extracellular mineral	ECM	= ECF x 0.009	0.13	0.01‡
Bone mineral	ECB	= (1.252 x Ht^2) -1.088; Ht in m	2.25	0.21†

*Median value of this data set (*n* 60 adults); †SD of the difference (predicted - measured); ‡Estimated error of measured variable (5 %).

Recent interest in bioelectrical impedance analysis (BIA) has highlighted its potential to permit rapid estimation of TBW and ECF at the bedside. Although BIA is not the subject of the present study, it is assumed that TBW and ECF (or TBK) measurements will be available in groups of subjects. Multicompartmental body composition modelling may then facilitate nutritional assessment in studies of health and disease. The full NAA database contains several hundred multi-elemental analyses that can aid future rigorous statistical evaluation of prediction equations generated by this preliminary study. As VCM essentially describes the cells responsible for resting O_2 consumption, which approximates to the 'metabolic body size' (Kinney, 1988), its introduction may facilitate normalization of metabolic data.

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Waist circumference : height ratio is a strong predictor of intra-abdominal fat as measured by computed tomography. By M.A. ASHWELL[1], T.J. COLE[2] and A.K. DIXON[3], [1]*Ashwell Associates, Ashwell Street, Ashwell SG7 5PZ,* [2]*MRC Dunn Nutrition Unit, Cambridge CB4 1XJ and* [3]*University Department of Radiology, Addenbrooke's Hospital, Cambridge CB2 2QQ*

The anthropometric measurements of waist : hip circumference ratio (WHR) (Ashwell *et al.* 1985), waist circumference (Lean *et al.* 1995), waist : thigh circumference ratio (WTR) and waist circumference : height ratio (WHTR) (Ashwell *et al.* 1996; Cox *et al.* 1996) have variously been proposed as indicators of abdominal obesity, and some have been associated with cardiovascular risk factors. The present study was designed to investigate which of these simple anthropometric indices was associated most closely with the total amount of intra-abdominal (IA) fat as determined from computed tomography (CT) images.

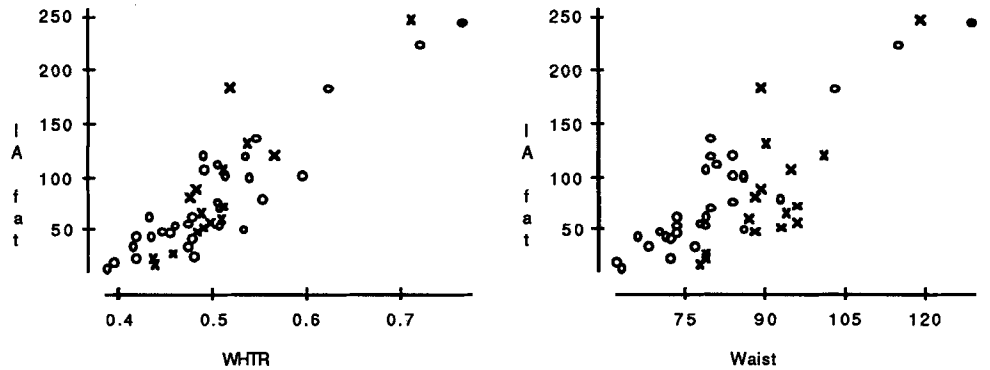
Thirty one women and sixteen men presenting for routine CT had their weight (kg), height, waist and thigh circumferences (cm) measured; all but three men and three women also had hip circumference (cm) measured. The cross-sectional areas (cm^2) of both IA and subcutaneous (SC) fat were calculated from CT images taken immediately cranial to the iliac crests as previously described (Ashwell *et al.* 1985).

The Table shows the Pearson product-moment correlations for the logarithms of selected anthropometric variables and ratios with CT estimates of total fat, IA fat and SC fat.

	Age	Weight	Height	BMI	Total fat	IA fat	SC fat	IA:SC	Waist	WHR	WTR
Weight	-0.12	1									
Height	-0.30	0.63	1								
BMI	0.07	0.79	0.03	1							
Total fat	0.34	0.52	-0.15	0.79	1						
IA fat	0.46	0.50	-0.06	0.69	0.79	1					
SC fat	0.25	0.47	-0.18	0.75	0.96	0.60	1				
IA:SC	0.33	0.17	0.10	0.14	0.06	0.66	-0.21	1			
Waist	0.13	0.87	0.38	0.82	0.63	0.75	0.50	0.44	1		
WHR	0.04	0.45	0.34	0.29	0.15	0.54	-0.05	0.68	0.73	1	
WTR	0.27	0.27	0.29	0.12	0.11	0.45	-0.07	0.61	0.57	0.81	1
WHTR	0.29	0.63	-0.08	0.87	0.75	0.83	0.63	0.43	0.89	0.60	0.48

$r > 0.29$, $P < 0.05$; $r > 0.37$, $P < 0.01$; $r > 0.47$, $P < 0.001$.

WHTR showed the largest correlation with IA fat (r 0.83), and WHR the largest correlation with the IA : SC fat ratio (r 0.68). The Figs. show the relationship between IA fat, WHTR and waist circumference for the two sexes (M=X, F=O). Multiple regression was used to find the best predictor of IA fat, adjusted for covariates. The effects of sex and SC fat were insignificant, so the sexes were combined. Even after adjusting for age (t 2.9, P=0.006) and BMI (t 0.2, P=0.8), WHTR remained by far the best predictor of IA fat (t 4.2, P=0.0002). Waist circumference alone was less significant (t 3.3, P=0.002).



We conclude that WHTR is the best simple anthropometric predictor of IA fat in men and women, and that WHTR is better than waist circumference alone because of the correlations between waist circumference, height and IA.

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Covert manipulation of the energy density of mixed diets: effect on *ad libitum* food intake in "free-living" humans. By L. M. O' REILLY, R.J. STUBBS, A.M. JOHNSTONE, O.M. MARA and K.A. ROBERTSON, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB*

High-fat, higher-energy dense (HF, HED) diets promote excess energy intakes (EI) relative to low-fat, lower energy dense (LF, LED) diets. This effect appears to be due to the energy density (ED) of the diet rather than its fat content *per se*, since changing the ED of high-carbohydrate (HC) diets led to similar changes in EI. Work in rats (Walls & Koopmans, 1992) and human subjects showed that energy compensation was more accurate when animals were intravenously infused with a mixture of glucose, fat and carbohydrate, in similar proportions to a mixed diet, than when nutrients were infused alone. This raises the possibility that subjects may compensate more accurately if the ED of a mixed diet is altered. The present study examined the effects of changing the ED of a mixed diet on *ad libitum* EI in men.

Six men, mean age 30.0 (SD 12.76) years; weight 71.67 (SD 19.8) kg; height 1.79 (SD 0.22) m, were each studied three times during a 14 d dietary treatment throughout which they had *ad libitum* access to one of three covertly-manipulated medium-fat (MF) diets. The fat, carbohydrate (CHO) and protein in each diet (expressed as a percentage of the total energy content) and ED were: LED, 38:49:13, 373 kJ/100 g; medium-ED (MED), 40:47:13, 549 kJ/100 g) and HED, 39:48:13, 737 kJ/100 g. The diets were homogenous in composition and were formulated in such a manner that they had (as far as possible) similar taste, texture and appearance. Every item was of similar composition both within and between diets. Diets were offered on a 3 d rotating menu and the order was randomized across subjects in a counterbalanced design. Subjects received a MF maintenance diet, calculated at 1.6 x RMR for 2 d before each dietary treatment. Nutrient composition was calculated using the tables from *McCance and Widdowson's Composition of Foods* (Holland *et al.* 1991). Subjects resided in, but were not confined to, a hotel metabolic suite throughout the study. ANOVA was conducted on the intakes of energy, fat, carbohydrate, protein, change in body weight and subjective hunger and pleasantness of the food, using diet and run as factors and subject as blocking factor. Mean daily energy and nutrient intakes are given in the Table.

Dietary treatment	Energy intake (MJ/d)		Fat intake (MJ/d)		Carbohydrate intake (MJ/d)		Protein intake (MJ/d)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LED	10.19	0.41	3.80	0.15	4.97	0.20	1.35	0.05
MED	12.80	0.59	5.03	0.23	6.00	0.28	1.68	0.08
HED	16.17	0.60	6.23	0.23	7.64	0.28	2.11	0.08

ANOVA confirmed that diet significantly influenced *ad libitum* intakes of energy ($F(2, 10)$ 16.08; $P<0.001$); fat, ($F(2, 10)$ 15.36; $P<0.001$); CHO, ($F(2, 10)$ 13.61; $P<0.001$); and protein ($F(2, 10)$ 15.90; $P<0.001$). Rated pleasantness of food (measured on visual analogue scales) was not significantly different between diets ($F(2, 8)$ 1.18; $P<0.365$), giving mean values of 80, 84 and 84 mm on the LED, MED and HED diets respectively. Diet significantly affected body weight ($F(2, 10)$ 6.52; $P=0.025$), producing changes of -1.11, +0.24 and + 0.95 kg over the 14 d.

These data complete a series of studies which suggest that dietary ED can be a major factor overriding EI regulation under conditions in which subjects eat unfamiliar diets of fixed composition, and diet selection is precluded. The role of learning and of nutrient selection in facilitating better energy compensation is currently under investigation.

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The effect of monoacylglycerols and triacylglycerols on appetite and day-to-day food intake in humans. By A.M. JOHNSTONE[1], R.J. STUBBS[1], N. MURPHY[1] and K.A. ROBERTSON[2], [1]*Rowett Research Institute, Aberdeen AB21 9SB and* [2]*Biomathematics and Statistics, Scotland, Aberdeen AB21 9SB*

High-fat (HF), energy-dense diets have been shown repeatedly to promote excess energy intakes (EI) and to increase body weight. We have previously found that not all fats exert the same effects on EI since when high levels of medium-chain triacylglycerols (which are preferentially oxidized) are incorporated into the diet they suppress food and EI (Stubbs & Harbron, 1996). It has also been shown that infusion of monoacylglycerols (Mono) into the duodenum of pigs is a potent appetite suppressant (Rayner, 1992). The present study was the second of two aimed at determining whether dietary Mono influences appetite and EI and if so whether these effects are attributable to pre-absorptive and absorptive phase (i.e. meal to meal) or postabsorptive oxidative events. This study examined whether overfeeding a Mono-rich, HF diet (*v.* an isoenergetically dense Tri-rich diet) affected appetite and nutrient oxidation throughout the day it was given, and the subsequent day's *ad libitum* food intake.

Six men, mean weight 76.89 (SD 7) kg; height 1.77 (SD 0.05) m; and age 26.4 (SD 6) years, were each studied twice on a 3 d protocol. On day 1 they were given a medium fat (MF) maintenance diet calculated at 1.6 x resting metabolic rate (RMR). During day 2 they were given a MF diet at 1.6 x RMR with an additional 0.45 x RMR as either Mono (Dimodan, Grinstead Products Ltd, Suffolk - information not available on positioning) or Tri incorporated into it, giving a total of 2.05 x RMR. On day 3, (outcome day), subjects had *ad libitum* access to isoenergetically dense MF (40% fat, 47% carbohydrate and 13% protein by energy) foods (550 kJ/100 g). Nutrient composition was calculated using The composition of foods (Holland *et al.* 1991). Subjects entered the calorimeter at 08.00 hours on day 2 for 48 h. Fat and carbohydrate oxidation rates were calculated from non-protein gaseous exchange, using the coefficients of Livesey & Elia (1988); protein oxidation was estimated from urinary N excretion. Subjective hunger was tracked hourly during waking hours. ANOVA was conducted on the intakes, oxidation and balances of energy, fat, carbohydrate, protein, subjective hunger and pleasantness of the food, using diet and run as factors and subject as blocking factor.

	Mono diet				Tri diet			
	Energy (MJ)	Protein (MJ)	CHO (MJ)	Fat (MJ)	Energy (MJ)	Protein (MJ)	CHO (MJ)	Fat (MJ)
Day 2, manipulation day								
Intake	15.5	1.8	5.9	7.8	15.8	1.9	6.2	7.7
Oxidation	11.3	1.2	5.2	4.9	11.7	1.5	5.3	5.0
Balance	4.2	0.6	0.7	2.9	4.1	0.5	0.9	2.7
Day 3, *ad libitum* day								
Intake	15.9	2.1	7.4	6.4	15.6	2.1	7.3	6.2
Oxidation	11.4	1.4	5.7	4.3	11.7	1.5	6.4	3.8
Balance	4.6	0.8	1.7	2.1	3.9	0.6	0.9	2.4

Surprisingly, subjects preferred the Mono diet over the Tri diet ($F(1, 20)$ 8.49; P=0.03). The ANOVA showed that there were no significant diet effects for the intake, oxidation or balance of the macronutrients and hence energy, on either day 2 (the manipulation day) or day 3. Subjective hunger was not affected by diet on either day ($F(1, 5)$ 1.15; P=0.332). This study suggests that when Mono is covertly incorporated into a diet it behaves in a manner that is very similar to Tri, in terms of its affects on appetite, feeding behaviour and nutrient balance.

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Breakfasts high in mono- or triacylglycerol: no differential effect on within-day appetite and energy intake. By L.M. RYAN, R.J. STUBBS, A.M. JOHNSTONE, H.E. LYONS and K.A. ROBERTSON, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB*

High-fat, higher-energy dense (HF, HE) diets typically induce excess energy intake (EI) relative to low-fat, lower-energy dense (LF, LE) diets. However, it has been shown that large loads of fat infused into the small intestine have a potent effect in suppressing appetite and subsequent EI (Welch *et al.* 1985). These different effects of site of fat administration on appetite have been termed the "fat paradox" by Blundell and can probably be explained by infused lipid producing supraphysiological saturation of small-intestinal receptors with emulsified fat. This effect may be limited by gastric emptying when fat is fed orally, reducing its capacity to suppress appetite. Infusion of monoacylglycerol into the duodenum of pigs has also been shown to have very potent effects in suppressing subsequent food intake (Rayner, 1992). However it is difficult to determine whether this is a particular property of monoacylglycerol or an effect of the fat paradox. The present study attempted to determine whether dietary monoacylglycerol differentially influences appetite and meal-to-meal EI in humans. Six men, mean age 25.83 (SD 5.52) years; weight 75.07 (SD 6.18) kg; height 1.86 (SD 0.05) m, and six women mean age 30.50 (SD 13.60) years; weight 64.30 (SD 6.57) kg; height 1.67 (SD 0.05) m, were each studied twice in a protocol that compared the effect of isoenergetically-dense, high-mono (HM), *v.* high-triacylglycerol (HT) breakfasts (at 08.30 hours) on subjective hunger (measured hourly on a 100 mm visual analogue scale) and *ad libitum* EI. EI was monitored at a test meal (13.00 hours) and subsequently throughout the rest of the day, ending at 23.00 hours.

Dietary treatment	EI-breakfast fixed (MJ)		EI-lunch *ad libitum* (MJ)		EI-*ad libitum* (MJ)		EI-total (MJ)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
HM	5.25	0.03	6.42	1.19	6.62	1.04	18.29	2.01
HT	4.89	0.03	5.84	0.86	7.75	1.54	18.48	2.30

The breakfasts were designed to match 80-85% of resting metabolic rate. The breakfasts (order randomized) each comprised 10% protein, 56% fat and 34% carbohydrate by energy. In the HM breakfast 65% of the fat energy was monoacylglycerol (Dimodan, Grinstead Products Ltd, Suffolk). Every item of the *ad libitum* diet comprised 13% protein, 40% fat and 47% carbohydrate by energy, with an energy density of 550 kJ/100 g. Nutrient composition was calculated using the tables from *McCance and Widdowson's Composition of Foods* (Holland *et al.* 1991). ANOVA was conducted on EI, subjective hunger and pleasantness of the food, using diet, sex and run as factors and subject as blocking factor. Subjective hunger was analysed in two periods (after breakfast, until lunch and after lunch).

Subjects found the diets to be similarly pleasant with mean values of 81 and 76 mm for the HM and the HT respectively ($F(1, 10)$ 1.55; P=0.24). There was no effect of diet on EI (see Table) at lunch or in the *ad libitum* period. There was no difference in the effect of the two diets on subjective hunger ($F(1, 10)$ 0.71; P=0.420) over the whole day (values =18.4 and 18.6 mm, respectively) or in the morning or afternoon time periods. Thus a large dose of monoacylglycerol or triacylglycerol at breakfast had similar effects on subjective hunger and EI at lunch and throughout the rest of the day, suggesting that orally ingested monoacylglycerol has no specific effects on appetite which are distinguishable from the those of dietary triacylglycerol.

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Effects of substitution of medium-chain triacylglycerol for long-chain triacylglycerol on energy balance in obese women on a weight-reducing diet. By L.M. MARTIN[1], M.B.E. LIVINGSTONE[1], H. McNULTY[1] and R.J. STUBBS[2], [1]*Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA and* [2]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Recent research has shown that high fat diets are associated with increased energy intake and adiposity (Miller *et al.* 1990; Warwick & Schiffman, 1992). However, most studies which demonstrate high-fat hyperphagia do not discriminate between possible differential effects of different kinds of fat on appetite and energy intake. Animal studies have demonstrated a low adipogenic tendency for medium-chain triacylglycerol (MCT) (C8-C10) relative to long-chain triacylglycerol (LCT). In addition, MCT limits hyperphagia in rats and humans at high doses (Stubbs, 1995). The aim of the present study was to compare the effects of MCT and LCT on weight loss and body composition in obese female human subjects as part of a weight-loss regimen.

Obese (BMI 28-58 kg/m^2) adult female subjects were recruited (*n* 60) and randomly divided into LCT and MCT groups. On every day of the 12-week study, each subject replaced any two of their usual daily meals with two liquid meals (2.5 MJ) containing either 60% energy as MCT or LCT (28% as CHO and 12% as protein). For the remainder of the day, subjects were instructed to eat *ad libitum* from a recommended selection of low-fat–high-fibre foods. Changes in body composition assessed by densitometry and anthropometry before, and following intervention, were compared in eighteen subjects who completed the protocol.

	LCT (*n* 8)		MCT (*n* 10)		*P* value †
	Mean	SD	Mean	SD	
Body weight (kg)					
Pre	95.8	20.6	97.7	20.9	NS
Post	88.7**	21.4	90.4**	17.7	NS
Change	-7.1	3.8	-7.2	5.3	NS
Fat mass (kg)					
Pre	36.1	11.8	38.3	10.9	NS
Post	31.4**	11.7	33.0***	8.1	NS
Change	-4.7	2.6	-5.7	4.8	NS
Fat-free mass (kg)					
Pre	60.4	10.4	59.5	11.0	NS
Post	58.5*	11.1	57.9**	10.9	NS
Change	-1.9	2.0	-1.6	5.0	NS
Body fat %					
Pre	36.7	5.7	38.9	3.5	NS
Post	34.1*	5.5	36.0**	2.5	NS
Change	-2.6	1.8	-3.2	2.2	NS
Fat-free mass %					
Pre	63.3	5.7	60.9	3.3	NS
Post	65.9*	5.5	64.0***	2.5	NS
Change	+4.4	2.6	+5.8	4.8	NS

*P<0.05, **P<0.01, ***P<0.001 (Student's paired *t* test). † Student's unpaired *t* test.

Within-group differences in pre v. post supplementation body weight, fat mass, % fat free mass and % body fat were significant. There was no significant differences between groups. These data indicate that this obese population did not respond better to MCT than to LCT at the dietary doses given.

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The effect of dietetic advice on incorporating altering meal frequency for hyperlipidaemic patients on low-fat diets. By S. M. KING and M.J. GIBNEY. *Unit of Nutrition and Dietetics, Department of Clinical Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Republic of Ireland*

Health promotion messages favour the consumption of three meals daily and tend to place between-meal snacks in an unfavourable light. Studies investigating the effect of meal frequency on plasma lipids, however, have suggested that an increased meal frequency may be beneficial (Fabry *et al* 1966). Many of these studies used highly controlled procedures on normal healthy subjects (Jenkins *et al* 1989). The present study investigated the effect of advice about altered meal frequency on a specific target group i.e. hyperlipidaemic males, in a free living situation. A significant end-point was therefore the patient's interpretation and implementation of the advice.

Seventy-eight subjects completed the study. Meal frequency (by food diary), nutrient intake (by dietary history and food atlas) and fasting lipid profiles were measured pre-trial and post-trial. Subjects were allocated to one of four intervention groups for a 4-week intervention period. Dietary advice was carried out in a dietetic outpatient setting. Allocation to the intervention groups was determined by the subject's meal frequency pre-trial. The four dietary groups were as follows:

	Pre-trial meal frequency	Pre-tial dietary fat	Post-trial meal frequency	Post-trial dietary fat
Group 1	High	Normal	Low	Low
Group 2	High	Normal	High	Low
Group 3	High	Normal	Low	Normal
Group 4	Low	Normal	High	Normal

Subjects expressed difficulty in adopting any alteration to meal frequency into their lifestyle. The magnitude of the alteration to meal frequency prescribed, was not achieved by any of the relevant groups. Both of the low-fat groups achieved significant decreases in total cholesterol (Group 1 0.52mmol/l (±0.76), Group 2 0.44mmol/l (±0.76); $P<0.01$and $P<0.02$ respectively). Group 1 had a significant decrease in energy intake, 1.79MJ (±1.4), and body weight 1.0kg(±1.5) $P<0.0003$ and $P<0.01$ respectively. Group 2 achieved a significant increase in HDL_2 (0.16mmol/l (±0.54) $P<0.05$) and a significant decrease in LDL:HDL (1.35mmol/l (±1.66) $P<0.05$). Of the two groups which were asked to alter meal frequency but maintain nutrient intake, the group which decreased its meal frequency (Group 3) achieved a significant reduction in total cholesterol (0.58mmol/l (±0.74) $P<0.001$) together with a significant decrease in both body weight 1.96kg(±1.51), and energy intakes 2.3MJ (±3.0), $P<0.0001$ and $P<0.001$ respectively. Group 4, which increased its meal frequency achieved significant increases in HDL_2 (0.09mmol/l(±0.17), $P<0.04$).

Group 2 which adapted a low fat diet and maintained its meal frequency acheived the best adherence to the study protocol, encountered the least difficulty and achieved the most favourable lipid results. This study would therefore suggest that the diet recommended for cardiac patients should be incorporated into the existing meal frequency of the patients and not concentrated on three meals daily.

This work was funded by MARS Incorporate.

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Dietary fat reduction achieved by increasing consumption of a starch-rich food: a 12-week follow-up by T.R. KIRK, S. BURKILL, M. CURSITER and J. LANDMAN, *Centre for Food Research, Queen Margaret College, Edinburgh EH12 8TS*

The purpose of the present study was to determine if simple advice to increase consumption of starch-rich foods, e.g. breakfast cereals, can achieve a reduction in percentage energy from fat, without increasing total energy intake. The preliminary findings of the study have been reported to the Society (Burkill *et al.* 1995) and the results from subjects who completed the long-term follow-up are presented here.

Students recruited to the study were matched for age, sex and smoking, and randomly assigned to an intervention group (*n* 26, one male, twenty-five females, mean age 19.7 (SEM 1.9) years) and a control group (*n* 22, one male, twenty-one females, mean age 19.7 (SEM 1.9) years). The intervention group were provided with ready-to-eat breakfast cereals and were asked to increase their consumption by approximately 420 g/week for 12 weeks. The breakfast cereal was to be accompanied by semi-skimmed or skimmed milk. No other dietary advice was given. Dietary assessments (7 d weighed inventory method) were made at baseline, 4 and 12 weeks. Data were analysed using COMP-EAT 4 and SPSS for Windows computer program. Results for total energy, and percentage energy from fat, carbohydrate (CHO), protein and starch are shown in the Table.

	INTERVENTION GROUP						CONTROL GROUP					
	Baseline		Week 4		Week 12		Baseline		Week 4		Week 12	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Energy (MJ)	7.82	2.47	7.87	2.41	7.92	2.40	8.01	2.62	8.44	2.85	7.75	2.60
% energy:												
Fat	34.8	1.07	29.4**	0.94	29.3*	1.01	36.0	0.99	37.1	0.71	34.6	1.01
CHO	45.6	1.37	51.1**	1.33	52.1*	1.25	46.7	0.97	45.2	0.82	46.9	1.06
Starch	26.2	0.95	31.3*	1.08	30.9*	0.99	26.5	1.03	26.4	0.77	26.6	1.16
Protein	12.3	0.40	13.1	0.37	12.9	0.29	13.3	0.31	13.0	0.47	12.9	0.40

Mean values were significantly different from control groups: * $P < 0.05$, ** $P < 0.001$ (independent t test).

The Table shows that, at baseline, there were no significant differences between the intervention and control groups, either in total energy or in percentage energy from macronutrients. However, by week 4 of the intervention period, there was a significant reduction in percentage energy from fat (-5.4%) in the experimental group, and this reduction was maintained at the 12-week follow-up. There was a corresponding increase in percentage energy from CHO: a significant increase of 5.5% after 4 weeks had reached 6.5% by the end of the 12-week intervention period. Total energy remained virtually unchanged, indicating a replacement of fat energy by CHO and starch energy. This confirms that the short-term energy changes (reported previously) were sustained for the duration of the 12-week study.

Preliminary dietary analysis indicates that the change in percentage fat energy in the intervention group can be partly attributed to a reduced consumption of spreading fats and of biscuits, cakes and confectionery; these dietary changes were not found in the control groups.

These results suggest that simple advice to increase consumption of a starch-rich food, breakfast cereal, led to isoenergetic replacement of fat by starch. This may provide an effective population strategy for achieving targets for dietary fat reduction.

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The effect of covertly manipulating the energy density of high-carbohydrate diets on *ad libitum* food intake in "pseudo free-living" humans. By R.J. STUBBS, C.G. HARBRON and A.M. JOHNSTONE, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB*

It has been demonstrated that excess energy intake (EI) occurs when subjects feed *ad libitum* on high-fat, higher-energy dense (HF, HED) diets, compared with low-fat, lower-energy dense (LF, LED) diets. These observations have been taken to suggest that EI regulation is driven by glucostatic or glycogenostatic mechanisms (e.g. Flatt, 1987) and that excess EI would be difficult to achieve if subjects fed *ad libitum* on high-carbohydrate (HC) diets. The present study examined the effects of covert changes in the energy density of HC diets (achieved primarily by using maltodextrin supplements), on *ad libitum* energy intake, subjective hunger and body weight in normal-weight men.

Six men, mean age 32.17 (SD 12.88) years; weight 69.74 (SD 6.74) kg; height 1.76 (SD 0.05) m, were each studied twice during 14 d dietary treatments throughout which they had *ad libitum* access to one of two covertly-manipulated diets. The fat, carbohydrate (CHO) and protein in each diet, expressed as a percentage of the total energy content, and energy density were low-ED (LED), 22:65:13, 348 kJ/100 g and high-ED (HED), 23:65:12, 617 kJ/100 g. Within each diet every item was of the same composition. Diets were offered on a 3 d rotating menu and the order was randomized in a counterbalanced design across subjects. Subjects received a maintenance medium fat (MF) diet for 2 d before each dietary treatment. Energy and nutrient intakes were calculated using the tables from *McCance and Widdowson's Composition of Foods* (Holland *et al.* 1991). Subjects resided in, but were not confined to, a hotel metabolic suite throughout the study (hence "pseudo free-living"). ANOVA was conducted on the intakes of energy, fat, carbohydrate and protein and subjective hunger and pleasantness of the food, using diet and run as factors and subject as blocking factor. Body-weight change was analysed by regression. The results of mean daily energy and nutrient intakes are given in the Table.

Dietary treatment	Energy intake (MJ/d)		Fat intake (MJ/d)		Carbohydrate intake (MJ/d)		Protein intake (MJ/d)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
HED	14.56	0.41	3.01	0.08	9.72	0.27	1.84	0.02
LED	8.56	0.23	1.83	0.05	5.57	0.15	1.17	0.03

Diet significantly influenced *ad libitum* intakes of energy ($F(1, 5)$ 50.74; $P<0.001$); fat, ($F(2, 5)$ 92.26; $P<0.001$); CHO, ($F(1, 5)$ 38.46; $P=0.002$); and protein ($F(1, 5)$ 77.44; $P<0.001$). Subjects felt significantly more hungry on the LED diet than the HED diet (30.4 mm *v.* 25.7 mm ($F(1, 160)$ 30.28; $P<0.001$)). The subjects found the diets to be similarly pleasant (75.5 mm *v.* 74.7 mm ($F(1, 160)$ 0.31; $P=0.579$)). Mean body weight increased on the HED diet at a rate of 0.07 kg/d and decreased at 0.1 kg/d on the LED diet ($F(1, 131)$ 86.60; $P<0.001$).

Thus changing the energy density of HC diets or the energy density and fat content of the diet (Stubbs *et al.* 1995) has led to parallel changes in EI and body weight. These results suggest that excess EI are possible on HC, HED diets, at least under conditions where diet selection is precluded.

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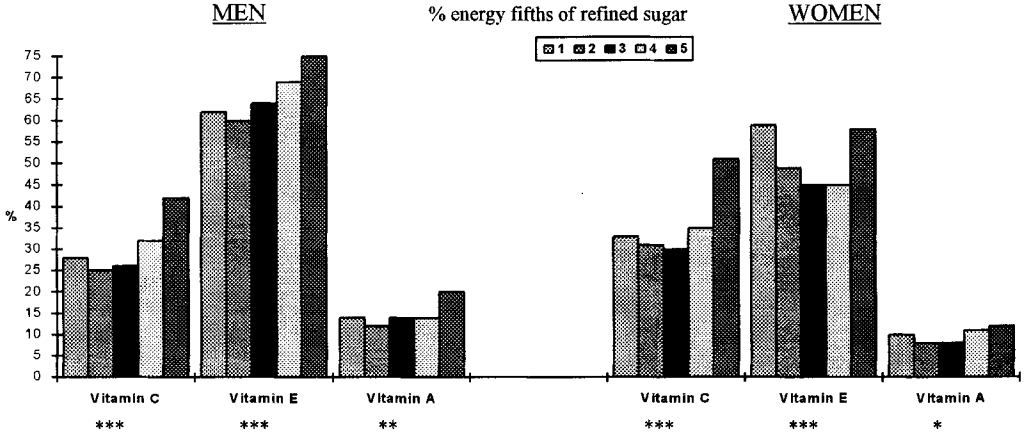
Refined versus non-milk extrinsic sugar: is there a different relationship to antioxidant vitamin adequacy ?. By C. BOLTON-SMITH. *Cardiovascular Epidemiology Unit, University of Dundee, Ninewells Hospital and Medical School. Dundee DD1 9SY.*

Before the recent report (Department of Health, 1989), sugars tended to be classified as either natural/raw or added/refined sugars. These were practical definitions which most people readily understood. The new definitions for sugars, as milk-, intrinsic- or non-milk extrinsic (NMES)-sugars are less easy for the non-specialist to understand, and do not benefit from being based on unambiguous chemical characteristics. With respect to micronutrient intakes in particular, the 'new' group to be given a recommended restricted intake (NMES), includes fruit and vegetable juices: one of the most important sources of vitamin C in today's diet, especially for women (Gregory *et al.* 1990). Hence, the left-hand arm of the U-shaped relationship between antioxidant vitamin adequacy and NMES intake by fifths (Bolton-Smith & Woodward, 1995) may have reflected the inclusion of these natural juices.

The same Scottish Heart Health Study/MONICA data set of 11 626 men and women aged 25-69 years was used to determine whether antioxidant vitamin adequacy differed by fifths of refined sugar intake, derived from the food-frequency questionnaire. The Fig. shows that the trend for higher prevalences of intakes below the recommended values at either extremes of intake, which were reported previously for NMES, are far less obvious by fifths of refined sugar intake. The exception is for vitamin E intake in women, where a clear U-shaped relationship is still observed.

Fig. Percentage not meeting the 'RNI' for the antioxidant vitamins by fifths of refined sugar intake



RNI, reference nutrient intake for vitamin C, 40 mg/d; for vitamin A (retinol equivalents), 600 μg/d for women, 700 μg/d for men; Vitamin E (α-tocopherol equivalents) USA recommendations for women 5 mg/d, for men 7 mg/d. Significance of Chi-squared test * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

It appears that, generally, it is the top 20 % of refined sugar consumers who may be at greater risk of inadequate antioxidant vitamin intakes in this population. For men this was above 15 %, and for women above 10 % of dietary energy as refined sugar. Care should be taken when deciding on the sugar definitions for analysis, particularly in relation to micronutrient adequacy, since NMES includes fruit and vegetable juices whilst refined sugar does not.

This work was supported by The Sugar Bureau.

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Micronutrient intakes by pre-school children in Britain: association with consumption of non-milk extrinsic sugars. By SIGRID A. GIBSON, *21 Orchard Rd, Burpham, Guildford GU4 7JH*

Non-milk extrinsic sugars (NMES) contribute approximately 19% of the total energy consumed by young children (Gregory *et al.* 1995). It has been suggested that relatively high intakes of NMES may potentially compromise micronutrient intakes in those with low energy requirements and intakes (Department of Health, 1991), and children could arguably be included in this category. The present study examined data from the *National Diet and Nutrition Survey* of children aged 1.5 - 4.5 years (Gregory *et al.* 1995) for evidence of compromised micronutrient intakes in children who derived a high proportion of daily energy from NMES. The 848 boys and 827 girls were classified by fifths of energy from NMES. Quintiles were at 12.2, 15.8, 19.9 and 24.9 % energy from NMES for boys and 11.9, 16.4, 20.2 and 24.5% energy from NMES for girls. Results were similar between the sexes and have been combined in the Table. Differences between the fifths were evaluated by one-way ANOVA and the Bonferroni test.

Boys and girls	Fifths of % energy from non-milk extrinsic sugars					All children		ANOVA
	1	2	3	4	5	(*n* 1675)		*P*=
	n 335	*n* 335	*n* 336	*n* 335	*n* 334	Mean	SE	
Energy (kJ/d)	4658	4755	4870	4823	4886	4798	27	0.04
Calcium (mg/d)	784^{d}	667^{c}	647^{c}	582^{b}	509^{a}	638	6	<0.0001
Iron (mg/d)	5.6	5.9^{b}	5.7^{b}	5.6	5.1^{a}	5.6	0.1	0.0013
Zinc (mg/d)	5.1^{c}	4.7	4.4^{b}	4.2^{b}	3.7^{a}	4.4	0.1	<0.0001
Thiamin (mg/d)	0.86^{b}	0.87^{b}	0.82^{b}	0.79^{a}	0.73^{a}	0.81	0.01	<0.0001
Riboflavin (mg/d)	1.40^{c}	1.28^{c}	1.21^{b}	1.14^{b}	1.02^{a}	1.21	0.01	<0.0001
Vitamin C (mg/d)	39^{a}	45^{a}	49^{b}	54^{b}	72^{c}	52	1	<0.0001
NSP (g/4184 kJ)	5.7	5.8^{c}	5.4^{b}	5.3^{b}	4.6^{a}	5.4	0.1	<0.0001

[a,b,c] Values in the same row with different superscript letters were significantly different at $P<0.05$.

Mean intakes of most micronutrients were well in excess of the reference nutrient intake (RNI) in all sugar groups, although several (Ca, Zn, B_1, B_2, B_3) showed an inverse relationship with sugars concentration ($P<0.0001$). Conversely, vitamin C intakes increased with NMES concentration ($P<0.0001$), reflecting fruit juice consumption. The only nutrients of which intakes were marginal in comparison to the dietary reference values were Fe and Zn, which were low across all groups (mean values 81% and 88% of the RNI respectively). Significant reduction in Fe intake only occurred above the highest quintile (> 24% NMES energy). It was largely attributable to the lower meat consumption by these children: 313 g/week (SE 12) for those with >24% NMES energy; 362 g/week (SE 6) for all children. The lower intakes of Fe and Zn in the upper NMES groups may possibly be mitigated by their higher vitamin C and lower fibre intakes respectively. Further work may be warranted: (1) to explore in more depth the dietary and non-dietary factors underlying low nutrient intakes and (2) to use data on co-consumption of foods, and bioavailability, to evaluate dietary intakes more precisely.

This study was supported by The Sugar Bureau.

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Bowel habit: the perceived influence of diet and lifestyle. By SARAH W. MIAN and G. JILL DAVIES, *Nutrition Research Centre, South Bank University, 103 Borough Road, London SE1 0AA*

Idiopathic constipation is a common digestive complaint affecting up to 20% of the population (Devroede, 1991). Absence of set criteria for normal bowel function has led to confusion and a poor understanding of what is meant by 'normal' and 'abnormal' bowel habit; such that one individual's 'constipation' may be anothers 'normal bowel habit' (Moore-Gillon, 1984). Thus, making a diagnosis of constipation difficult.

Subjects were recruited by placing advertisements in the media. Of the respondents who completed the health screening questionnaire and bowel habit diary, 153 Caucasian adults aged between 18 and 82 years (median 32.42 years) living in the United Kingdom were selected. All subjects were asked to identify and record their bowel habit, any unwanted symptoms experienced, and factors perceived to influence bowel habit over a period of seven consecutive days.

Factors influencing bowels	No. of recorded incidences* (% of subjects) 'bowel habit type' 'Normal' (*n* 122)	'Constipated' (*n* 19)	'Other' (*n* 12)	Stool frequency (decrease or increase)
Irregular eating pattern	12 (10)	3 (11)	1 (8)	Decreased
Decreased intake of:				
Specific foods†	17 (3)	2 (11)	2 (8)	Decreased
Fibre	8 (5)	7 (11)	1 (8)	Decreased
Ignored call to stool	20 (15)	2 (11)	2 (17)	Decreased
Stress	10 (3)	6 (26)	0	Decreased
Lack of exercise	4 (3)	4 (16)	0	Decreased
Travelling ‡	3 (2)	1 (5)	0	Decreased
Increased intake of:				
Fibre	28 (15)	0	2 (8)	Increased
Alcohol	12 (10)	2 (11)	8 (50)	Increased
Fluid	9 (4)	4 (5)	1 (8)	Increased
Spicy food eaten	19 (14)	3 (16)	0	Increased
Overeating	18 (11)	1 (5)	4 (33)	Increased
Specific foods†	17 (2)	2 (11)	0	Increased
Increased exercise	8 (5)	2 (11)	1 (8)	Increased
Feeling:				
Nervous	5 (4)	0	5 (17)	Increased
Relaxed	2 (1)	3 (11)	0	Increased

* More than one response given. †Bran, brown bread, beans, prunes, grapefruit, grapes, chocolate, hot coffee
‡Need to pass a motion before travel.

The Table shows that 80% of subjects thought their bowel habit was 'normal'. In contrast, 12% of subjects complained of 'constipation' and the remaining 8% did not identify their bowel habit and categorized it as 'other'. Both dietary and lifestyle factors were perceived to either increase or decrease stool frequency. Subjects with a 'normal' bowel habit associated the increased intake of dietary fibre with an increase in stool frequency whereas ignoring the call to stool was perceived to decrease stool frequency. Both a lack of fibre in the diet and stress were associated with a decreased stool frequency by subjects with self-reported constipation.

The findings suggest that individuals who do not seek medical advice for constipation may attribute this condition to inadequate dietary fibre intake and stress arising from every day events. Therefore, the need for careful assessment of factors associated with constipation, particularly diet and lifestyle is required.

Financial support from Reckitt & Colman Products Ltd is gratefully acknowledged.

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The effect of meat and milk proteins on *in vitro* dialysability of iron. By B. MULVIHILL and P. A. MORRISSEY, *Department of Nutrition, University College, Cork, Republic of Ireland*

Meat is a known enhancer of non-haem Fe bioavailability. While the exact mechanism of enhancement remains unclear, it has been suggested that a relationship exists between the sulphydryl content of meat and Fe dialysability (Mulvihill & Morrissey, 1995). The present study was designed to examine the effects of meats from different species and of ß-lactoglobulin, the major sulphydryl-containing protein in milk, on *in vitro* dialysability of Fe from a semi-purified meal.

Samples (containing 1 g protein) were incorporated into 100 g semi-purified meal containing glucose (11.5 g), maize oil (5.9 g), $CaHPO_4$ (0.11 g), KH_2PO_4 (0.22 g) and Fe (0.6 mg) as $FeCl_3$. The meals were labelled with ^{59}Fe and *in vitro* dialysability of Fe was determined by the method of Miller *et al.* (1981). This method measures the release of soluble low-molecular-weight (dialysable) Fe after digestion with pepsin (*EC* 3.4.23.1) and pancreatin, under simulated gastrointestinal conditions. Egg albumin was used as a reference protein. Radioactivity was measured in the pepsin digest and dialysate allowing calculation of % dialysable Fe.

	Mean n 6	SEM
Egg albumin	3.58	0.39
Beef	11.55*	0.35
Pork	12.09*	0.62
Lamb	16.11*	0.83
Lambs liver	22.79*	0.66
Chicken leg	5.65*	0.36
Chicken breast	7.79*	0.59
Venison	16.17*	0.50
Salmon	8.88*	1.03
Whey	1.22*	0.10
ß-Lactoglobulin	9.83*	0.45

* Significantly different from egg albumin, $P<0.05$ (Student's t- test).

When compared with egg albumin, all samples significantly enhanced Fe dialysability, except for whey protein which was inhibitory. ß-Lactoglobulin significantly enhanced Fe dialysability, indicating that ß-lactoglobulin is not the inhibitory component of whey protein. Lamb's liver gave the greatest enhancement of Fe dialysability.

Incorporation of the sulphydryl blocking agent, N-ethylmaleimide (NEM) (0-10 mM), into the semi-purified meal containing lamb's liver significantly reduced Fe dialysability in a dose related manner. These present results further confirm that the free sulphydryl content of meat plays an important role in enhancing Fe bioavailability in foods.

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Growth hormone and calcium absorption in aged female rats. By K. CASHMAN[1] and J.C. FLEET[2] *[1]Department of Nutrition, University College, Cork, Republic of Ireland, [2]USDA Human Research Center on Aging, Tufts University, Boston, USA*

Ageing is associated with reduced intestinal Ca absorption and circulating 1, 25 $(OH)_2$ cholecalciferol (D_3) levels. Aged rats (>16 months) respond to parathyroid hormone (PTH) and growth hormone (GH) by increasing Ca absorption and intestinal levels of calbindin D9k (CaBP) (Fleet *et al.* 1994). PTH, but not GH, increases levels of 1, 25 $(OH)_2$ D_3 in aged animals, suggesting that the GH response is not dependent upon 1, 25 $(OH)_2$ D_3. Thus, the objective of the present study was to evaluate the effects of GH on Ca absorption in aged rats and to investigate the mechanism by which GH increases Ca absorption.

Two studies were carried out in 16-month-old female Sprague-Dawley rats. In study A, sixteen rats were randomized into two treatment groups (*n* 8/group). Treatment groups received either 100 μg GH/100 g body weight (BW) per d or vehicle (control group) via subcutaneous injection in the abdomenal area. Each treatment was administered for 12 d. On days 9 - 12, food consumption, and faecal and urinary Ca output were monitored for determination of Ca balance. On day 12, animals were killed and tissues were harvested and frozen at -70° until analysed. Study B involved an identical design but rats were used only to supply tissues in an attempt to understand the mechanism of action of GH.

	Vehicle		Growth hormone	
Variable	Mean†	SE	Mean†	SE
Study A				
Food intake (g/d)	11.6	0.8	13.4*	0.2
Ca absorption (mg/d)	17.4	2.9	25.7*	1.6
1,25 $(OH)_2$ D_3 (pg/ml serum)	20.1	3.4	30.6	3.8
CaBP (μg/mg protein)	1.9	0.6	4.5*	0.5
Study B				
Body wt changes (g)	-20.5	4.5	-4.8*	3.6
Duodenal wt (g)	1.01	0.05	1.19*	0.04
Mucosal wt (g)	0.47	0.04	0.53	0.03
IGF-1 (μg/ml serum)	0.74	0.39	1.12*	0.38
Vitamin D receptor (fg/mg protein)	1142	465	1083	371

* Significantly different from the vehicle, $P<0.05$ (by Student's *t* test) † Values are given as the mean for eight rats.

GH administration increased food intake and reduced weight loss in these aged rats. GH did not alter mucosal weight or vitamin D-receptor concentration. Rats treated with GH had higher insulin-like growth factor-1 (IGF-1) levels, the physiological mediator of GH action, when compared with the control rats. GH administration significantly increased net Ca absorption and intestinal CaBP levels despite the fact that serum 1, 25 $(OH)_2$ D_3 was not significantly elevated. Thus, GH stimulates Ca absorption in aged rats by a mechanism independent of 1, 25 $(OH)_2$ D_3, which is mediated by IGF-1. GH increased duodenal weight which may have influenced Ca absorption via increased absorptive surface and number of CaBP-producing cells.

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Fleet, J.C., Bruns, M.E., Hock, J.M. & Wood, R.J. (1994). *Endocrinology* **134**, 1755-1760.

The effect of sugar alcohols on calcium absorption in the rat. By T. BENNETT, D. BUCKLEY, A. FLYNN and K. CASHMAN, *Department of Nutrition, University College, Cork, Republic of Ireland*

The purpose of the present study was to investigate the effect of sugar alcohols on Ca absorption using a rat model which has been shown to be useful for studies on Ca bioavailability (Cashman & Flynn, 1994; Harrington *et al.* 1994).

Two studies were carried out using 7-week-old male rats, Wistar strain, average weight 201 g. In study A, forty rats were randomized into five groups of eight rats each and fed on a purified diet (AIN-76) for 2 weeks. Each group was then given a meal (10 g of same diet) containing xylitol or sorbitol at levels of 0, 50 or 150 g/kg (in replacement of sucrose), 5 g Ca as ^{47}Ca-labelled $CaCO_3$ and 0.2 g Fast Green FCF as a faecal marker. Study B involved an identical design but the rats were given a meal (10 g of same diet) containing galactitol or maltitol at levels of 0, 50 and 100 g/kg. Fractional absorption of ^{47}Ca in both studies was determined by the ^{47}Sc:^{47}Ca ratio method of Brommage & Binacua (1991).

Study A				Study B			
		Ca absorption (%)				Ca absorption (%)	
Sugar alcohol	g/kg diet	Mean	SE	Sugar alcohol	g/kg diet	Mean	SE
Control	0	32.1	1.9	Control	0	38.3	2.4
Xylitol	50	57.7*	1.6	Galactitol	50	73.1*	2.7
Xylitol	150	60.2*	1.1	Galactitol	100	76.2*	2.3
Sorbitol	50	45.3*	3.3	Maltitol	50	48.7*	3.8
Sorbitol	150	62.1*†	2.7	Maltitol	100	51.1*	2.5

* Mean values within a column were significantly different from control, $P<0.05$ (ANOVA).
† Mean value was significantly different from 50 g sorbitol/kg, $P<0.05$ (ANOVA).

Increasing the sugar alcohol concentration of the meal from 0 to 50, 100 or 150 g/kg significantly increased Ca absorption. The greatest enhancement of Ca absorption was seen with addition of galactitol and the lowest with addition of maltitol to the meal. The enhancement of Ca absorption by these poorly absorbed sugar alcohols may be due to increased passive absorption of Ca arising from increased permeability of the gap junctions between the enterocytes (Bronner 1987).

This work was supported by the Department of Agriculture, Food and Forestry, Dublin.

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Excretion of urinary pyridinium crosslinks in the rat: day-to-day variations and variations due to age and sex differences. By A. CREEDON, K. CASHMAN and A. FLYNN, *Department of Nutrition, University College Cork, Republic of Ireland*

Urinary pyridinium crosslinks of collagen, pyridinoline (Pyr) and deoxypyridinoline (Dpyr, occurring primarily in bone), show promise as specific and sensitive biochemical indices of bone resorption in the human (Eyre, 1992). They may have considerable potential for monitoring changes in rates of bone resorption and how such changes are influenced by diet. In order to use these crosslinks in studies assessing the influence of diet on the rate of bone resorption, a number of potential variables affecting the excretion of these compounds must be evaluated. The objectives of the present study were to examine the day-to-day variations in crosslink excretion and to establish any sex- or age-related differences in crosslink excretion in a rat model.

Twelve 6-week-old rats (six female, six male), Wistar strain, average weight 131 g, were housed individually in metabolism cages and fed *ad libitum* on a chow diet for 7 weeks. Total 24 h urine samples were collected and stored at -20 ^{0} until required for analysis, three times each week throughout the study and for five consecutive days during week 3 when rats were 9 weeks old. Urinary pyridinium crosslinks were measured using a three-step procedure: each urine sample (0.25 ml) was hydrolysed with an equal volume of 12M-HCl at 110 ^{0} for 18 h, the crosslinks were then extracted by CF1 cellulose chromatography and were measured by reversed-phase HPLC with fluorescence detection (Eyre *et al.* 1984).

	Female (n 6)		Male (n 6)		
	Mean	SE	Mean	SE	P value*
Final body weights (BW) (g)	198	8	313	6	≤ 0.001
Mean Pyr (nmol/d)†	7.5	0.3	8.4	0.3	≤ 0.05
Mean Pyr (nmol/d per 100 g BW)‡	3.8	0.3	2.7	0.1	≤ 0.01
Mean Dpyr (nmol/d)†	8.1	0.6	10.6	0.5	≤ 0.001
Mean Pyr (nmol/d per 100 g BW)‡	4.1	0.4	3.4	0.2	≤ 0.05

* Comparison of means between males and females by Student's t test.
† Values calculated from data obtained in the last week of the study period i.e. mean of 3 measurements per rat.
‡ Values corrected for body weights of the animals.

Urinary excretion of Pyr and Dpyr was greater in males than in females; however, when adjusted for body weights urinary crosslink excretion was lower in the males than in females. Urinary excretion of Pyr and Dpyr did not change with age in either male or female rats; however, when expressed on a body weight basis crosslink excretion decreased with age (data not shown).

The CV for day-to-day variation for Pyr excretion over five consecutive days was 16% for both males and females and for Dpyr excretion was 9% and 17% in males and females respectively. Day-to-day variation was significant only when less than three consecutive urine collection days were used, indicating that urinary crosslink excretion should be measured over at least three consecutive days.

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Effect of calcium supplementation on biochemical markers of bone formation and bone resorption in healthy young adults. By F. GINTY, K. CASHMAN and A. FLYNN, *Department of Nutrition, University College Cork, Republic of Ireland*

Recent advances in the study of bone metabolism have resulted in the development of rapid and sensitive assays for circulating and urinary markers that specifically reflect bone formation or resorption. These biochemical markers have considerable potential in the investigation of the influence of diet and other factors on bone metabolism. The objective of the present study was to examine the effect of short-term Ca supplementation on biochemical markers of bone turnover in healthy young adults.

Eighteen adults (thirteen females and five males), with a mean age of 23 years, were recruited. They had no history of bone disease, and had not taken any medication that could affect bone or cartilage metabolism. Subjects received 800 mg Ca/d as calcium lactate gluconate and $CaCO_3$ (Sandocal®) in two equal doses, morning and evening, for 14 d in addition to their usual diet. Fasting morning void urine samples were collected for three consecutive days immediately pre-supplementation, for each day during the supplementation period, and for three consecutive days immediately post-supplementation. A fasting blood sample (10 ml) was drawn on the day immediately preceding supplementation, once each during weeks 1 and 2 of the supplementation period, and on the third day after supplements were withdrawn. Urine was analysed for Ca (Ur Ca) by atomic absorption spectrophotometry, creatinine (Cr) by colorimetry and urinary deoxypyridinoline (Ur Dpyr) by the method of Ginty *et al.* (1995). The values of Ur Dpyr and Ca were expressed relative to urinary Cr. Serum osteocalcin and serum bone-specific alkaline phosphatase (EC 3.1.3.1; Alkphase-B) were determined by enzymeimmunoassay (EIA). Assessment of dietary Ca was carried out by means of daily completion of food diaries from which nutrient intakes were estimated using a food composition database (Microdiet).

	Supplementation periods					
	Pre-		Ca Supplementation		Post-	
Variable	Mean	SE	Mean	SE	Mean	SE
Dietary Ca intake (mg/d)	877[a]	79	1600[b]	64	762[a]	88
Serum osteocalcin (μg/l)	9.7[a]	1.1	12.5 [b]	1.2	13.2[b]	1.4
Serum Alkphase-B (U/l)	23.6[a]	2.2	25.5[a]	1.9	27.6[b]	2.1
Ur Dpyr (nmol/mmol Cr)	12.1[a]	0.8	10.5[b]	0.7	14.3[c]	0.9
Ur Ca (mmol/mmol Cr)	0.25[a]	0.02	0.33[b]	0.03	0.25[a]	0.03

[a,b,c] Values are means for eighteen subjects. Significant differences were recorded at $P < 0.05$; values within a row not sharing a common superscript were not significantly different (by repeated measures and Student's *t* - test as follow up).

Dietary Ca supplementation (800 mg elemental Ca daily for the 14 d) reduced the excretion of Ur Dpyr, a biomarker of bone resorption. Serum osteocalcin, but not Alkphase-B, (both biomarkers of bone formation) was elevated by Ca supplementation. These results indicate that short-term Ca supplementation suppresses bone resorption and may also stimulate bone formation.

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Relationship between present and past dietary intake and bone mass using broadband ultrasound attenuation. By SUSAN A. NEW[1], ALISON STEWART[2], DAVID A. GRUBB[3] and DAVID M. REID[2], [1]*Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH,* [2]*Osteoporosis Research Unit, Woolmanhill Hospital, Aberdeen AB9 8AU,* [3]*Computing Department, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Broadband ultrasound attenuation (BUA) is one of the most recently developed methods for assessment of bone health. Attenuation of ultrasound and its velocity are believed to relate to calcaneus bone mass and possibly trabecular structure, although this has yet to be fully defined (Peel & Eastell, 1993). The aim of the present study was to investigate dietary influences on bone mass.

Calcaneus bone mass was measured using BUA (Walker Sonix UBA 575) in 165 pre-menopausal women aged 45-49 years who had been randomly selected from the community health index. The coefficient of variation of this technique in the author's hands was 2.6%. No women had taken any medication or suffered from any condition likely to affect their bone metabolism. A food-frequency questionnaire, which had previously been developed and validated against 7 d weighed records was used for assessment of dietary intake (Lanham & Bolton-Smith, 1993). Before analysis nutrient intakes were adjusted for total energy intake by calculating the residual from regression analysis. Past intakes of milk during childhood (up to 12 years) and early adulthood (20-30 years) were recorded and answers categorised into low, medium and high intakes.

Energy and nutrient intakes were well within the estimated average requirements (EAR) and reference nutrient intake (RNI) for the UK female population aged 19-50 years as shown in the Table (Department of Health, 1991). The mean energy equivalent (energy intake:calculated BMR) (EI:BMR) was 1.4. No significant relationships were found between energy-adjusted nutrient intakes and calcaneus bone mass (mean: 87.4 SD: 18.3 dB/MHz), and no differences were found in women who said they consumed a low intake of milk in their childhood and early adulthood compared with a medium or high intake.

Intake/day	Mean	SD	Median	EAR/RNI	Intake/day	Mean	SD	Median	RNI
Energy (MJ)	8.1	2.3	7.9	8.1	Calcium (mg)	1058	367	1014	700
EI:BMR	1.41	0.42	1.37	---	Iron (mg)	12.5	4.3	12.1	11.4
Protein (g)	80.1	21.8	76.8	45.0	Potassium (mg)	3301	815	3260	3500
Fat (g)	74.3	31.4	69.2	65.8	Sodium (mg)	2607	904	2533	1600
Carbohydrate (g)	240	70.4	237	193	Vitamin C (mg)	122	82	101	40
NSP (g)	14.9	5.7	14.3	12.5	Vitamin D (μg)	3.3	1.9	3.0	---

The findings in this study did not demonstrate a positive relationship between nutritional factors and bone health, which has previously been reported in both hip and spine bone mineral mass (New *et al.* 1995,1996). Further investigations of the BUA technique as a method for bone mass assessment are required.

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Little evidence of ethnic differences in bone mineral status between British and Gambian adults resident in the United Kingdom. By BAKARY DIBBA, ANN PRENTICE, M. ANN LASKEY and DOROTHY M. STIRLING, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

The incidence of osteoporotic fracture is low among people of African ancestry, despite low Ca intakes. Studies of African-Americans suggest this is related to ethnic differences in bone mineral content. However, our investigations in The Gambia have failed to demonstrate higher bone mineral or lower menopausal bone loss in West African women (Prentice *et al.* 1991). To explore this more fully, we have used whole-body dual energy X-ray absorptiometry, a technique unavailable in The Gambia, to compare the bone mineral status of African men and women, born and brought up in The Gambia but currently resident in the UK, with those of British adults of similar weight and height.

Table 1	Gambian men		British men		Gambian women		British women	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ca intake (mmol/d)	20.3	11.4	24.3	11.1	16.6*	8.0	24.2	6.1
Weight (kg)	67.4	7.6	70.7	10.2	56.8	11.8	64.6	11.4
Height (m)	1.74	0.06	1.76	0.05	1.63	0.05	1.65	0.06

* $P \leq 0.05$.

The study involved twenty-two men (twelve Gambian, ten British; 18-48 years) and seventeen premenopausal women (seven Gambian, ten British; 22-46 years). Whole-body bone mineral content (BMC, g) and bone area (BA, cm^2) were measured using an Hologic QDR/1000w, with separate regional scans of the lumbar spine, hip and radius. Ethnic differences were examined by ANOVA or ANCOVA with Sheffé post-hoc tests; men and women were considered separately. Percentage differences were obtained from these analyses with data transformed to natural logarithms; bone data were adjusted for size using BA, weight and height (Prentice *et al.* 1994). Bone data were expressed as BMC, BMD (BMC/BA, g/cm^2) and size-adjusted BMC. Table 1 gives subject characteristics. The Gambian women had lower Ca intake than the British as measured by prospective 7 d diary; there was no difference between the men. The Gambians reported a lower past Ca intake than the British.

Table 2	Men			Women		
	BMC	BMD	size-adj BMC	BMC	BMD	size-adj BMC
Whole-body	0 (-14, +13)	+1 (-6, +8)	+2 (-3, +8)	-8 (-23, +8)	-1 (-8, +6)	+2 (-6, +8)
Spine L1-4	-6 (-26, +15)	+2 (-12, +16)	+11 (-3, +25)	-16 (-34, +2)	-5 (-17, +8)	+6 (-8, +20)
Radius shaft	-4 (-16, +8)	-1 (-7, +6)	+1 (-4, +6)	-3 (-18, +12)	-4 (-13, +6)	0 (-9, +9)
Radius wrist	-5 (-21, +11)	+1 (-10, +12)	+4 (-8, +16)	-6 (-24, +12)	-4 (-15, +8)	+2 (-11, +14)
Femoral neck	+10 (-5, +25)	+21* (+8, +33)	+21* (+6, +36)	0 (-26, +27)	0 (-17, +17)	+5 (-6, +16)
Trochanter	-7 (-24, +10)	+1 (-15, +18)	0 (-19, +19)	-20 (-43, +3)	-8 (-23, +6)	+2 (-10, +14)

Data are mean (95% confidence interval) for the percentage difference between Gambian and British subjects.* $P \leq 0.01$.

There were no statistically significant ethnic differences at the whole-body, radius, spine or trochanter. Differences were close to zero or lower in Gambians; there was a trend towards a higher size-adjusted BMC in Gambians at the lumbar spine (Table 2). At the femoral neck, in men only, Gambians had a higher BMD and size-adjusted BMC, associated with a smaller BA (-11%, $P \leq 0.01$).

African-Americans have higher BMC and BMD than Caucasians by 5-15%. In contrast, this study found little evidence of higher bone mineral status in Gambian adults, except at the femoral neck in men.

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Influence of nutrient intake on bone mass in hip osteoarthritis and hip fracture patients. By SUSAN A. NEW[1], ALISON STEWART[2], DAVID A. GRUBB[3] and DAVID M. REID[2]. [1]*Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH,* [2]*Osteoporosis Research Unit, Woolmanhill Hospital, Aberdeen AB9 8AU,* [3]*Computing Department, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Hip osteoarthritis (OA) and hip fracture (HF) are both important causes of locomotor morbidity in the elderly population. OA is a disease of joint cartilage associated with secondary changes in the underlying bone whereas osteoporosis (HF) is the loss of bone tissue, resulting in bones which are brittle and liable to fracture (Cooper *et al.* 1991). The aim of the present study was to examine the influence of dietary intake on bone mineral density (BMD) in a group of OA and HF patients. Fifteen women with osteoarthritis of the hip who were awaiting surgery, and twelve women who had sustained a hip fracture at least 3 years previously were recruited for the study. All patients were aged between 62 and 79 years. Bone mineral density of the hip (femoral neck (FN); femoral trochanter (FT); femoral Wards (FW)) and total body (TB) was measured using dual energy X-ray absorptiometry (DXA; Norland XR-26). Bone mass of the os calcis was measured using ultrasound attenuation (BUA; McCue Electronics CUBA). Dietary intake was assessed using a food-frequency questionnaire previously developed and validated against 7 d weighed records (Lanham & Bolton-Smith, 1993). Weight (Wt), height (Ht) and years post menopause (YPM) were also recorded. Before analysis, nutrient intakes were adjusted for total energy intake by calculating the residual from regression analysis.

No differences were found in the age, YPM, Wt, Ht, BMI, BUA or TB BMD between the two groups, but BMD at the FN, FT and FW sites was lower in the HF patients ($P<0.01$). No differences were found in energy and nutrient intakes between the two groups although in general mean values were slightly higher in the HF group. For the OA group, significant correlations were found between energy-adjusted intakes of Ca and TB, FN, FT and FW BMD as shown in the Table. Correlations were also found between protein, K and Mg intake and BMD. These relationships remained significant after adjustment for age, YPM, Wt and Ht. No relationships were found between nutrient intake and BMD in the HF group.

	OA group (*n* 15) Bone mineral mass measurement					HF group (*n* 12) Bone mineral mass measurement				
Energy-adjusted nutrient intake	TB	FN	FT	FW	BUA	TB	FN	FT	FW	BUA
Calcium	0.78***	0.84***	0.74***	0.76***	0.44	0.23	0.27	0.18	0.26	0.04
Protein	0.43	0.54*	0.65**	0.42	0.53	0.48	0.37	0.37	0.30	0.06
Potassium	0.73***	0.35	0.37	0.27	0.11	0.12	0.04.	0.08	0.14	0.23
Magnesium	0.65**	0.35	0.34	0.26	0.27	0.00	0.09	0.31	0.03	0.4
Vitamin C	0.37	0.21	0.21	0.20	0.25	0.13	0.18	0.17	0.05	0.16

Partial correlation coefficients * $P<0.05$; ** $P<0.01$; *** $P<0.001$

These results show there to be no differences in current dietary intake in OA or HF patients. However, a positive relationship between current intakes of Ca, protein, K and Mg bone mass in patients with OA was found. Analysis of differences in past dietary habits between the two groups are required.

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Erythrocyte folate analysis; effect of pH of ascorbic acid diluent, point of deconjugation, and length and temperature of haemolysate storage. By ANTHONY J.A. WRIGHT[1], JUAN M. ROMERA[2], PAUL M. FINGLAS[1] and SUSAN SOUTHON[1], *[1]Institute of Food Research, Norwich NR4 7UA and [2]UNIASA, R&D Department, Granada, Spain*

Using synthesized polyglutamates of folic acid (PGA-glu_{2-7}), Goli & Vanderslice (1992) concluded that deconjugation of folate polyglutamates with human plasma folate conjugase (*EC* 3.4.12.10) at an acidic pH could result in degradation and loss of folates, while between pH 6.0 and 7.0 optimum results could be obtained. This raises doubts about the accuracy of most erythrocyte folate analysis since it is common practice to dilute blood samples tenfold with 1% ascorbic acid (pH about 2.8) before 'self-deconjugation', as a means of protecting against oxidative damage and ensuring complete lysis.

Pressure for a change in blood diluent pH also comes from a second direction. Most blood-folate analysis is currently conducted using commercially available radioisotope folate-binding protein assay (RFBP) kits. These kits use milk folate-binding protein(s) which exhibit different pH-dependant affinity curves for folic acid (assay calibrant) and 5-methyltetrahydrofolic acid which is the folate form found, post-deconjugation, in erythrocyte haemolysates. Equal affinity for both folate forms has been demonstrated at ca. pH 9.3 and this is the pH of most diagnostic kit assays. However, small differences in assay pH can strongly affect RFBP assay results and a number of factors that may cause pH shifts have been noted or suggested by Van den Berg *et al.* (1994). Because of the fear that 'acidic' samples might reduce RFBP assay pH, it is commonly proposed that the 1% AA-pH 2.8 diluent should be adjusted to a more neutral pH, or that it should be replaced by its more neutral salt (sodium ascorbate).

An experimental protocol was designed to investigate the effect of: (a) erythrocyte diluent pH, (b) the point of folate deconjugation, (c) lysate storage temperature, and (d) lysate storage time. A sample of fasting venous whole-blood was divided and diluted tenfold with either 1% AA-pH 2.8 or 1 M-NaOH-adjusted AA (pH 6.0) and stored for up to 9 months in either liquid N_2 or at -20°. Samples were deconjugated either pre-storage or just before microbiological folate assay with *Lactobacillus rhamnosus* NCFB 243 (= ATCC 7469, NCIB 6375) on 96-well tissue-culture plates. Results were assessed by ANOVA, regression analysis and t-test. Deconjugation, pre- or post-storage, did not affect folate values. There was no effect of storage temperature and appropriate results were pooled and statistically examined by 2-way ANOVA, with variables 'time', 'pH' and 'time x pH' interaction.

Table. *Change in erythrocyte folate value (ng/ml whole-blood) with length of sample storage (d) and initial blood diluent, ascorbic acid or ascorbic acid adjusted to pH 6.0.*

Storage (d)...	1	8	22	176	267	Significance of variance ratio (F) for time, pH and time x pH interaction.		
No. of replicates...	32	24	32	32	32			
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Time	pH	Ti x pH
Blood diluent								
Ascorbic acid, pH 2.8	132.2[a] (11.8)	153.3[c] (14.7)	143.3[ac] (15.3)	105.5[b] (11.7)	146.2[ac] (12.9)	$P<0.001$	$P<0.001$	$P<0.001$
Ascorbic acid, pH 6.0	80.4[a] * (10.9)	95.1[ab] * (14.6)	86.8[ab] * (12.5)	97.6[b] (12.3)	126.2[c] * (18.4)			

a,b,c Within a row, values not sharing a common superscript were significantly different ($P<0.05$).
* Mean erythrocyte folate value for the 'AA-pH 6.0' blood diluent was significantly different ($P<0.001$) from the mean value for the 'AA-pH 2.8' blood diluent on the corresponding day of storage.

Folate values using AA-pH 6.0 were 40% lower than with AA-pH 2.8. Folate values with AA-pH 2.8 did not change systematically with storage, but values with AA-pH 6.0 increased with length of storage (r 0.892, $P<0.05$) suggesting that folate was initially bound rather than destroyed.

It was concluded that until further extensive and detailed investigations are undertaken, researchers should continue using 1% ascorbic acid (pH about 2.8) as the whole-blood diluent before 'self-deconjugation' and erythrocyte folate analysis.

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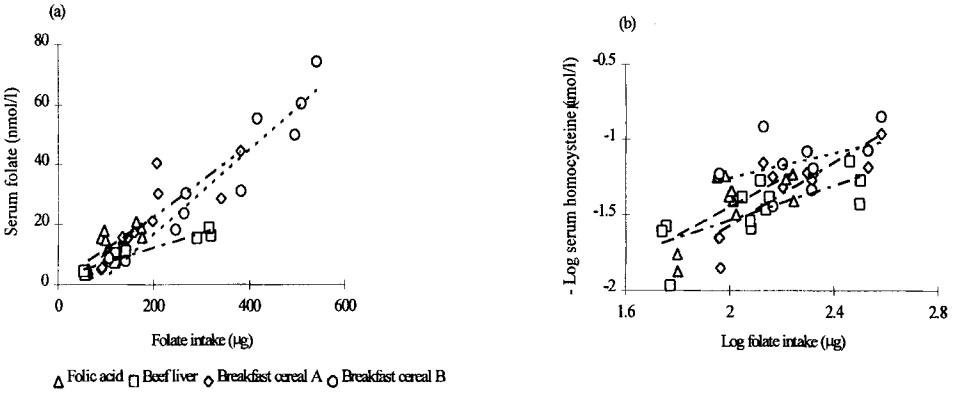
Response of static and functional status variables to folate repletion in depleted rats. By C. A. MARTIN and P.J.A. SHEEHY, *Department of Nutrition, University College, Cork, Ireland*

Laboratory methods for assessment of nutritional status can be divided into those which measure direct (static) and indirect (functional or dynamic) variables. For folate, static variables include circulating folate levels and tissue stores, while functional variables include formiminoglutamic acid (FIGLU) tests, plasma homocysteine, and various forms of DNA synthesis tests. Selection of appropriate folate status variables is important in order to study the relationship between folate intake and disease risk, and to study folate bioavailability from foods. The objective of the present study was to evaluate the change in concentration of a range of folate status variables during repletion of folate-depleted rats.

Sixty weanling male Wistar rats were divided into two groups of equal mean weight. One group (n 56) was fed on a folate-deficient diet containing 1% succinyl sulphathiazole (to suppress microbial folate synthesis) for 28 d. A control group (n 4) was fed on the same diet supplemented with 500 μg folic acid/kg. After depletion, three rats from the folate-deficient group were randomly assigned to each of sixteen repletion diets containing folic acid, lyophilized beef liver, breakfast cereal A (maize-based) or breakfast cereal B (rice-based) calculated to provide 100, 250, 375 or 500 μg folate/kg diet. After a further 28 d, rats were killed by cardiac puncture under urethane anaesthesia.



After 28 d, serum folate concentration measured by microbiological assay in depleted rats was significantly lower (1.10 (SE 0.31) *v.* 19.8 (SE 0.00) nmol/l $P < 0.001$) and serum total homocysteine determined by HPLC was significantly higher (92.5 (SE 6.36) *v.* 18.8 (SE 1.18) μmol/l, $P < 0.001$) than in control rats. After the repletion phase, concentrations of folate in serum (Fig. a), as well as in liver and kidney (data not shown) were directly related to folate intake. Responses to diets containing folic acid and breakfast cereal A were similar, while the lowest response was observed in rats fed on the beef-liver diet. The inverse log of serum total homocysteine concentration was directly related to log folate intake (Fig. b). Responses to diets containing folic acid and breakfast cereal A were similar, that to beef liver was lower, while the lowest response was to breakfast cereal B. In this depletion-repletion rat model, serum, liver and kidney folate and serum total homocysteine clearly reflected differences in intake in the low and moderate ranges. Homocysteine was a less useful indicator of folate status at high intakes, probably because the folate deficiency which led to hyperhomocysteinaemia during the depletion period had been corrected.

Changes in erythrocyte folate status in response to increasing levels of pteroylglutamic (folic) acid supplements; implications for preventing neural tube defects. By GERALDINE J. CUSKELLY[1], HELENE MCNULTY[1] and JOHN M. SCOTT[2], *[1]University of Ulster, Coleraine BT52 1SA and [2]Department of Biochemistry, Trinity College Dublin, Ireland*

Maternal erythrocyte folate (RCF) concentrations above 400 μg/l (908 nmol/l) are associated with lowest risk of neural tube defects (NTD; Daly *et al.* 1995). Current Department of Health (1992) recommendations for the prevention of NTD with no previous history is an extra 400 μg folic acid or folate/d above usual dietary intake. However, it remains to be confirmed whether equally optimal protection (in terms of RCF concentration) might be achieved by levels of intake lower than this amount. The objective of the present study was to investigate the effect on RCF status of supplemental folic acid in the range 100 - 400 μg/d.

Following recruitment, females (non-pregnant, non-consumers of folic acid-fortified foods, non-sufferers of a NTD, nor mothers of a baby with NTD, non-users of medication or suffering from a chronic illness known to interfere with folate metabolism, not taking supplements containing folic acid; *n* 50), were randomized into groups to receive either placebo, or 100, 200, 300 or 400 μg supplemental folic acid/d for 12 weeks. RCF was measured pre- and post-intervention by microbiological assay. Dietary intakes (estimated using the diet history method supplemented with a food frequency questionnaire) were assessed pre- and mid-intervention. Responses were assessed by paired *t* test in those who completed the study (*n* 28), following transformation of data where appropriate.

	Folic acid supplement (μg/d)									
	Placebo		100		200		300		400	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Erythrocyte folate (μg/l) *n*	4		7		6		5		6	
Pre-intervention	236	62	327	52	304	51	307	46	314	74
Post-intervention	262	105	460**	104	449***	74	674**	149	613**	119
Mean % change	8	23	41	28	49	13	115	69	98	50
Dietary folate intake (μg/d) *n*	4		7		6		5		6	
Pre-intervention	215	116	271	78	201	85	203	105	212	114
Mid-intervention	173	55	202	31	213	74	173	67	225	84
Mean % change	-11	22	-21	21	11	18	-8	26	26	48

Mean values were significantly different from pre-intervention: , ** $P < 0.01$, *** $P < 0.001$.

Before intervention, all subjects had RCF concentrations within the normal range. There were no significant changes in dietary folate intake over the period of intervention, indicating that the response of RCF to intervention was most probably due to folic acid supplementation. Intervention at all levels caused a significant increase in mean RCF concentrations reaching levels above 400 μg/l. However, within the 100 μg/d and 200 μg/d supplementation groups, individual subjects failed to achieve this optimal concentration (*n* 2 in both cases). These results suggest that levels of supplemental folic acid as low as 100 μg/d increase mean RCF concentrations above 400 μg/l, the level associated with lowest risk of NTD but in order to ensure optimal folate status in all subjects, 300 μg/d of additional folic acid is required. This level is lower than the current Department of Health (1992) target of 400 μg/d and the result may therefore have implications for a folic acid fortification policy. Further studies are needed to examine the response to intervention in groups with lower RCF status.

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The response of plasma homocysteine to low-dose pteroylglutamic (folic) acid supplementation in healthy male subjects. By M. WARD[1], H. McNULTY[1], J.M. Mc PARTLIN[2], J.J. STRAIN[1], D. WEIR[2] and J. M. SCOTT[3], *[1]Human Nutrition Research Group, University of Ulster, Coleraine BT52 ISA and Departments of [2]Clinical Medicine and [3]Biochemistry Trinity College, Dublin, Ireland*

It is now widely accepted that even moderate elevations in plasma homocysteine constitute an independent risk factor for coronary heart disease (Boushey *et al.* 1995). Since intracellular homocysteine is metabolized by remethylation to methionine by the methionine synthase (*EC* 2.1.1.13) reaction requiring 5-methyltetrahydrofolate as a co-substrate, the possibility of reducing plasma homocysteine levels by administration of folic acid is of interest. Previous studies have shown a homocysteine-lowering effect of folic acid at doses ranging from 0.5 to 5 mg/d (Den Heijer *et al.* 1995), however the effect of lower doses is currently unknown. The purpose of this pilot study was to investigate the effect of low-dose folic acid (200, 400 µg/d) on plasma homocysteine concentrations in a group of healthy, normohomocysteinaemic male subjects (*n* 8).

Subjects aged 44 - 51 years (non-consumers of folic acid-fortified breakfast cereals, not taking medication or vitamin supplements, and free from vascular, hepatic and renal disease and haematological disorders) received folic acid supplements at a dose of 200 µg/d, until weekly analysis of serum folate (SF;microbiological assay) concentration indicated that a maximum response had been achieved. The daily dose was then increased to 400 µg and SF continued to be analysed until a new plateau was established. Fasting samples collected at baseline, in response to folic acid supplementation at 200 µg/d, at 400 µg/d and post-intervention were analysed for SF and plasma homocysteine concentrations by HPLC. Data were transformed as appropriate and compared with baseline values using the paired *t* test.

	Baseline		Supplementation				Post-supplementation			
			6 weeks (200µg/d)		14 weeks (400µg/d)		5 weeks		10 weeks	
	n 8		*n* 8		*n* 8		*n* 5		*n* 8	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Serum folate (µg/l)$^{\Delta}$	4.3	1.8	8.1**	1.7	23.7***	4.4	9.0**	2.3	6.8**	2.4
Plasma homocysteine (µmol/l)	8.40	2.40	6.94**	1.86	6.51**	2.09	8.05**	2.60	8.27	1.71

Significantly different from baseline : ** $P<0.01$, *** $P<0.001$ (paired *t* test). $^{\Delta}$ µg/l ÷ 441.4 = µmol/l

All subjects had SF concentrations within the normal range on recruitment. The maximum response to folic acid supplementation at 200 µg/d was reached at week 6; however, when the dose was increased, analysis of SF levels showed that a new plateau was not established until week 14 of supplementation at 400 µg/d (results not shown). Supplementation with 200 µg folic acid/d for a period of 6 weeks resulted in a significant decrease of 1.46 µmol/l in mean plasma homocysteine concentration. While increasing the dose of folic acid to 400 µg/d for a further 14 weeks resulted in an additional mean decrease of 0.43 µmol/l, this was not significantly different from the response to 200 µg/d. By 5 weeks post-supplementation plasma homocysteine concentration had increased, but did not return to baseline until 10 weeks post-supplementation. These results indicate that a significant homocysteine-lowering effect can be achieved by folic acid intervention at levels as low as 200 µg/d, which would be achievable through food fortification. The present pilot study offers support to recent arguments for a more aggressive public health initiative towards folic acid fortification (Bower & Wald, 1995), however confirmation of these results is currently being pursued in a larger study by this research group.

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The effects of oral vitamin supplementation on cardiovascular risk factors. By J.V. WOODSIDE[1], J.W.G. YARNELL[1], I.S. YOUNG[1], D. McMASTER[1], K.F. GEY[2], C. MERCER[1], C.C. PATTERSON[1], E.E. McCRUM[1] and A.E. EVANS[1], [1]*School of Clinical Medicine, The Queen's University of Belfast, Grosvenor Road, Belfast BT12 6BJ and* [2]*Department of Biochemistry, University of Berne, Switzerland.*

It is now recognised that elevated plasma homocysteine concentration and the susceptibility of LDL to oxidation are independent risk factors for premature vascular disease. The aim of this present study was to assess the effects of vitamin supplementation on these risk factors. A total of 610 men aged 30-49 years were recruited from a local workforce. Those taking dietary supplements (16.6%) were excluded from further study. Fasting plasma samples were collected and lifestyle details noted. Total homocysteine was assayed by HPLC according to the method of Ubbink *et al.* (1991). The homocysteine distribution was positively skewed with a median of 7.18 μmol/l and an interquartile range of 2.69. Log-transformed homocysteine was inversely correlated with both log serum folate and log vitamin B_{12} levels (r -0.45, -0.38 respectively, $P<0.001$).

Men with a homocysteine level >8.34 μmol/l (roughly the top 30% of the distribution) were invited to join the intervention study. The 132 willing men were randomly assigned using a factorial design to one of four groups: supplementation with B group vitamins alone (1 mg pteroylglutamic acid, 7.2 mg pyridoxine, 0.02 mg cyanocobalamin), antioxidant vitamins (150 mg ascorbic acid, 100 mg α-tocopherol, 9 mg β-carotene), B vitamins with antioxidant vitamins, or placebo. Intervention was double-blind. Baseline vitamin levels are shown in the table.

	Mean	SE
Vitamin B_{12} (pmol/l)	273.73	10.42
Vitamin B_6 (nmol/l)	50.67	5.26
Folic Acid (nmol/l)	11.09	0.47
Vitamin C (μmol/l)	28.35	1.91
Vitamin E (μmol/l)	31.52	1.00
β-carotene (μmol/l)	0.216	0.019

A total of 101 men completed the 8-week intervention period. When the homocysteine levels were analysed by group, statistically significant ($p<0.05$) decreases were observed in the two groups receiving pteroylglutamic acid, pyridoxine and cyanocobalamin (with and without antioxidants). Pooling the data showed that there was no interaction between the B-group vitamins and the antioxidants. The effect of B-group vitamins alone over 8 weeks was a reduction of 27.9% (95%CI 22.0%, 33.0%, $P<0.001$) while antioxidants alone produced a non-significant increase of 5.1% (95%CI -2.8%, 13.6%, $P=0.21$).

Resistance to LDL oxidation was measured as described by McDowell *et al.* (1995) and preliminary results show that the lag time to oxidation was increased in the two groups receiving antioxidants whether with (7.76±1.03 min) or without (8.65±1.03 min) B vitamins (Mean±SE, $P<0.001$).

In the absence of any obvious interaction between the two groups of vitamins, this study shows that a supplement containing B-group vitamins and antioxidants may be effective in reducing cardiovascular risk.

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Macronutrient intake of Glaswegian adults compared with Scottish Nutrient Targets. By ANNIE S.ANDERSON, LINDA MAHER and M.E.J. LEAN, *University of Glasgow Department of Human Nutrition, Glasgow Royal Infirmary, Glasgow G31 2ER*

Following the publication of The Scottish Diet Report (Scottish Office 1993) there has been widespread health promotion encouraging diets high in fibre-rich carbohydrate and low in fat (especially saturated fat).

In the present study a random sample of 160 free-living pre-dominantly middle class Glaswegian adults recruited from the Community Health Index completed 7 d weighed dietary records during 1995 which were analysed by the COMP-EAT dietary analysis programme (Nutrition Systems, London).

Almost half (49%) the men were achieving the current nutrient target for dietary fat, but only a quarter (24.5%) were achieving targets for NSP and a quarter (24.5%) for saturated fat. The proportions of women who achieved targets were only a third (32.7%) for fat, 15.9% for saturated fat and 8.4% for NSP. No individual achieved the recommendation of 40% energy from starch. There was a significant, inverse linear correlation between percentage energy from fat and percentage energy from sugars (R -0.44, $P < 0.001$), but no significant relationship between percentage energy from fat and percentage energy from starch (R -0.10, NS).

Total energy intake was rather low with 25% of men and 42% of women reporting energy intakes less than 1.2 x BMR.

	Men (N 53)		Women (N 107)		Nutrient target
	Mean	SD	Mean	SD	(Scottish Office, 1993)
Energy (MJ/d)	9.9	2.5	7.4	1.8	Not detailed
% energy from protein	15.5	2.4	14.7	2.7	Not detailed
% energy from fat	36.2	6.3	37.3	5.0	<35
% energy from saturates	13.8	3.8	14.0	4.6	<11
% energy from carbohydrates	42.7	6.8	44.2	5.7	
% energy from starch	23.9	5.4	25.2	4.6	>40
% energy from sugars	17.8	5.9	17.8	6.0	<10
% energy from alcohol	5.3	6.2	3.4	4.4	
NSP (g/d)	13.0	5.6	10.4	3.8	>16

These cross-sectional results suggest some improvements in nutrient intake since the weighed survey of Gregory *et al.* (1990). Focusing on fat alone in health promotion may result in failure to achieve other targets such as starch, especially if intake of sugars is inversely related to fat.

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Differences in macronutrient intake after changing to a vegetarian diet. By FRANCES ROBINSON and ALLAN HACKETT, *School of Education and Community Studies, Liverpool John Moores University, Barkhill Road, Liverpool L17 6BD*

There is a considerable amount of information supporting the notion that consuming a vegetarian diet confers health benefits. For example, non-meat eaters have rather lower standardized mortality ratios than meat eaters for ischaemic heart disease (Thorogood *et al.* 1994). It is unclear, however, whether a person changing from a mixed to a vegetarian diet would enjoy the same benefits. An investigation of the effects of changing to a vegetarian diet for a period of 12 months (Johansson *et al.* 1992) found that energy intake decreased, but this study included substantial guidelines on what to exclude from the diet for its duration. The effects of changing to a non-prescribed vegetarian diet have received little attention. In the present study, adults (mean age 30.8 years) who were about to change to a vegetarian diet kept a record of their food intake for 3 d whilst still consuming a mixed diet. (Several subjects (*n* 18) who had become vegetarian up to 3 months prior to recruitment had their pre-vegetarian diet assessed by a retrospective diet history.) Questionnaires and anthropometric measurements were also completed. A second 3 d food intake record was collected 3 months after subjects had changed to their vegetarian diet. The differences in intakes were examined by use of a paired *t* test.

	Males (*n* 9)				Females (*n* 30)			
	Pre vegetarian		Post vegetarian		Pre vegetarian		Post vegetarian	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Energy (kJ)	11143	665	10303	420	8271	263	7977	387
% Protein energy	13.6	0.9	12.3	0.9	14.0	0.5	13.0	0.5
% Carbohydrate energy	44.9	1.3	45.6	2.1	45.0	1.1	48.6*	1.2
% Sugars energy	17.8	1.3	15.1	1.4	17.5	1.0	19.8**	1.0
% Fat energy	37.7	1.4	36.1	1.9	35.7	0.9	33.3	1.1
% Alcohol energy	4.1	1.4	6.1	1.3	5.3	1.3	5.2	1.0
EI : BMR	1.4	0.1	1.3	0.1	1.4	0.1	1.3	0.1
P : S	0.50	0.07	0.62	0.12	0.53	0.05	0.58	0.06

Significantly different from pre-vegetarian : * P = 0.005 ** P = 0.004

The results show that after 3 months of following a self-selected vegetarian diet, only the females showed marked differences in their macronutrient intake. The percentage energy from carbohydrate was significantly higher for females after 3 months on a vegetarian diet, but it would appear that most of this difference could be explained by the increase in percentage energy from total sugars. The reductions in energy intake and in the proportion of energy from fat and protein and the rise in the polyunsaturated : saturated fatty acid ratio (P:S) of both males and females were not statistically significant.

As current recommendations endeavour to increase dietary carbohydrate and reduce fat intakes, it would appear that changing to a vegetarian diet, even when the diet was self-chosen, had beneficial effects on both of these variables, although not significantly so for the fat (P= 0.07), at least for the women in the study.

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Dietary changes made by vegetarians and non-vegetarians during pregnancy. By RANA E. DRAKE[1], SHEELA REDDY[2], and G. JILL DAVIES[1], *[1] Nutrition Research Centre, South Bank University, 103 Borough Road, London SE1 0AA, [2]Leatherhead Food RA, Randalls Road, Leatherhead, KT22 7RY*

Vegetarian diets are being adopted by a growing number of young women, yet little is known about the effect this could have upon pregnancy and its outcome. Slightly lower birth weights have been reported among vegetarians than non-vegetarians (Reddy *et al.* 1994). The reason for this has not been found, however results from studies of non-pregnant vegetarians suggest they may be at increased risk of poor nutritional status with regard to Fe, folate and/or vitamin B_{12} (Sanders, 1995). These studies can only act as a rough indicator since pregnancy is associated with a variety of dietary changes which may exert a considerable effect upon diet quality.

Magazine adverts were used to recruit women for a postal questionnaire. Of the 197 questionnaires distributed 162 were returned. Questionnaires completed by 133 white primiparous women living in the UK were analysed. Five of the respondents who were excluded had started to eat meat or fish since becoming pregnant although they had not consumed these for between 6 and 10 years before pregnancy.

Information was obtained about dietary changes made since becoming pregnant, including cravings and aversions as well as any intentional changes. Sources of dietary advice were also investigated and respondents were asked about the usefulness of advice received.

	Lacto-ovo-vegetarians (*n* 32)	Pesco-vegetarians (*n* 18)	Non-vegetarians (*n* 79)
% who wanted to know about diet in pregnancy	91	100	84
% adding/increasing certain items	88	94	89
Most common items added/increased*	fruit, milk, dairy produce, breakfast cereals, vegetables, yeast extract	fruit, fruit juice, breakfast cereals, milk, dairy produce	fruit, milk, vegetables, dairy produce, confectionery, fruit juice
% decreasing/stopping certain items	97	89	94
Most common items decreased/stopped*	alcohol, soft cheeses, tea/coffee, soft eggs	soft cheeses, alcohol, soft eggs, snacks†, tea/coffee	alcohol, soft cheeses, pate, soft eggs, tea/coffee, liver

* Items mentioned by more than 10% of the dietary group are listed in rank order.
† Biscuits, cakes, chocolate, crisps.

Books, Department of Health publications and magazines were reported to be the most useful sources of dietary advice. Almost half (44 %) of lacto-ovo-vegetarians and 39 % of pesco-vegetarians believed the advice available to them was inadequate, compared with 15 % of non-vegetarians.

These findings suggest that whilst some vegetarians are making beneficial dietary changes particularly those that enhance intakes of Fe, vitamin B_{12} and vitamin C during pregnancy others may require specifically targeted advice to help facilitate such changes.

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Sanders, T.A.B. (1995). *Pediatric Nutrition* **42**;955-965.

An index of fruit and vegetable consumption: data from the UK lone-parent nutrition survey. By ELIZABETH A. DOWLER[1] and CLAIRE CALVERT[2]. *[1]Human Nutrition Unit, London School of Hygiene and Tropical Medicine, 2 Taviton Street, London WC1H 0BT and [2]Division of Public Health Medicine, Nuffield Institute for Health, 71-5, Clarendon Road, University of Leeds, Leeds LS2 9PL*

The recent survey of nutrition and diet in lone-parent households in London used a food-frequency questionnaire (FFQ) to investigate food variety; results are presented elsewhere (Dowler & Calvert, 1995). FFQ data were obtained from 189 parents. Answers to the fifty-nine questions listing fruits and vegetables (pulses and fruit juices were omitted) were used to calculate the probability that parents were eating at least five different fruits or vegetables daily. Frequency responses of "most days" were weighted as 0.5, "once or twice a week" as 0.145 and all other responses as "0". The weighted responses were summed; the counts obtained ranged from 1 to 27 with a mean of 2.0. This count was compared with intakes of NSP, folate, vitamins A, C and E from a 3-d intake survey from 128 parents using regression and correlation; weak correlations between 0.2 and 0.3 were obtained ($P<0.01$).

The counts were grouped as "below 2.5", "2.5 - below 5" and "5 and over", to produce a crude fruit-vegetable indicator. These categories were also compared with nutrient intakes; the results are shown in the Table. The main differences in nutrient intake occurred between those eating fewer than 2.5 fruits or vegetables daily, and the rest of the group.

	<2.5 fruit or veg/d (*n* 40)		2.5-<5 fruit or veg/d (*n* 45)		5+ fruit or veg/d (*n* 43)	
Nutrient	Mean	SE	Mean	SE	Mean	SE
NSP (g)	9.3*	0.60	10.6	0.74	11.2	0.68
Zinc (%RNI)	100*	5.3	108	6.1	124	6.9
Folate (%RNI)	83*	6.0	102	6.1	104	7.2
Vit A (ret.equiv.) (%RNI)	86	†	110		122	
Vitamin E (%RNI)	154	†	162		200	
Vitamin C (%RNI)	74**	†	134		142	

RNI, reference nutrient intake (Department of Health, 1991).
Significant differences between groups *$P<0.05$, **$P<0.01$;
† Geometric mean - no SE.

People eating at least five fruits or vegetables a day were more likely to be black Africans, black British or Caribbean than white Europeans ($P<0.002$). They were also more likely to come from non-manual classes ($P<0.005$), to have tertiary education qualifications ($P<0.01$), and not be in receipt of income support ($P<0.002$). Those who said they always looked for "fresh" food when shopping were much more likely to be eating more than five fruits or vegetables daily ($P<0.0001$), particularly if they were not poor. There was no relationship with parental smoking, being a vegetarian, age or family size.

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Department of Health (1991). *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects* no. 41. London: H.M. Stationery Office.

Do the increased weight-gain recommendations for pregnancy predispose parous women to obesity? By HELEN E. HARRIS[1], GEORGE T. ELLISON[1], MARY HOLLIDAY[2] and EMY LUCASSEN[1], [1]*Maternal and Child Health Research Programme, and* [2]*School of Health, University of Greenwich, Wellington Street, London SE18 6PF*

In the last few decades, advice given by health care professionals to pregnant women on weight gain has changed dramatically, with recent trends showing an increase in weight-gain recommendations. These recommendations have raised concerns that mothers may be placed at greater risk of obesity (Institute of Medicine (IoM), 1990). Following the most recent increase in the US weight-gain recommendations for pregnancy (IoM), 1990), the present study aimed to investigate whether the higher BMI observed at higher parities is the result of cumulative increases in body weight that occurred during successive pregnancies, or whether women of higher parity simply gain more weight in association with pregnancy. To investigate these possible explanations, we used a repeat pregnancy study to examine the change in body weight displayed by women from the beginning of one pregnancy to the beginning of the next. Detailed information on sociodemographic, clinical and behavioural characteristics was abstracted from the medical records of women who gave birth to their second or higher order singleton children in the principal maternity ward of a South Thames hospital between 1992 and 1993. Any mothers whose obstetric records were available for their 1992/1993 pregnancy and the previous one were eligible for inclusion in the study. Complete data were available for 597 mothers. The relationship between parity and BMI amongst the women was initially examined using analysis of covariance (ANCOVA) with BMI at the start of the 1992/1993 pregnancy as the dependent variable. The independent role of both parity and weight gain during pregnancy on the increase in maternal BMI was then assessed using a second ANCOVA with change in BMI from one pregnancy to the next as the dependent variable.

When the sociodemographic, anthropometric, behavioural and obstetric variables were entered into the ANCOVA, they explained 80.4% of the total variance in BMI at the start of the 1992/1993 pregnancy, with parity (r^2 0.029, P <0.001) explaining a significant proportion of the variance. The second ANCOVA explained 19.5% of the variation in BMI-change between pregnancies (see Table).

Covariates	*B*	β	SEM	*P*	r^2
Inter-pregnancy interval (days)	0.001	0.313	0.000	<0.001	0.076
Maternal age (years)	-0.024	-0.057	0.019	0.211	0.002
Socio-economic group	-0.063	-0.055	0.046	0.169	0.003
Nulliparous BMI (kg/m^2)	0.074	0.161	0.018	<0.001	0.024
Weight gain in pregnancy (kg)	0.110	0.255	0.017	<0.001	0.054
Factors			*F*	*P*	r^2
Parity (1, 2, 3 or 4)			3.70	0.012	0.015
Smoking (non-smoker, ex-smoker, 0-10 cigarettes/d or more than 10/d)			2.39	0.068	0.010
Ethnicity (white-European or Asian-Indian)			2.77	0.097	0.004
Marital status (single or married)			4.81	0.029	0.006

Mothers in the present study gained or retained, on average, 0.92 kg/m^2 after their first pregnancy, 1.03 kg/m^2 after their second, 1.20 kg/m^2 after their third and 2.75 kg/m^2 after their fourth pregnancy, after accounting for sociodemographic and clinical confounders. Even after correcting for the increase in weight associated with ageing, both parity (r^2 0.016, P 0.012) and pregnancy weight gain (r^2 0.059, P < 0.001) were still independently associated with the change in BMI between pregnancies. After correcting for the increase in BMI associated with ageing, mothers' BMI was seen to increase, on average, by 0.11 kg/m^2 for every kg weight gained during pregnancy. Consequently, mothers of normal BMI (19.8-26.0 kg/m^2; IoM, 1990) who gain the 16 kg-upper limit of recommended gestational weight gain (IoM, 1990) could expect to retain 1.76 kg/m^2 following the birth of their children. For women of average height (1.65 m), such gains would represent an increase in weight of about 4.8 kg. Clearly, gains of this magnitude will place a number of women at risk of obesity, particularly if they are already of high parity.

Institute of Medicine (1990). *Nutrition During Pregnancy*. Washington, DC: National Academy Press.

Food and nutrient intakes of council nursery-school children in Sheffield. By RACHAEL S. BOND and JANE EARLAND, *Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Sheffield S5 7AU*

The nutrient intakes of fifty pre-school children aged 2-5 years, attending two council-run nursery schools in Sheffield were assessed using a 4 d estimated dietary diary. Parents were supplied with a set of household measures to assist in the estimation of portion sizes. The diaries were checked on the second day and at the end of the reporting period which included one weekend day and 2 d at the nursery school. The investigator measured all the food and drink consumed by the children at nursery school. Height and weight were measured to assess current nutritional status. A questionnaire was used to assess eating habits of the children and the parents' ability to identify 'healthy' foods from a list of twenty-three foods. The children were from social classes III, IV and V and consisted of 69% White and 31% Afro Caribbeans. The mean school leaving age of the mothers was 16.1 years and 63% of the sample were from single parent families.

According to the dietary analyses, the majority of the children had adequate and well-balanced diets. Of the sample, 22% had Fe intakes which were below the reference nutrient intake (RNI). As shown in the Table, individual intakes were also below the RNIs for Zn, vitamin A and vitamin C, with all other nutrients being above requirements.

Nutrient	Main dietary sources	% of sample consuming less than RNI (n 50)	% of sample consuming less than LRNI (n 50)
Iron	Fortified breakfast cereals & meat	22	1
Calcium	Milk & dairy products	0	0
Zinc	Milk & meat	56	4
Vitamin A	Milk & meat	30	0
Vitamin C	Potatoes, fruit juice & fruit	34	0
Folate	Fortified breakfast cereals, milk & meat	0	0

Although mean intakes of NSP were over twice those reported by Gregory *et al.* (1995), fruit and vegetables were eaten on a daily basis by only 17% and 13% respectively. The main sources of fibre for the children in this survey were breakfast cereals, bread and potatoes. There was a significant positive correlation between energy and fibre intakes (r 0.47; $P<0.01$) showing that the energy intakes had not been compromised by the high fibre intakes. Of the total food energy 15% was from added sugars and 37% was derived from fat. There were significant positive correlations between fat and total sugar intakes (r 0.48; $P<0.001$) and between fat and Na intakes (r 0.46; $P<0.01$). Na intakes were up to four times the RNI, with the main food sources being savoury snacks such as crisps and ready-made meals. Anthropometric measurements were within the normal range for age. The values for BMI were similar to those found by Gregory *et al.* (1995). A significant positive relationship was found between energy intake and height (r 0.49; $P<0.001$) and weight (r 0.54; $P<0.001$).

The ability of the parents to differentiate between 'healthy' and 'unhealthy' foods was encouraging, with 56% of parents correctly categorizing twenty-one or more of the foods. However there were no significant correlations between the number of correct answers and their children's intakes of individual nutrients.

In conclusion, the results of this survey are encouraging, particularly with respect to the relatively high intakes of fibre and the ability of the parents to identify 'healthy' foods. However, the high intakes of salt and sugar and low consumption of fruit and vegetables need to be addressed.

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Should caution be exercised in advocating reducing intakes of sugars in children? By T.R. KIRK and C.H.S. RUXTON, *Department of Dietetics and Nutrition, Queen Margaret College, Edinburgh EH12 8TS*

To meet UK dietary reference values (DRV) for fat and carbohydrate would require simultaneous decreases in percentage energies derived from fat and non-milk extrinsic (NME) sugars, and a corresponding increase in percentage energy derived from starch (Department of Health, 1991). However, there is evidence that simultaneous reductions in both fat and NME sugars may be difficult to achieve (Cade & Booth 1990). The present paper reports the relationships between percentage energy from fat and NME sugars in a sample of 136 7-8-year-old children recruited from five Edinburgh primary schools. A 7 d weighed inventory, carried out by parents after training, was used to estimate daily energy and nutrient intakes. Data were analysed using COMPEAT 4.0 and SPSS for Windows.

Mean energy and nutrient intakes have been reported elsewhere (Ruxton *et al*, 1996). Mean values for percentage energy from fat, saturated fat, total sugars and starch were 37%, 14%, 26% and 23% respectively. Energy intakes derived from fat and saturated fat were in excess of DRVs (35% and 11%). An estimate of NME sugar intake was made by subtracting percentage energy from lactose from percentage energy from total sugars (22%) and subtracting from this an assumed 4% energy for intrinsic sugars. The resulting estimate was 18% energy derived from NME sugars. This exceeds the DRV for NME sugars of 11% of food energy.

Thus it would appear, assuming adult DRV for fat and carbohydrate are applicable to children, that there is a need to reduce intakes of both fat and sugar. However, when percentage energies from fat and NME sugars were correlated using Pearson's correlation coefficient, an inverse relationship was found (r -0.65, $P<0.001$) consistent with that reported elsewhere in older (Gibson, 1993) and younger (Payne, 1991) children. A similar inverse relationship was seen when percentage energy from saturated fat and NME sugars were correlated (r -0.24, $P<0.01$). In addition to the negative correlations between fat and sugar intakes found in cross-sectional studies, there is some evidence, in adults, that covert removal of sugar from the diet results in an increase in total fat intake (Naismith & Rhodes, 1995). In view of the inverse relationships described, and assuming that reduction in dietary fat is of higher priority than reduction in dietary sugars, caution should perhaps be exercised in recommending reductions in percentage energy from NME sugars in children. A tentative conclusion can be made, that reducing percentage energy from sugars may not be feasible or desirable, since it is unlikely that children would be able to replace energy from sugars with energy from starch alone, and the result may be an increase in percentage energy from total and saturated fat.

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Frequency of consumption of biscuits, cakes and confectionery: relationship with nutrient intake.

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There is concern that a high consumption of sweet foods such as biscuits, cakes and confectionery, may be associated with low intakes of vitamins and minerals and an increase in susceptibility to obesity. We recently reported that high consumption frequency of biscuits, cakes and confectionery is not associated with increased body weight or BMI (New & Grubb, 1996a). The relationship between consumption frequency and nutrient intake is now reported.

We analysed data from a recent study examining the relationship between nutrition and bone mass, details of which have been reported previously (New *et al.* 1996b). Briefly, 994 randomly selected pre-menopausal women aged 45-49 years were asked to complete a food-frequency questionnaire which had previously been developed and validated against 7 d weighed records (Lanham & Bolton-Smith, 1993). Frequency of consumption (times/d and number d/week) for savoury biscuits (SAB), sweet biscuits (SWB), cakes (C), sugar confectionery (SC) and chocolate confectionery (CC) were divided into low, medium and high categories as follows: SWB and SAB (low 1 t/week or less; medium 2-6 t/week; high 7t/week or greater): C, SC, CC (low < 1 t/week; medium 1-4 t/week; high 5 t/week or greater).

With increasing frequency of consumption of these food groups, intakes of protein, fat, NSP, Ca and Fe increased significantly ($P<0.01$) as shown in the Table. Vitamin C was found to be slightly lower in the high frequency consumption group for CC and SC, but differences were not significant, and intakes were well above the reference nutrient intake.

			Relationship between nutrient intake and frequency of consumption									
	Consumption		Fat (g)		Fibre (g)		Calcium (mg)		Iron (mg)		Vitamin C (mg)	
	Frequency	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
SWB	Low	220	56.5[a]	23.6	14.7[a]	6.4	942[a]	320	11.2[a]	4.0	122.1	74.0
	Medium	318	69.8[b]	24.0	15.0[a]	5.6	1025[b]	310	12.0[b]	3.9	118.2	68.1
	High	457	84.5[c]	29.3	16.0[b]	5.3	1108[c]	348	13.4[c]	4.0	113.1	62.7
SAB	Low	374	69.9[a]	26.4	14.0[a]	5.9	967[a]	317	11.4[a]	4.0	109.0[a]	71.0
	Medium	440	80.9[b]	30.3	15.6[b]	5.2	1044[b]	311	12.7[b]	3.8	117.2[b]	59.9
	High	181	89.1[c]	35.0	17.6[c]	5.4	1201[c]	381	14.2[c]	4.1	132.1[c]	77.0
C	Low	241	60.7[a]	23.5	14.4[a]	5.7	936[a]	300	11.3[a]	3.8	116.9	69.7
	Medium	569	72.3[b]	26.7	15.4	5.7	1049[b]	338	12.4[b]	3.8	117.3	69.7
	High	185	93.8[c]	29.3	16.5[b]	5.4	1163[c]	332	14.3[c]	4.5	115.0	59.5
CC	Low	338	62.8[a]	24.7	14.9	5.8	979[a]	321	11.7[a]	3.9	120.3	77.1
	Medium	540	76.3[b]	28.1	15.7	5.6	1078[b]	343	12.8[b]	4.1	116.4	65.9
	High	117	91.4[c]	30.4	15.1	5.3	1076[b]	328	13.2[b]	4.3	110.1	65.9
SC	Low	697	69.9[a]	26.4	15.4	5.9	1013[a]	327	12.3[a]	4.0	120.7	72.8
	Medium	234	80.7[b]	30.3	15.2	5.0	1100[b]	332	12.8	4.0	109.7	54.2
	High	64	89.1[b]	35.0	15.6	5.0	1189[b]	400	14.1[b]	4.5	103.0	53.1

a,b,c Values with unlike superscripts within a food group were significantly different, $P<0.01$.

These data support previous findings in children (Gibson, 1996), and provide further evidence that nutrient quality of the diet is not affected in individuals who are frequent consumers of sweet foods.

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The relationship between confectionery consumption, physical activity and body mass index (BMI) in a Scottish population. By C. BOLTON-SMITH and M-K. McCLUSKEY, *Cardiovascular Epidemiology, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY*

High confectionery consumption (CC) is commonly presumed to be a major contributing factor to overweight and obesity in developed countries. One of the major contentions against sugary foods in relation to obesity has been that they "draw fat into the diet". Recent analyses indicate that the top five sources of sugars (68 % total) in the UK diet contribute only 11 % of dietary fat (Gibney et al. 1995), and that rising prevalence of obesity is most closely associated with trends in inactivity, not with diet (Prentice & Jebb 1995). A more positive view of confectionery is that it provides a valuable source of energy, especially for the more physically active.

The extent to which frequency of CC was related to levels of physical activity at work and leisure, and to BMI (kg/m^2) was investigated in 1812 men and women aged 25-69 years, who participated in the third Scottish MONICA cross-sectional study in Glasgow in 1991. Physical activity at work and leisure was categorized as low, moderate or high based on tertiles of physical activity levels (multiples of BMR taken from James & Schofield, 1990) derived from hours per week at each level of physical activity reported on the Scottish-MONICA questionnaire. Body weight and height were measured by the study nurse. CC consisted of all types of sweets and chocolates, but not chocolate biscuits which were included as "sweet biscuits". The frequency options on the questionnaire ranged from never, through fortnightly to 1 - 7 times per week.

Table. *Mean BMI and weekly frequency of confectionery consumption by level of physical activity.*

	Work activity level							Leisure activity level						
	Low		Moderate		High			Low		Moderate		High		
n men....	437		234		221			349		270		274		
n women....	447		248		251			385		281		283		
	Mean	SD	Mean	SD	Mean	SD	P	Mean	SD	Mean	SD	Mean	SD	P
BMI														
Men	25.8	4.3	25.7	4.7	25.8	4.4	NS	25.9	4.9	25.9	4.9	25.5	5.1	NS
Women	26.5	5.8	26.1	5.5	25.6	5.8	NS	25.6	5.6	26.3	5.4	26.7	6.2	*
CC														
Men	1.7	2.14	2.0	2.10	2.1	2.1	**	1.7	1.94	1.8	2.10	2.1	2.35	NS
Women	2.1	2.16	2.5	2.27	2.6	2.24	**	2.4	2.31	2.4	2.12	2.3	2.20	NS

Significant differences between the activity groups * $P<0.05$; ** $P<0.01$ by ANOVA (CC data $\sqrt{}$-transformed).

The Table shows that mean BMI did not differ significantly across work physical activity groups for men or women, but CC increased with increasing physical activity at work for men ($P=0.01$) and women ($P=0.002$). Conversely for leisure-time physical activity, BMI was highest in the high activity group for women ($P=0.035$), but no significant differences in frequency of CC occurred. For men, but not women, consumption of other sweet snacks (sweet biscuits and ice cream+yoghurts) was also significantly greater in the higher activity groups at work ($P \leq 0.005$).

These data suggest that both men and women who are active at work tend to eat more confectionery, but this has little bearing on their BMI. Women who are active at leisure tend to have a higher BMI, but report eating confectionery neither more nor less frequently than their leaner counterparts. This may well reflect a change in lifestyle after putting on weight.

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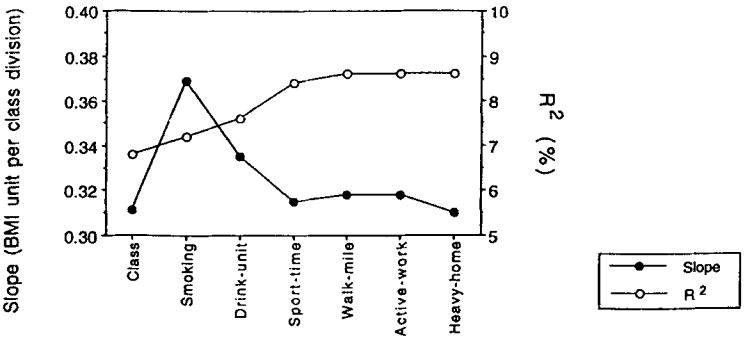
Obesity and social class in women: effects of smoking, drinking and physical activity. By SUSAN A. JEBB, ANDREW M. PRENTICE and TIMOTHY J. COLE, *MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH*

There is a strong social class gradient for obesity in women in the UK. The prevalence of obesity (BMI >30 kg/m^2) rises from 10.7% in social class I to 25.3% in social class V (Bennett *et al.* 1995). The *Health Survey for England* (HSE) 1993 measured a number of behavioural variables which are significantly related both to social class and to BMI (Economic and Social Research Council,1995). The purpose of the present investigation was to test the extent to which the social class gradient for obesity can be explained by interactions between these variables.

The HSE database contains anthropometric measurements from a nationally representative sample of 17687 subjects and associated questionnaire data on smoking, drinking and physical activity. We have analysed data from 5685 women aged 16-60 years with complete data. Variables were analysed as: 'Class', 1=I, 2=II, 3=IIInm, 4=IIIm, 5=IV, 6=V (Office of Population Censuses & surveys,1990); Smoking, Yes/No; Drink-unit, total alcohol units per week; Sport-time, min per month; Walk-mile, sum of miles per month in excess of 5 min; Active-work, 0-4 from none to very active occupation; Heavy-home, days per month heavy household, DIY or garden work. Variables were entered into multiple linear regression after appropriate power transformations.

Smoking, drink-unit, sport-time and walk-mile were all negatively associated with BMI ($P<0.0005$). Active-work and heavy-home were unrelated. Smoking and heavy-home were less common in women of higher social class ($P<0.0001$). Drink-unit and sport-time were more common in women of higher social class ($P<0.0001$), and walk-mile and active-work were unrelated.

The Fig. shows changes in the slope and R^2 values for lnBMI (adjusted for age) *v.* class with step-wise addition of the potential explanatory variables. Adjustment for smoking strengthened the BMI *v.* class relationship since smoking (more prevalent in low social class) tends to reduce BMI. Introduction of drink-unit and sport-time reversed this effect. The remaining variables had no additional effect. After all adjustments the BMI *v.* class relationship remained the same as when unadjusted. Even after adjustment, only 8.7% of the variance in BMI could be explained.



This surprising finding could have arisen from imprecision in quantifying the behavioural variables. However, their strong independent influences on BMI suggest that this is unlikely. A more likely explanation may be that the social class gradient is mediated by other factors not recorded by HSE (eg diet composition, television viewing, obstetric history or cognitive restraint).

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