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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at the University of Southampton on Tuesday–Friday, 2–5 August 1994, when the following papers were presented.

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Body composition, resting energy expenditure and fitness in female professional and student contemporary dancers. By M. KHALOUHA[1], M. COX[1], N. GIBSON[1], Y. KOUTEDAKIS[2] and P.J. PACY[1], [1]*Nutrition Research Unit, St Pancras Hospital, 4 St Pancras Way, London NW1 2PE and* [2]*School of Health Sciences, Wolverhampton University, Wolverhampton WV1 1DJ*

Professional dancers are prone to a myriad of musculo-skeletal injuries which may have serious consequences to the dancer and company. The cause for injury remains to be established but may relate to abnormal nutritional status and lack of fitness which has been found in ballet dancers (Koutedakis, unpublished results). The present study was performed to examine components of nutritional status in female professional (group A) and third year students (group B) of contemporary dance and compare results with matched sedentary females (group C). Resting energy expenditure (REE) was measured for 30-45 minutes after a 10 h overnight fast by indirect calorimetry with a ventilated hood. Percentage body fat (%BF) was determined by underwater weighing (UWW) and bioelectrical impedance (Bodystat 1500, BIAb). Fitness was characterized from the predicted VO_2max which was calculated by regressing O_2 consumption, measured by indirect calorimetry and ventilated hood, against heart rate to predicted maximum (220 minus age) during a submaximal step test (Cox *et al.* 1995). Group A consisted of twelve females, age 27 (SD 4) years, weight 57 (SD 5) kg, body mass index 21 (SD 1) kg/m^2, professional status 6 (SD 4) years; group B 13F, age 22 (SD 2) years, weight 55 (SD 5) kg, body mass index 20 (SD 2) kg/m^2 and group C 14F, age 24 (SD 8) years, weight 57 (SD 7) kg, body mass index 21 (SD 1) kg/m^2.
The results are shown below.

	REE (kJ/d)		UWW (% fat)		BIAb (% fat)		fitness (VO_2max/kg)	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A	5128	414	19.9	7.7	21.2	2.9	33.8	6.4
B	5346	473	21.6	5.0	20.9	2.3	34.0	7.4
C	5271	443	26.4*	3.9	26.4*	3.2	28.6*	3.9

* Mean values were significantly different from A and B, $p < 0.05$ (unpaired t test).

We demonstrated that there were no significant differences between professional and student contemporary dancers although both groups were fitter and had less fat than sedentary non-athletic women. However the fitness level of the dancers was considerably lower than that of elite international oarswomen n 5,age 25 (SD 2) years, weight 62 (SD 2) kg, body mass index 21 (SD 1) kg/m^2 at 47.2 (SD 5.4) VO_2max/kg (p<0.01). Overall %BF of oarswomen determined by UWW and BIAb was not only very similar but was higher than the value of about 15% reported in ballet dancers. If our suggestion that lack of fitness is an important factor in dance-related injury is correct, these results suggest that there is considerable potential to reduce this by improving fitness of contemporary dancers.

This work was supported by Dance UK.

Cox, M., Gibson, N.R., Khalouha, M., Mills. K. & Pacy, P.J. (1995). *Proceedings of the Nutrition Society* (In the Press).

Factors predicting weight loss during the first 4 weeks of treatment in obese adults on three weight-reducing diets. By C. J. WATTS, C. D. SUMMERBELL and J. S. GARROW, *Rank Department of Human Nutrition, St Bartholomew's Hospital Medical College, London EC1M 6BQ*

For certain subgroups of obese patients some weight-reduction treatments may be more effective than others. Identifying reliable predictors of these subgroups would mean that patients could be matched to appropriate treatments.

Forty-one subjects were recruited to the study and randomly allocated to one of three weight-reducing diets (Watts *et al.* 1994). The dietary treatments were:

Group 1. Milk aimed to provide 3472 - 4644 kJ, (in the form of milk, yoghurt or cottage cheese), plus *ad libitum* quantities of one nominated food daily. Seven foods were selected, three fruit or vegetables, two high protein foods and two "favourite" foods. These foods were repeated on a weekly basis.

Group 2. Milk aimed to provide 3472 - 4644 kJ (in the form of milk, yoghurt or cottage cheese).

Group 3. Energy-controlled diet, aimed to provide 3347 - 4184 kJ.

Data are available for twenty-six subjects who attended visit 1 (baseline) and visit 2 (after 4 weeks). The mean age of these subjects was 45 (SD 14) years, mean body mass index (BMI) was 44.0 (SD 9.0) kg/m^2. Eighteen (69%) of the subjects were women. Eight (31%) reported participating in regular exercise. There was no significant difference at baseline between the three groups in terms of age, BMI, sex, reported participation in regular exercise, self-esteem (Rosenberg, 1965) or eating self-efficacy (Glynn & Ruderman, 1986). It was hypothesized that four factors, initial self-esteem, initial eating self-efficacy, participation in regular exercise and group allocation would be predictive of weight loss at visit 2. Eating self-efficacy refers to a persons' belief that they can control their eating.

As expected there was a significant positive correlation between initial BMI and weight change $r = 0.35$; (P=0.04). There was a significant inverse correlation between initial BMI and both initial self-esteem $r = -0.49$; (P=0.005) and initial eating self-efficacy $r = -0.42$; (P=0.016). Of the four predictive factors chosen, only the group allocation was significantly associated with weight loss. Mean weight loss was 2.8 (SD 2.3) kg in group 1, 7.9 (SD 2.1) kg in group 2 and 2.0 (SD 3.3) kg in group 3. Mean weight loss in group 2 was significantly larger than that in both group 1 (P=0.01) and group 3 (P=0.001). Neither initial self-esteem nor initial self-efficacy were significantly correlated with weight change. The analysis was repeated using multiple regression so that the effect of initial self-esteem and initial eating self-efficacy on weight change could be evaluated while controlling for initial BMI. Results still showed no significant relationship between these variables. There was no significant association between change in weight and participation in regular exercise.

There was a significant positive correlation $r = 0.37$; (P=0.033) between weight loss and change in eating self-efficacy score between visit 1 and visit 2. This may be an important factor in the final analysis of the present study since changes in eating self-efficacy within the first month of treatment have been shown to predict successful weight loss (Glynn & Ruderman, 1986).

Preliminary results show that the only factor which is significantly associated with weight loss is the dietary treatment. It is possible that the strong effect of the diet has masked other effects which are actually present.

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Rosenberg, M. (1965). *Society and the Adolescent Self Image*. Princeton, NJ: Princeton University Press.
Watts, C.J., Summerbell, C.D. & Garrow, J.S. (1994). *Proceedings of the Nutrition Society* (subsequent abstract).

Estimation of body composition in children using skinfold thickness: a cross-validation study. By J.J. REILLY, J. WILSON and J.V.G.A. DURNIN, *University of Glasgow Department of Human Nutrition, Yorkhill Hospitals, Glasgow G3 8SJ*

Body composition methodology in children is limited by variability within and between subjects in the composition of fat-free mass (FFM). While this problem is common to all "two component" methods, changes in fat distribution and, possibly, variability in skinfold compressibility pose particular problems for the use of skinfold thickness (SFT) in children. In addition there are now several published equations for the prediction of body density and/or body fatness (as a percentage of body weight, BF%) from SFT in children, but little information on their general applicability.

In the present study BF% was estimated from SFT in ninety eight children (sixty four boys, thirty four girls; mean age 9.1 (SD 1.7 years) by using the equations of Brook (1971), Johnston *et al* (1988), Slaughter *et al* (1988), Deurenberg *et al* (1990), and Durnin and Rahaman (1967). In eighty one of the children BF% was measured by underwater weighing (UWW). In all cases measured (UWW) and predicted densities were converted to BF% using the modifications to the Siri (1961) equation proposed by Westrate and Deurenberg (1989). Mean BF% by densitometry was 13.2 (SD 8.0) % in boys and 19.8% (SD 7.9) % in girls. Differences between methods, and between the various methods and densitometry, were determined by calculation of bias and limits of agreement.

In the girls the equations of Brook (1971), Durnin and Rahaman (1967) and Johnston *et al* (1988) tended to underestimate BF%: 95% CI for predicted-measured BF% -3.1 to -9.1 (Brook 1971); -3.7 to -9.3 (Johnston *et al* 1988); -0.2 to -5.9 (Durnin and Rahaman 1967). The equations of Slaughter *et al* 1988 (95% CI -2.5 to +3.0) and Deurenberg *et al* 1990 (95% CI -3.9 to +2.0) had no significant bias relative to densitometry, but in both cases limits of agreement with the reference method were wide. Magnitude and direction of bias was significantly related to body fatness for all equations tested with the exception of Brook (1971).

In the boys the equations of Durnin and Rahaman (1967) (95% CI -1.2 to -3.8), and Johnston *et al* 1988 (95% CI -3.4 to -5.9), also tended to underestimate BF% relative to densitometry. The equations of Slaughter *et al* (95% CI +1.4 to +3.8) and Deurenberg *et al* (1990) (95% CI + 1.8 to +4.7) tended to overestimate BF% relative to densitometry. The equation of Brook (1971) had no significant bias (95% CI -0.8 to +1.4), but for all five equations limits of agreement between the reference method and the prediction equations were wide, as in the girls. The magnitude and direction of bias was significantly related to body fatness for all of the equations tested with the exception of the Brook (1971) equation.

Differences between measured and predicted fatness at the individual level are large and use of these "standard" equations can produce large systematic errors. While these differences are partly the inevitable result of errors/invalidity of the reference method (Siri, 1961), the magnitude of the differences observed suggests that the prediction of fatness in children from SFT requires caution.

The study was supported by the Scottish Office Home and Health Department.

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The effect of dietary energy density on energy intake, weight gain and change in subcutaneous fat depots in 7-month-old infants. By K.F. MICHAELSEN, *Research Department of Human Nutrition, Royal Veterinary and Agricultural University, Copenhagen, Denmark*

There is general agreement that fat intake of infants and toddlers should not be too low. The concern is that energy intake will be reduced if the food is too bulky, and thereby affect growth. In a recent survey from England of home-prepared weaning foods (Morgan *et al.* 1993) concern was expressed that 48% of the meals had an energy density less than that of human milk (2.9 kJ/g). However, only a few studies have examined the association between energy density, energy intake and growth (Fomon *et al.* 1977; Sanchez-Griñan *et al.* 1992).

In an intervention study with a crossover design we compared the effect, in 7-month-old infants (n 17), of two study diets, of different energy densities, with their normal diet. The parents could choose from a selection of foods, and each food was prepared in a high- and a low-energy-density version by adjusting the contents of water, oil and maltodextrin. The parents were asked to feed the infants *ad lib*. Each diet was served for a 10 d period, and the parents were blind to the study design. The levels of energy density in the two diets were chosen to resemble the 10th and 90th percentiles found in a study of diet of Danish infants (K.F. Michaelsen, unpublished results). The study was approved by the Ethics Committee of Copenhagen and Frederiksberg.

		Normal diet		Low-energy-density diet		High-energy-density diet	
		Mean	SEM	Mean	SEM	Mean	SEM
Diet:	Energy density (kJ/g)	2.9	0.1	2.3	0.1	3.8***	0.1
Intake:	Amount (g/d)	999	29	1040	35	942**	22
	Energy (kJ/d)	2833	110	2419	96	3525***	80
Growth:	Weight gain (g/d)	17	3	10	4	28*	4
	Δ skinfolds (mm/d)	0.001	0.03	-0.09	0.04	0.12**	0.03

Mean values were significantly different from low-energy-density: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

The large difference in energy density between the two periods was only compensated to a small degree by changes in the amount eaten. The resulting significant differences in weight gain and fat deposition are most probably explained by the difference in energy intake, since there were only small differences in other nutrients between the two diets.

Despite the fact that the parents were told to feed the infants *ad lib*, it cannot be excluded that part of the difference in energy intake between the two periods was caused by the parents' perception of how much an infant can eat.

In conclusion, a low-energy-density diet, which is often a diet with a low fat content, has a negative impact on weight gain during the weaning period.

The project was supported by the Danish Research and Development Program for Food Technology (FØTEK) and the Danish Heart Foundation. The assistance of dietician Pia S. Larsen, and home-economist students Kirsten Lund Nielsen and Janne Helweg-Jørgensen is acknowledged.

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The effects of exercise on postprandial lipaemia: apolipoprotein B-48 and retinyl palmitate as markers of chylomicron particles. By S. G. ISHERWOOD, S. SETHI, B. J. GOULD and C M. WILLIAMS, *Nutritional Metabolism Research Group, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

Physical exercise has been shown to have a favourable effect on some of the risk factors associated with coronary heart disease (CHD), however the effects of exercise on chylomicron (CM) particle metabolism have not been clearly shown. Due to the potentially atherogenic nature of chylomicron remnants (CMR), factors which increase the magnitude of postprandial lipaemia may be associated with an increased risk of CHD (Zilversmit, 1979). Currently CM metabolism can be followed by using retinyl palmitate (RP) as a marker for CM particles, however it would be preferable to use apolipoprotein B-48 (apo B-48) as a marker as this protein is uniquely associated with the CM and remains with the particle throughout its lifetime. The availability of a specific antibody to apo B-48 (Peel *et al.* 1993) enables both these markers to be measured, and their responses compared, in experimental studies of postprandial lipaemia in man.

Fourteen, healthy, young, male subjects were recruited and allocated into active (regular, moderate exercise) and inactive (no regular exercise) groups according to their habitual exercise levels. Each subject was randomly allocated to receive, on separate occasions, each of three test meals containing 20, 40 or 80 g fat (168 g CHO, 44 g protein). Blood samples were collected in the fasted state and hourly for an 8 h period after eating the meal. 100 mg of RP (170,000 IU retinol equivalents) was taken with the meal. Triacylglycerol (TAG) and non-esterified fatty acid (NEFA) concentrations were measured in plasma and apo B-48, RP and TAG concentrations were measured in a CM-enriched fraction isolated from plasma by ultracentrifugation. For the analysis of apo B-48 a postprandial CM sample was used as an internal standard. The results are shown in the Table as total area under the time response curve (AUC) with seven subjects in each group.

		Area under the curve (AUC)					
		20 g Fat		40 g Fat		80 g Fat	
		active	inactive	active	inactive	active	inactive
CM-TAG	Mean	115	208*	163	281*	237	330*
(min.mmol/l)	SEM	5	26	24	40	24	26
CM-apo B-48	Mean	7602	11725	7969	18977	7903	23046*
(% standard.min)	SEM	2881	3083	1311	5231	1343	5734
CM-RP	Mean	143	436**	284	618**	413	817**
(min.µg/ml)	SEM	17	129	31	69	43	110
Plasma-TAG	Mean	456	546	461	603*	526	676**
(min.mmol/l)	SEM	31	30	38	43	32	13
Plasma-NEFA	Mean	154	149	155	168	177	195
(min.mmol/l)	SEM	21	24	36	22	27	21

mean values were significantly different compared with the active group for the same meal: * $P<0.05$, ** $P<0.01$.

The CM-TAG, CM-apo B-48, CM-RP and plasma-TAG results all showed a significantly lower AUC in the active group after the high-fat meal. The active group also showed a significantly lower AUC for CM-TAG and CM-RP responses after the medium- and low-fat meals. The NEFA results did not show a statistically significant difference between the two groups, although there was a different pattern in their responses.

These results suggest that CM particle metabolism occurs at a faster rate in active subjects, which is in agreement with other studies, although it is not entirely clear whether this effect is due to effects on lipoprotein lipase (*EC* 3.1.1.34) or CMR uptake by the liver.

We acknowledge financial support from the Agricultural and Food Research Council.

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Olive oil and postprandial lipaemia: a study on the effects of meals of different olive-oil content on postprandial plasma-lipid levels in healthy men. By A. ZAMPELAS, C.C. CULVERWELL, J.M.E. KNAPPER, K. JACKSON, B.J. GOULD, J. WRIGHT, and C.M. WILLIAMS, *Nutritional Metabolism Research Group, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH.*

Postprandial lipaemia is the fed state of the lipid transport system, the magnitude of which has been correlated with the presence or absence of coronary heart disease (CHD; Patsch *et al.* 1992). It has been suggested that dietary fatty acid composition can modify the extent of postprandial lipaemia (Sethi *et al.* 1993) but the effects of *n*-9 monounsaturated fatty acids (MUFA) (commonly found in olive oil) on postprandial plasma-lipid responses have not been fully investigated.

The present study was carried out to investigate the effect of three test meals of different MUFA content (12, 17, and 24% energy) on postprandial plasma triacylglycerol (TAG), non-esterified fatty acid (NEFA) and cholesterol (TC) levels in fifteen healthy male volunteers. The meals were designed so that polyunsaturated fatty acid contents were comparable for the three meals (5%), with variations achieved by substituting MUFA for saturated fatty acids (SFA). Subjects attended the Clinical Investigation Unit of the Royal Surrey County Hospital on three separate occasions and were randomly allocated to the three test meals. On each study day, a fasting blood sample was taken (0 min) and the test meal was consumed immediately after the collection of the fasting blood sample. The test meal consisted of 135 g white bread, 36 g jam and a milk shake in which the oil under investigation was incorporated (40 g dried skimmed milk powder, 40 g strawberry flavour, 40 g test oil). The total energy intake was 4.18 MJ (150 g carbohydrate, 24 g protein, 42 g fat). Further blood samples were taken half-hourly for the first 2 h and hourly thereafter, until 9 h postprandially.

Plasma TAG responses to the 24% MUFA test meal showed a small biphasic response, reaching peak levels 180 and 420 min postprandially but no statistically significant differences were found in postprandial responses to the three test meals. Plasma TAG responses are given in the following Table.

	TAG (mmol/l)											
Time (min)...	0		60		180		300		420		540	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12%MUFA	1.1	0.3	1.2	0.4	1.7	0.6	1.6	0.5	1.5	0.4	1.1	0.3
17%MUFA	1.1	0.4	1.3	0.4	1.7	0.6	1.5	0.5	1.3	0.6	1.0	0.3
24%MUFA	1.1	0.3	1.3	0.4	1.7	0.8	1.5	0.6	1.6	0.2	1.0	0.4

Plasma NEFA responses to the three meals were also identical, reaching lowest levels approximately 2 h after the meal, and rising above the fasting values at 6 h postprandially. There was no response of TC to the three meals with postprandial levels being similar to the fasting values.

In conclusion, substituting MUFA for SFA, in the acute test meal situation, at levels found in human diets, does not alter the magnitude of postprandial lipaemia, but further work is necessary to determine whether long term dietary modification of this type influences long term postprandial lipaemia.

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The effects of starvation and the insulin/glucose clamp on plasma fatty acid turnover and oxidation. By J.WEBBER[1], J.TAYLOR[1], H.GREATHEAD[2], J.DAWSON[2], P.BUTTERY[2] AND I.A.MACDONALD[1], *[1]Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and [2]School of Agriculture, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD.*

During starvation plasma non-esterified fatty acid (NEFA) concentrations increase and there is a progressive rise in the utilization of fat as an energy source by the body. The relative contributions of plasma and tissue fatty acids to whole-body energy metabolism in the postprandial state and during starvation is not clear. The present study was designed to examine the effects of starvation on plasma fatty acid turnover and oxidation and to assess the influence of insulin on these processes.

Ten healthy subjects (six women, mean age 24.6 (SE 1.5) years, weight 67.0 (SE 2.7) kg, body mass index 23.8 (SE 0.8) kg/m^2) were recruited. They each attended on three occasions, after 12, 36 and 72 h fasting. At each visit a bolus of $NaH^{13}CO_3$ was given followed by an infusion of [1-^{13}C]-palmitate in albumin and after 45 min of the infusion, four arterialized venous blood samples and four expired-air samples were obtained for later measurements of plasma palmitate concentration, [1-^{13}C]-palmitate enrichment and $^{13}CO_2$ enrichment by mass spectrometry. A 100 mU/m^2 per min insulin infusion was then commenced and blood glucose was clamped at 4 mmol/l with a variable infusion of 200 g/l dextrose (derived from potato starch). Further blood samples were obtained after 90 min of the clamp, the [1-^{13}C]-palmitate infusion and indirect calorimetry being continued throughout the study.

		Duration of fasting (h)					
		12		36		72	
		Mean	SE	Mean	SE	Mean	SE
Plasma NEFA turnover	Basal	4.36	0.49	6.16	0.68	7.19	0.68
(μmol/min per kg)	Clamp	1.21	0.13	1.79	0.29	3.87	1.04
Plasma NEFA oxidation	Basal	2.24	0.13	2.62	0.16	3.63	0.24
(μmol/min per kg)	Clamp	0.42	0.02	0.71	0.12	1.35	0.35
Total lipid oxidation	Basal	2.74	0.13	3.61	0.21	3.86	0.18
(μmol/min per kg)	Clamp	1.45	0.27	2.08	0.38	3.06	0.35
Percentage of turnover	Basal	53	4	47	5	52	4
oxidized	Clamp	36	4	43	5	37	3
NEFA oxidation as % of	Basal	83	6	76	6	96	7
total lipid oxidation	Clamp	43	9	39	11	47	10

Fasting increased plasma NEFA turnover (lipolysis) and plasma NEFA oxidation. However, fasting had no effect on the proportion of turnover which was oxidized. The insulin/glucose clamp decreased both NEFA turnover and oxidation as well as reducing the percentage of turnover oxidized. Insulin and glucose infusion also reduced plasma NEFA oxidation, favouring intratissue lipid oxidation, which actually appeared to increase during the clamp.

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The intra- and inter- individual variability in the gastrointestinal handling of [1-^{13}C]palmitic acid in healthy adults. By J.L. MURPHY[1], A. E. JONES[1], A. HOUNSLOW[1], S. BROOKES[2], M. GRIFFITHS[2] and S. A. WOOTTON[1], [1]*Institute of Human Nutrition, Southampton SO16 7PX and* [2]*Europa Scientific Ltd., Crewe CW1 1ZA*

Measurements of gross faecal lipid losses made during balance studies have led to the general belief that the absorption of dietary lipid is very efficient in normal health. Such an approach assumes that all dietary fatty acids are handled equally across the gastrointestinal tract. Jones *et al.* (1985) showed that [1-^{13}C]stearic acid was relatively poorly absorbed in comparison with [1-^{13}C]oleic or [1-^{13}C] linoleic acids and the proportion of label recovered in stool varied markedly between subjects (8% to 35%). The purpose of the present study was to examine the extent to which the gastrointestinal handling of [1-^{13}C]palmitic acid and dietary lipid may vary both within and between normal adults.

Following an overnight fast six healthy women aged 21-30 years ingested [1-^{13}C]palmitic acid (10 mg/kg) with a standardized test meal (1660 kJ) of low natural ^{13}C abundance. All subjects recorded weighed food intakes for 5 d and collected all stools between carmine markers administered simultaneously with [1-^{13}C]palmitic acid. Enrichment of ^{13}C in stool samples was analysed by mass spectrometry (ANCA system, Europa Scientific Ltd., Crewe). Lipid intake was estimated using computerized food composition databases and weighed stools were analysed for lipid (Gompertz & Sammons, 1963). The trial was repeated in the same subjects. The results are given in the Table as individual and median values.

	Stool ^{13}C (% dose)		Stool lipid (% ingested)	
Subject number	Trial 1	Trial 2	Trial 1	Trial 2
1	7.4	31.5	13.7	13.9
2	13.1	8.3	5.6	5.4
3	17.6	10.0	1.9	3.1
4	7.2	18.9	2.3	4.2
5	32.4	49.9	2.8	4.8
6	8.0	71.0	5.1	3.8
Median	10.6	25.2	4.0	4.5

A greater range in the excretion of ^{13}C label in stool was observed between subjects in trial 2 compared with trial 1 (Murphy *et al.* 1993)(not significantly different) with poor agreement within each subject. In both trials there was no relationship between the excretion of ^{13}C label and lipid in stool. The present study demonstrates that the gastrointestinal handling of [1-^{13}C]palmitic acid (when ingested as the free acid) varies widely both between and within a given individual and is not associated with the relatively efficient absorption of dietary lipid in healthy adults.

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Effect of monounsaturated fatty-acid-enriched test meals on plasma insulin and GIP concentrations. By J.M.E. KNAPPER, N. FURLONGER, C.C. CULVERWELL, A. ZAMPELAS, K. JACKSON, L.M. MORGAN, J.A. TREDGER and C.M. WILLIAMS. *Nutritional Metabolism Research Group, University of Surrey, Guildford GU2 5XH*

The importance of post-prandial lipaemia in the pathogenesis of atherosclerosis has been recently reviewed (Sethi *et al.* 1993). Lipoprotein lipase (*EC* 3.1.1.34; LPL) is the rate-limiting enzyme responsible for the clearance of plasma triacylglycerol. It is known that insulin stimulates adipose tissue LPL postprandially and we have shown that the insulinotropic gut hormone GIP (glucose-dependent insulinotropic polypeptide) directly stimulates adipose LPL activity both on its own and in combination with insulin (Knapper *et al.* 1993). Alteration in plasma insulin or GIP concentrations postprandially could therefore in turn affect clearance of dietary triacylglycerols.

We have investigated the effect of acute substitution of saturated fatty acids (SFA) with monounsaturated fatty acids (MUFA) within a mixed meal on plasma glucose, insulin and GIP concentrations. Healthy men (age range 18-30 years, body mass index 20-25, *n* 15) attended the Clinical Investigation Unit at a local hospital on three separate occasions 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 compositions of the three test meals were modified to provide 12, 17 and 24 % energy as MUFA at the expense of SFA. The PUFA content was maintained at approximately 6%. Meals were provided in a random order. Two basal plasma samples were collected and the meal was consumed over a 20 min period. Blood was then sampled every 30 minutes for the first 2 h and then hourly until 9 h after the meal. Fluid intake (water, diet drinks or decaffeinated tea and coffee) was strictly controlled during the investigation period. Plasma glucose was measured by an automated hexokinase method and insulin and GIP measured by specific radioimmunoassay. Incremental areas under the curve are shown in the Table.

	12% Energy as MUFA		17% Energy as MUFA		24% Energy as MUFA	
	Mean	SD	Mean	SD	Mean	SD
Plasma glucose (mmol/l.min)	144	151	103	194	146	208
Plasma insulin (nmol/l.min)	115	50	125	81	146	82
Plasma GIP (nmol/l.min)	63	11	70	15	70	19

Fasting levels for plasma glucose, insulin and GIP were superimposible at the start of the three test meals. No significant differences in incremental areas under the graph were seen. Plasma insulin responses did show differences in the time to mean peak insulin concentration which were reached at 30 min following the 12 % MUFA meal, 60 min following the 17 % MUFA meal, and 120 min following the 24 % MUFA meal. However differences in insulin concentration at individual time points never achieved statistical significance. Plasma GIP and glucose concentration curves following the three test meals were largely superimposible. We therefore conclude that physiological amounts of MUFA, as a substitute for saturated fatty acids in an acute test meal, had no modifying effect on plasma glucose, insulin or GIP responses in healthy individuals. Whether this would also be true in the chronic situation is unknown, and we intend to investigate this shortly.

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Dietary cholesterol may modify the modulatory effects of dietary fats on the metabolic responses to endotoxin in rats. By H. T. BESLER and R. F. GRIMBLE, *Department of Human Nutrition, University of Southampton, Southampton SO16 7PX*

Dietary fat modulates cytokine-mediated metabolic changes which follow exposure to inflammatory agents (Grimble,1994). In a previous study we examined the effects of diets containing 50, 100 and 200 g fat/kg fat predominantly in the form of maize oil (rich in linoleic acid, (18:2*n*-6)), olive oil (rich in oleic acid, (18:1*n*-9), poor in 18:2*n*-6) or butter (rich in 18:1*n*-9, poor in 18:2*n*-6), on metabolic response to endotoxin. Maize oil was included in the butter and olive-oil diets at 10 g/kg. Metabolic response to endotoxin increased in maize-oil-fed animals. Responses were blunted in animals fed on the olive-oil diets. Responses were blunted only in the butter-fed groups receiving diets containing 50 and 100 g fat/kg. The butter diet containing 200 g fat/kg permitted similar responses to endotoxin to those seen in animals on 50 g/kg maize-oil diets (Besler & Grimble,1992, 1993 *a*,*b*).It has been shown recently that cholesterol addition to the diet of rabbits, containing saturated fat, increased the expression of mRNA for interleukin 1α and ß (IL-1α and ß) and tumour necrosis factor α (TNFα) in the aorta wall following endotoxin injection (Fleet *et al.*1992). Thus, the cholesterol content of butter may be contributing to the response of rats fed on 200 g/kg butter diets. We therefore tested this hypothesis by feeding male Wistar rats on diets containing 90 or 190 g butter/kg (1/BU and 2/BU respectively) or a cholesterol-supplemented butter diet (1/BC) containing 90 g butter/kg and the same amount of cholesterol as in the 190 g/kg butter diet i.e., 440 mg cholesterol. A 100 g/kg maize-oil diet (1/MO) was also included to compare the results of butter- and maize-oil-fed animals. All diets were adequate in all nutrients. In all butter diets, 10g maize oil/kg was added to prevent essential fatty acid deficiency. At the end of the 4-week feeding period, animals received either endotoxin (END) (Difco strain, 055:B9) at a dose of 800 μg/kg body weight or sterile non-pyrogenic saline (SAL). Saline-injected animals were pair-fed the intakes of the endotoxin-injected animals over the 24 h period following injection.

Diet...		1/MO		1/BU		1/BC		2/BU	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Serum albumin (mg/ml)	(SAL)	39.1	0.2	34.4	0.2	35.2	0.1	36.7	0.1
	(END)	28.2*	0.4	33.8	0.1	30.6*	0.3	29.4*	0.2
Caeruloplasmin (U/l)	(SAL)	38.7	0.3	40.7	0.5	43.1	0.6	39.7	0.4
	(END)	93.7*	0.4	42.5	0.4	78.7*	0.7	86.3*	1.6
Serum zinc (μg/ml)	(SAL)	2.60	0.1	2.54	0.2	2.45	0.1	2.55	0.1
	(END)	1.62*	0.1	2.33	0.1	1.98*	0.1	1.82*	0.2
Liver protein (mg/g)	(SAL)	213.	4.9	207.	6.2	202.	4.9	208.	5.6
	(END)	277.*	6.4	218.	5.9	249.*	4.5	269.*	4.8
Liver zinc (μg/g)	(SAL)	33.1	0.9	33.4	0.7	32.9	1.1	34.1	1.0
	(END)	39.6*	1.0	35.3	1.0	38.7*	0.7	41.2*	0.9

Each observation represents the mean and standard error for four animals. * significantly different from saline control (ANOVA): $P<0.05$.

The results confirm our earlier observations that a diet containing 100 g fat/kg, predominantly in the form of butter inhibits responses to endotoxin. The inhibition ceases when diets containing a higher content of butter are fed. Data from the 1/BC group suggest that while butter has an intrinsically anti-inflammatory fatty acid content, cholesterol in this dietary fat may exert a proinflammatory effect at high levels of dietary intake.

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Dietary lipids modulate systolic blood pressure in the rat. By A. G. CLAMP, S. C. LANGLEY, R. F. GRIMBLE and A. A. JACKSON, *Institute of Human Nutrition, University of Southampton, Southampton SO9 3TU*

The role of dietary fat in the regulation of blood pressure has been previously evaluated in both human and animal studies. However, the results of such studies are largely inconclusive. The present study was performed to determine whether feeding diets with differing fatty acid content and composition had an influence on systolic blood pressure in the rat.

Weanling male Wistar rats were fed for 5 weeks on a diet composed of either standard rat chow (23 g fat/kg diet) or one of four synthetic diets (100 g fat/kg diet) containing butter fat, coconut oil, maize oil or fish oil as the main lipid source. In all synthetic diets 100 g/kg of the fat was provided as maize oil to prevent essential fatty acid deficiency. At the end of the 5-week period, systolic blood pressure was determined using an indirect tail-cuff method as previously described (Langley & Jackson, 1994). The systolic blood pressures of twenty male weanling rats were determined as a baseline comparison.

There were no significant differences in the initial or final body weights between the five dietary groups. Food intake did not vary between the groups, with the exception of fish oil fed rats, which consumed 10% less food per day than chow-fed controls.

Diet...	Chow		Butter		Coconut		Maize		Fish	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
SBP	146[a]	1	169[b]	2	178[b]	8	126[c]	4	153[a]	3

SBP, systolic blood pressure (mm Hg); a,b values with unlike superscripts were significantly different (ANOVA) $P<0.01$ (n 4 for each dietary group).

At weaning the systolic blood pressure of the rats was 119 (SE 5) mm Hg. The blood pressure of chow-fed animals increased by 27 mm Hg in the period between weaning and 5 weeks of age, when the study ended. Feeding the butter-fat, coconut-oil or maize-oil-based diets was found to have significant effects upon systolic blood pressure. A positive relationship was observed between blood pressure and saturated fatty acid intake (r 0.77, $P<0.0005$). Systolic blood pressure was negatively correlated with unsaturated fatty acid intake (r -0.68, $P<0.001$) and the unsaturated : saturated fatty acid intake ratio (r -0.916, $P<0.00001$).

In conclusion, feeding a diet rich in saturated fatty acids was shown to increase blood pressure in the rat. A high intake of n-6 fatty acids, and in particular linoleic acid, appears to have a hypotensive effect.

Langley, S. C. & Jackson, A. A. (1994). *Clinical Science* **86**, 217-222.

The role of dietary protein restriction during pregnancy on the activity of placental 11ß-hydroxysteroid dehydrogenase. By G.J.PHILLIPS[1], S.C.LANGLEY-EVANS[1], R.BENEDIKTSSON[2], J.R.SECKL[2], C.R.W.EDWARDS[2] and A.A.JACKSON[1], [1]Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX and [2]Department of Medicine, University of Edinburgh, Edinburgh EH4 2XU

Epidemiological studies in humans have demonstrated an association between low birth weight and the development of hypertension in later life (Barker *et al.* 1990). Intrauterine growth retardation and hypertension has been recently demonstrated in an animal model (Langley & Jackson, 1994) following fetal exposure to maternal low-protein diets. Fetal exposure to exogenously administered glucocorticoids retards intrauterine growth (Reinisch *et al.* 1978) and results in increased blood pressure in the adult offspring (Benediktsson *et al.* 1993). Most maternal corticosterone crossing the placentae is converted to biologically inactive products by the placental enzyme 11ß-hydroxysteroid dehydrogenase (11ßOHSD; *EC* 1.1.1.146). It was hypothesized that exposure of the rat fetus to a low maternal protein intake would lead to altered placental 11ßOHSD activity thereby increasing the risk of fetal exposure to maternal glucocorticoids and the development of hypertension later in life.

Female Wistar rats (*n* 4-6) were given access to synthetic diets containing 180g (normal) or 90g (low) protein/Kg (Langley & Jackson 1994) 14 d before mating. Following mating, rats were maintained on their appropriate diets until day 20 of pregnancy, at which point half of the animals were killed by CO_2 asphyxiation and ten fetus's per dam with attached placentae were removed. Body and placental weights were measured and placental 11ßOHSD activity assessed (Bendiktsson *et al,* 1993). The rest of the animals were allowed to deliver naturally and at 24 weeks of age blood pressure was measured by the tail-cuff method (Langley & Jackson, 1994).

Dietary protein group (g/Kg)	11ßOHSD activity (10^{-9}moles/10 min per placentae)			Systolic blood pressure (mmHg)		
	Mean	SD	*n*	Mean	SD	*n*
90	46.6**	21.8	20	139*	3	9
180	71.3	21.3	30	127	3	10

Mean values were significantly different from 180g protein/Kg: * $p<0.05$, ** $p<0.005$.

No difference in placental or fetal weight was observed between animals exposed to 180 or 90g protein/Kg *in utero*. However, placental 11ßOHSD activity was significantly lower and blood pressure at 24 weeks of age significantly greater in animals exposed to 90g protein/Kg than equivalently aged animals exposed to 180g protein/Kg.

In conclusion exposure of the fetus to maternal low-protein diets results in a reduction in the activity of 11ßOHSD. This may expose the fetus to elevated maternal glucocorticoids and lead to hypertension in the adult offspring.

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***In utero* exposure to maternal low-protein diets induces hypertension in weanling rats, independently of maternal blood pressure changes.** By S.C. LANGLEY-EVANS, G.J. PHILLIPS and A.A. JACKSON. *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX.*

A strong association between maternal nutrition, fetal growth and the development of hypertension has been demonstrated in young adult rats (Langley & Jackson, 1994). This provides a model of a similar association noted in the human population (Barker *et al.* 1993). To determine the age of onset of the hypertensive state a total of seventy-seven rat pups were bred, having been exposed to two different levels of maternal dietary protein during fetal development.

Five female Wistar rats were fed on a diet containing 180g casein/kg (control diet) for 2 weeks before conception and during pregnancy. A further group of five females was fed on a diet containing 90g casein/kg (low-protein diet) over the same period. Blood pressures of the pregnant females were monitored during gestation. On giving birth all the animals were immediately transferred to a standard laboratory chow diet, which was also used to wean the offspring at the age of 3-4 weeks. These animals therefore differed only in terms of prenatal dietary experience. All litters were thinned to eight pups in order to standardize feeding in the suckling period. Blood pressures of the weanling animals were measured at 4 weeks of age.

Feeding of the low-protein diet had no effect upon the systolic blood pressures of adult female rats, either before conception or during pregnancy. Blood pressure decreased by 15-20 mm Hg during pregnancy in both groups of animals, with no observable differences between low-protein and control groups.

Maternal diet	Sex of offspring	Systolic blood pressure of offspring (mm Hg)			Birthweight (g)		
		Mean	n	SEM	Mean	n	SEM
Control	Male	91	20	5			
	Female	90	16	5			
	Both	91	36	5	5.20	22	0.1
Low-protein	Male	119*	21	5			
	Female	120*	20	4			
	Both	120*	41	5	4.70*	26	0.1

* $P<0.05$ relative to control group.

Systolic blood pressures of rats exposed to maternal low-protein diets *in utero* were significantly higher than those of control rats. Blood pressures at 4 weeks old were negatively correlated with maternal protein intake (r -0.55, $P<0.01$). The hypertension of the low-protein-exposed animals was associated with lower weight at birth.

Fetal exposure to maternal low-protein diets induces hypertension in the rat. The phenomenon is observed early in life and is independent of sex and the influence of maternal blood pressure. The low-protein diet itself did not alter the blood pressures of adult rats.

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Langley S.C., Jackson A.A. (1994). *Clinical Science* 86, 217-222.

Sexual dimorphism of macronutrient selection and regional adipose tissue accumulation following *in utero* exposure to maternal low-protein diet. By H.D. MCCARTHY, C.PICKARD, J.SPEED and A.A. JACKSON, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

Epidemiological studies have linked poor interuterine growth with adult non-communicable diseases such as hypertension and type 2 diabetes mellitus (Barker, 1993). Obesity is a major risk factor for both these and other diseases, with intra-abdominal obesity being particularly important. We now have an animal model whereby the mechanisms underlying the fetal origins of adult hypertension and type 2 diabetes can be studied. We used this model to investigate whether appetite (energy and macronutrient intake) and adiposity during growth and adulthood are affected by a poor maternal plane of nutrition.

Female Wistar rats were on fed semi-synthetic diets containing 180 or 90 g casein/kg, supplemented with DL-methionine for 14 d before mating and throughout pregnancy. Within 12 h of birth, dams were transferred to standard laboratory chow. Upon weaning, pups were separated by group and sex, and transferred to a self-selection regimen for carbohydrate (maize starch and sucrose mixture), protein (casein) and fat (lard) balanced for vitamins, minerals and fibre. Body weight and energy and macronutrient intake were measured at regular timepoints throughout the 100 d study. After this time, rats were killed, their nasoanal length recorded and nine selected regional adipose sites dissected and weighed. Intakes and body weights were compared using a Student's *t* test for unpaired data, *n* 7-10 per group.

Macronutrient intake differed between sexes and group. In male offspring, fat intake was significantly higher in the 90 g/kg group (by 32-158 %, $P<0.05$). Carbohydrate intake was consistently lower in this group (by 22-58 %, $P<0.05$) as was protein (by 11-35 %, $P<0.04$) although these differences did not reach statistical significance on all occasions. This resulted in a lower total energy intake in the 90 g/kg males (by 7-30 %, $P<0.05$). In the female offspring, fat intake was similar between groups. Carbohydrate intake was consistently lower (by 33-46 %) in the 90 g/kg group although this difference achieved significance ($P<0.01$) only up to 70 d. Protein intake was consistently higher in the 90 g/kg group (by 38-50 %, $P<0.05$). In spite of these changes in macronutrient consumption, total energy intake was unaltered throughout the study.

No significant difference in birth weight of pups was observed between groups. At weaning however, offspring from the 90 g/kg dams were significantly heavier (♂ by 15 %, $P<0.01$, ♀ 16 %, $P<0.001$). At all other timepoints body weight was similar in the male offspring, whereas in the females, this difference was maintained and at the end of the study female offspring from 90 g/kg dams were 40 g heavier (15 %, $P<0.01$). Final nasoanal length did not differ between groups for either sex.

In female offspring, adipose tissue weights were heavier in the 90 g/kg group in all regions studied, with the greatest increases observed in the four intra-abdominal sites (45-160 %, $P<0.02$). In male offspring, omental and mesenteric adipose sites were significantly heavier (by 36 %, $P<0.02$ and 44 %, $P<0.001$ respectively) in the 90 g/kg group. Mean wet weights of the other adipose sites were also increased (5-32 %) but these changes did not reach statistical significance.

In conclusion, these results indicate that exposure to inadequate nutrition *in utero* alters fetal programming which can manifest itself as a sex-dependent alteration in the pattern of macronutrient selection in adult life. Additionally, in these same offspring, regional adipose mass is increased, particularly intra-abdominally, which is far more pronounced in the female, resulting in an obese appearance. The mechanisms underlying these physiological changes are unknown.

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Glucose tolerance in rats exposed to maternal low-protein diets *in utero*. By R.F.BROWNE, S.C. LANGLEY-EVANS and A.A.JACKSON, *Human Nutrition Department, Biomedical Sciences Building, Southampton University, Southampton, SO16 7PX.*

It is now becoming clear that low birth weight is associated with an increased risk of non-communicable diseases of adulthood, including hypertension, diabetes, ischaemic heart disease and syndrome X (Langley & Jackson, 1994; Barker *et al.* 1989; Hales *et al.* 1991). Maternal malnutrition and intrauterine growth retardation have long been associated with low birth weight in animals and humans. In the rat impaired glucose tolerance may be induced by fetal exposure a low-protein diet (Dahri *et al.* 1991). In the present study we examine further the relationship between exposure to low-protein diets *in utero* and glucose tolerance.

Diets containing 180, 120, 90 and 60 g protein/kg were fed to female rats before conception and during pregnancy. Mothers were transferred to a chow diet (CRMX, 183g protein/kg) on giving birth. The offspring were weaned onto the same chow diet. At 9 weeks of age the pups were fasted for 24 h before an intravenous glucose tolerance test (IVGTT) under anaesthesia (sodium pentabarbitone) was performed. A 2 g/kg body weight load of glucose was administered at time 0 min via a cannulated jugular vein. Samples were taken from the other jugular at times -10, -5, 5, 10, 15, 20, 30, 40 and 60 min. Samples were stored in 10ml/l trichloroacetic acid and a heparin solution (10 U/ml + 9 g NaCl/kg). Glucose analysis was carried out using the glucose oxidase (EC 1.1.3.4) method.

In the recent study the intravenous glucose load was cleared in 60 min by the 180 g protein/kg exposed, control rats and 120 g protein/kg exposed rats. Peak blood glucose concentrations of 27.2 mM and 27.1 mM were observed respectively. Rats exposed to 90 g protein/kg also cleared the glucose load in 60 min, but a lower peak blood glucose concentration of 24.4 mM was noted. Rats in the 60 g protein/kg group cleared the glucose load in 40 min with a peak blood glucose concentration of 22.1 mM. The area under the glucose tolerance curve was reduced by 40% relative to the 180 g protein/kg control group.

	Area under glucose tolerance curve (mM)											
Maternal dietary protein...	180 g/kg			120 g/kg			90 g/kg			60 g/kg		
	Mean	n	SEM	Mean	n	SEM	Mean	n	SEM	Mean	n	SEM
	769.1	6	59.7	785.5	5	91.6	637.2	6	28.0	517.9	5	58.1

* Significantly different from 180 g protein/kg group, $P<0.05$; + significantly different from 120 g protein/kg group, $P<0.05$.

In this study the 60 and 90 g protein/kg exposed rats cleared the glucose load more rapidly than the 120 and 180 g protein/kg exposed groups.Examination of pancreatic weight revealed no differences between the four groups. However, this does not exclude the possibility that the function of the pancreatic ß cells or the size or number of these cells are altered by the low protein diet. The 60 and 90 g protein/kg exposed groups may have increased insulin secretion leading to the glucose being cleared more rapidly.

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Langley, S.C. & Jackson, A.A. (1994). Clinical Science **86**, 217-222.

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Energy expenditure and substrate metabolism after carbohydrate ingestion in relation to fetal growth in women born in Preston. By S.A.WOOTTON, J.L.MURPHY, F.WILSON and D.PHILLIPS, *Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX*

Impaired development in-utero and during infancy may be one of the factors which causes impaired glucose tolerance and non-insulin-dependent diabetes (NIDDM; Hales *et al*, 19910. Studies in insulin-resistant subjects have shown that the metabolic handling of glucose following ingestion is altered in skeletal muscle (DeFronzo, 1988) and may be associated with a reduced postprandial thermogenesis (Ravussin & Zawadki, 1987). The aim of the present study was to determine the influence of birth weight on the metabolic disposal of an oral glucose load labelled with ^{13}C and energy expenditure in a group of women without known alterations in glucose tolerance or NIDDM.

Sixteen female subjects aged 38-40 years were recruited to one of two groups by birth weight: HIGH (3.56-4.45 kg *n* 7); LOW (2.24-2.74 kg *n* 9). Following an overnight fast, each subject ingested 100 g naturally enriched maltodextrin in 500 ml water (Maxijul, Scientific Hospital Supplies UK; ^{13}C approx δ -11‰). Breath samples were collected before and at hourly intervals for 5 h and ^{13}C enrichment was determined by isotope ratio mass spectrometry (ABCA-*NT*, Europa Scientific, Crewe). Whole body energy expenditure and CO_2 excretion were determined by indirect calorimetry (Deltatrac, Datex Instrumentarium Corp., Helsinki) at the same time points. Excretion of the ^{13}C label on breath was expressed as a percentage of administered dose over the 5 h study period (% admin). The thermic response was calculated as the mean increment in energy expenditure above the basal metabolic rate (BMR) determined before carbohydrate ingestion.

	Body weight (kg)		BMR (MJ/d)		Breath excretion (% admin)		Thermic response (kJ/d)	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HIGH	69.9	7.7	5.962	0.487	31.2	4.9	301	46
LOW	59.9	9.5	5.410	0.595	24.7	3.1 *	285	15

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

The women in the LOW group tended to be lighter ($P>0.05$) and shorter ($P<0.05$) than those in the HIGH group but no difference in body composition was evident. No differences in BMR (either in absolute or relative to body weight) or fasting respiratory exchange ratio (RER) were observed between the groups. The increase in energy expenditure following carbohydrate ingestion was similar in both groups and returned to baseline within the 5 h study period, although thee was a more pronounced and sustained increase in RER in the HIGH group than in the LOW group ($P<0.05$ at 3 h). Significantly less ^{13}C label was recovered on the breath in the LOW group than in the HIGH group over the study period ($P<0.05$). These results suggest that birth weight may significantly alter the immediate metabolic handling of ingested carbohydrate.

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Fetal growth retardation and insulin resistance induced by a low-protein diet during pregnancy.
By M.J. HOLNESS and M.C. SUGDEN, *Department of Biochemistry, Queen Mary and Westfield College, University of London, London, E1 4NS.*

Permanent changes in metabolism occur as a result of a low-protein diet during early life (Dahri *et al.* 1991). The present study examined the effects of a low (80 g/kg) protein diet on glucose homoeostasis during insulin infusion in late pregnancy in the rat. Control rats received a diet containing 200 g protein/kg during pregnancy. Pregnant rats were examined at day 19 of gestation (term is 21-23 d) in the post-absorptive (6 h starved) state. Insulin was infused to achieve steady-state plasma insulin concentrations in the high-physiological range (see Table). Variable rates of glucose infusion were used to adjust glycaemia to about 4 mM during insulin infusion. In each dietary group, the glucose infusion rate required to maintain glycaemia (GIR) reached a plateau within 60 min, with steady-state glucose concentrations of 4.27 (SE 0.14, *n* 5) mM and 4.17 (SE 0.21, *n* 5) mM in the control and low-protein groups respectively ($P > 0.05$). Fetal glucose utilization was measured using the clamp technique in combination with 2-deoxy[1-^{3}H]glucose administration, using a lumped constant of 0.85 (see Leturque *et al.* 1986).

	Fetal weight (g)		Plasma insulin (μU/ml)		GIR (mg/min per kg)	
Diet...	Control	Low-protein	Control	Low-protein	Control	Low-protein
Mean	2.63	2.39*	141.2	136.8	31.95	26.25*
SE	0.02	0.07	15.2	17.8	0.96	1.06
n	5	5	5	5	5	4

* Significantly different from control, $P < 0.01$, Student's unpaired *t* test.

The administration of an 80 g protein/kg diet throughout pregnancy was associated with a significant (9 %) impairment in fetal growth, evident as a decline in mean fetal weight at day 19 of gestation. Steady-state plasma insulin concentrations reached during the clamp were not statistically different in pregnant rats maintained on control or low-protein diet, and insulin clearance rates (ml/min), calculated by dividing the insulin infusion rate (μU/min) by the plasma insulin concentration (μU/ml), were similar in the control and low-protein groups [control diet, 8.4 (SE 0.9) ml/min; low-protein diet, 8.5 (SE 1.1) ml/min; $P > 0.05$]. In contrast, the GIR required to maintain glycaemia at about 4 mM was significantly (18 %) lower in the pregnant rats maintained on the 80 g protein/kg diet. Although the developing conceptus makes a significant contribution to whole-body glucose clearance (Leturque *et al.* 1986), the decrease in glucose requirement observed in pregnant rats maintained on 80 g protein/kg diet could not be attributed entirely to decreased fetal use of glucose: fetal contributions to total glucose disposal were only 4.6 (SE 0.1) % and 4.2 (SE 0.1) % in pregnant rats maintained on the control and 80 g protein/kg diets respectively. The results thus suggest that a reduced glucose requirement during pregnancy in rats maintained on 80 g protein/kg diet reflects a decreased maternal response to insulin, which may be manifest either through inadequate suppression of hepatic glucose production or through reduced peripheral glucose disposal.

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Micronutrients and fetal growth retardation in Brazil. By P. RONDO[1], R. ABBOTT[2], L. RODRIGUES[3] and A.M. TOMKINS[2]. [1]*Nutrition Department, Public Health School, University of Sao Paulo, Brazil,* [2]*Centre for International Child Health, Institute of Child Health, London WC1N 1EH and* [3]*Epidemiology Department, London School of Hygiene and Tropical Medicine, London WC1E 7HT.*

A case-control study of 712 Brazilian mother-baby pairs was performed to assess the levels of vitamin A, folate and iron in maternal blood and cord blood samples from 356 babies who had intrauterine growth retardation (IUGR) and 356 who had appropriate birth weight (ABW). To control for confounding factors, stratification and logistic regression analyses were performed. The following groups of risk factors for IUGR were explored: nutritional, genetic/constitutional, obstetric/antenatal care, toxic exposures, infections, demographic, psychological and socioeconomic. IUGR in this population was significantly related to ten independent risk factors (Table).

	Adjusted odds ratio	95% CI	Attributable risk (%)
Coffee intake	2.45	1.65- 3.62	50.6
Cigarette smoking	3.22	2.25- 4.62	28.1
Per capita income	2.27	1.65- 3.13	27.6
Cord vitamin A	2.78	1.88- 4.12	20.1
Cord haemoglobin	3.78	2.22- 6.50	18.8
Maternal weight	5.11	2.96- 8.88	18.7
Maternal ferritin	1.92	1.37- 2.71	18.1
Maternal weight gain	5.12	2.51-10.71	16.8
History of low birth weight	3.33	2.02- 5.53	13.8
Beer intake	2.57	1.54- 4.33	10.9

The higher levels of maternal ferritin in IUGR, despite similar levels of maternal Hb may reflect increased prevalence of subclinical systemic infection in IUGR mothers. The higher cord levels of Hb in IUGR babies may reflect placental insufficiency and hypoxia. The lower levels of cord vitamin A, despite similar levels of maternal vitamin A, may reflect problems of placental transport, fetal binding, storage and utilization. Nutritional deficiency in IUGR babies may be the result of being born small, rather than vice versa.

The study was supported by the Overseas Development Administration.

Influence of early diet in full-term babies on their subsequent cognitive performance during adolescence. By L.C. GREENE[1], A. LUCAS[2], M.B.E. LIVINGSTONE[1] and P.S.E.G. HARLAND[3], *[1]Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA, [2]MRC Dunn Nutrition Unit, Cambridge CB4 1XJ and [3]Child Health Department, West Lane Hospital, Middlesbrough TS5 4EE*

The long-term consequences of infant nutrition on neurodevelopment remains an area of considerable controversy. Animal studies have shown that undernutrition in early life has an adverse effect on later behaviour and performance. However, similar studies in humans are inconclusive. Lucas *et al.*(1989, 1990) have shown that dietary manipulation in preterm infants has a significant impact on developmental quotient during infancy. A subsequent 8.3 point advantage in intelligence quotient (IQ) was also reported at 7.5-8 years follow-up (Lucas *et al.* 1992).

To examine the association between early diet and later achievement of full-term infants information on the method of infant feeding was collected for 432 subjects (208 males, 224 females) aged 11-16 years living within the South Tees area. Data were collected from 0-4 years hospital consultation records and health visitor reports. In addition, extensive data on the current family structure, social class and mother's education level were obtained. IQ was assessed using the Raven's Standard Progressive Matrices test combined with a battery of additional psychometric tests which measured verbal fluency, numerical ability, reasoning and primary mental ability.

	Breastfed group 1 (*n* 177)		Non-breastfed group 2 (*n* 255)		Advantage for breastfed subjects
	Mean	SEM	Mean	SEM	
Duration of breastfeeding (weeks)	12.7	0.9	0		-
Raven's IQ	99.2	0.9	94.9	0.7	4.3 ***
Verbal IQ	107.1	1.1	102.0	0.9	5.1 ***
Numerical IQ	106.5	1.2	101.8	0.8	4.7 ***
Reasoning IQ	112.7	1.2	107.8	0.9	4.9 ***
Primary mental ability IQ	110.5	1.2	104.5	0.9	6.0 ***

*** $P < 0.001$, group 1 *v.* group 2 (Student's *t* test).

The Table above presents the unadjusted IQ scores of children in the breastfed (group 1) and non-breastfed (group 2) groups. Group 1 children showed a highly significant advantage over group 2 children for all measures of IQ assessed. When regression analysis was used to adjust for possible confounding factors (social class, mother's education, birth weight, gestational age, birth rank, child's sex and mother's age) known to be related to developmental scores the beneficial effect of breastfeeding was statistically non-significant. However, when duration of breastfeeding (*n* 155) was considered, subjects breastfed for more than 12 weeks (*n* 52) had a significant advantage with respect to verbal IQ ($P<0.01$), reasoning IQ ($P<0.01$) and primary mental ability IQ ($P<0.05$) when compared to those breastfed for 1-12 weeks (*n* 103). After adjustment for possible confounding factors, this significant advantage in verbal and reasoning IQ persisted.

Although the impact of known and unknown confounding influences precludes firm conclusions, these results would suggest that duration of breastfeeding exerts a significant influence on the later cognitive achievement of full-term infants.

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Acute interleukin-1β administration induces anorexia and increases regional hypothalamic neuropeptide Y concentrations in the meal-fed rat. By H.D. MCCARTHY[1], S. DRYDEN and G. WILLIAMS, *Department of Medicine, University of Liverpool, P O Box 147, Liverpool L69 3BX,* [1] present address *Department of Human Nutrition, University of Southampton, Southampton, SO9 3TU*

Interleukin-1β (IL-1β) is thought to mediate the anorexia in inflammatory states such as infection and cancer, and potently reduces food intake in rats maintained on laboratory chow (Moldawer *et al.* 1988). The mechanism by which cytokines inhibit feeding is unclear but it is likely that they interact with hypothalamic appetite-regulating pathways. The neuropeptide Y (NPY) network within the hypothalamus is an endogenous feeding stimulatory pathway (Williams *et al.* 1991), arising, in part, from NPY-synthesizing cell bodies within the arcuate nucleus, whose projections travel to the medial preoptic area, paraventricular nucleus, ventromedial nucleus and dorsomedial nucleus. At these sites, exogenous NPY administration stimulates feeding. In the present study, we tested the effect of acute peripheral IL-1β administration in the rat, on feeding, and its interaction with the hypothalamic NPYergic system, by measuring regional NPY concentrations within selected NPY-containing hypothalamic areas.

Twenty-four male Wistar rats (starting weight 85 (SE 5) g) were trained over a 3-week period to consume their daily intake of standard laboratory chow in a 4 h block, beginning at 10.00 hours. On the day of the study, the rats were divided into three weight-matched groups. The first group was injected with sterile saline (9 g NaCl/l, given intraperitoneally) and the second with recombinant human interleukin-1β (rh IL-1β, 1 μg/100 g body weight, given intraperitoneally). Thirty minutes after injection, animals were allowed free access to preweighed food hoppers. The third group were saline injected and pair-fed to the level of intake of the IL-1β-treated rats. One hour later, rats were killed, plasma collected and eight selected hypothalamic areas microdissected and assayed for NPY content using a specific radioimmunoassay.

Food intake was suppressed in the IL-1β-treated rats by 83 % ($P<0.0001$) compared with saline-treated rats. Circulating corticosterone concentration was significantly elevated in both IL-1β-treated and pair-fed rats (by 344 % and 315% respectively, $P<0.001$ *v.* saline-treatment). Circulating glucose and insulin concentrations were unaffected by any treatment.

NPY concentrations in the IL-1β-treated group were significantly elevated in the medial preoptic area (by 86 %, $P<0.03$), paraventricular nucleus (94 %, $P<0.007$), ventromedial nucleus (40 %, $P<0.03$) and the dorsomedial nucleus (39%, $P<0.02$). NPY concentrations were not altered in the arcuate nucleus (the main hypothalamic site of synthesis), nor in any other region examined. In pair-fed animals, which consumed the same amount of food as the IL-1β-treated group, NPY concentrations were intermediate between the free-feeding, saline-treated rats and anorexic IL-1β-treated rats in the medial-preoptic area (increased by 41% compared with controls, $P<0.02$, $P<0.05$ *v.* IL-1β-treated), paraventricular nucleus (63 %, $P<0.0001$, not significant *v.* IL-1β-treated) and ventromedial nucleus (19%, $P>0.05$, not significant).

Since NPY concentrations were not increased in the arcuate nucleus, this suggests that NPY synthesis was not stimulated acutely. The increased NPY concentrations in other regions, all of which are implicated in appetite regulation, could have accumulated due to a decreased peptide release at the neuronal terminal. This being the case, such an acute inhibition of NPY release within critical appetite-regulating areas, might partly account for IL-1β-induced anorexia in the rat.

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Modulatory effects of butter and maize oil on the response of cholesterol metabolism in liver and serum to endotoxin in rats. By H. T. BESLER., P.S. TAPPIA and R. F. GRIMBLE, *Department of Human Nutrition, University of Southampton, Southampton SO16 7PX*

The cytokines interleukin 1 and 6 (IL-1 and IL-6) and tumour necrosis factor (TNF) have been reported to bring about a wide variety of metabolic changes (Grimble, 1994). These changes are collectively called the acute-phase response (APR). These include changes in hepatic glucose and glycogen utilization, triacylglycerol and lipoprotein metabolism and mineral metabolism. It has been reported that the administration of TNF or IL-1 increases serum triacylglycerol levels within 1-2 h due to an increase in hepatic lipoprotein production (Feingold *et al.* 1993). The purpose of the present study was to examine whether endotoxin administration increases serum and liver cholesterol concentration and secondly, whether dietary fats in the form of maize oil (rich in linoleic acid, 18:1*n*-6) and butter (rich in oleic acid, 18:1*n*-9 and poor in 18:1*n*-6) modulate IL-1, IL-6 and TNF in the circulation and subsequently cholesterol metabolism.

Male Wistar weanling rats from the Southampton University Medical School colony were fed *ad lib* on butter (BU) and maize oil (MO) at 100 g fat/kg diet for 4 weeks. All diets were adequate in all nutrients. Butter diet contained 10 g maize oil/kg to prevent essential fatty acid deficiency. At the end of the feeding period, each dietary group was divided in two. One half received a subcutaneous injection of 0.8 mg endotoxin/kg body weight (END)(Difco strain,055:B9). The second half of each group received an injection of sterile non-pyrogenic saline (SAL) and were pair-fed the intakes of the endotoxin animals over a 24 h period.

Diet...		MO		BU	
		Mean	SE	Mean	SE
Serum cholesterol	(SAL)	2.85	0.09	2.97	0.08
(μmol/l)	(END)	5.31*	0.14	3.10	0.07
Liver cholesterol	(SAL)	61.2	3.93	63.6	3.29
(μmol/g)	(END)	98.6*	3.12	74.2	3.93
IL-1	(SAL)	361.0	27.1	213.6	75.1
(pg/ml)	(END)	105.3*	32.8	197.2	58.5
(IL-6)	(SAL)	3.71	0.24	2.85	0.12
(ng/ml)	(END)	5.91	2.49	3.28	0.18
TNF	(SAL)	1.35	0.16	1.28	0.22
(ng/ml)	(END)	1.42	0.08	1.51	0.25

Each observation represents the mean and standard error for four animals. * Significantly different from saline control (ANOVA): $P<0.05$.

Endotoxin injection resulted in a substantial increase of cholesterol concentration in liver and serum of rats fed on maize oil, compared with saline injection. In the butter group no significant difference was apparent between saline- and endotoxin-injected animals. In maize-oil-fed animals, there were no significant differences in IL-6 and TNF, whereas IL-1 level was significantly lower after endotoxin injection in serum. In rats fed on the butter diet, there were no differences in any of the cytokines examined.

Many early studies described the presence of IL-1 inhibitory bioactivities in humans and animals (Arend, 1993). Thus, reduced IL-1 level in the group of animals fed on maize oil may be due to increased IL-1 inhibitory activities in response to endotoxin. In the present study, the increase in liver cholesterol induced by endotoxin may account for the increase in serum cholesterol concentration. An increased cholesterol concentration in the liver and serum may be due to increased activity of the rate-limiting enzyme, HMG-CoA reductase (EC 1.1.1.88), since HMG-CoA reductase is accepted as a member of the group of proteins that is positively regulated by inflammatory stimuli (Feingold, 1992, 1993).

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Fatty acids modulate the affinity of tumour necrosis factor-α (TNFα) receptors in cultured rat hepatocytes. By A. G. CLAMP and R. F. GRIMBLE, *Institute of Human Nutrition, University of Southampton, Southampton SO9 3TU*

The inflammatory response to tumour necrosis factor-α (TNFα) in cultured rat hepatocytes has been shown to be enhanced by supplementation with palmitic acid (16:0), and suppressed by docosahexaenoic acid (DHA; 22:6*n*-3) treatment (Clamp & Grimble, 1994). The aim of the present study was to assess whether these fatty acids could modulate the binding affinity and number of TNFα receptors, and thereby account for their effects on the acute-phase response of hepatocytes to TNFα *in vitro*.

Rat hepatocytes were incubated (CO_2-air; 5:95, v/v; 37°) in a medium containing 50ml foetal bovine serum/l, antibiotics, insulin and dexamethasone, supplemented with a bovine serum albumin (BSA)-fatty acid complex at a final concentration of 200 μM. After 24 h the binding affinity (K_d) and number (B_{max}) of TNFα receptors were assessed by competitive binding of ^{125}I-TNFα and unlabelled TNFα, followed by Scatchard analysis (Scatchard, 1949).

Fatty Acid	K_d ($\times 10^{-10}$ M)		B_{max} (sites/cell)	
	Mean	SEM	Mean	SEM
16:0	2.31	0.34	2590	56
22:6*n*-3	1.04*	0.12	2481	51

* Significantly different to palmitic acid value ($P<0.05$). *n* 4 for each fatty acid.

There was no significant difference between numbers of hepatocyte TNFα receptors when cells were cultured with either palmitic acid or DHA. However, the binding affinity of the receptors was significantly greater in hepatocytes supplemented with DHA than in those treated with palmitic acid.

The results of the present study indicate that the affinity of the rat hepatocyte TNFα receptor can be modulated by incubation with fatty acids. Although this result does not explain the attenuation of the hepatocyte inflammatory response to TNFα by DHA, it may provide some insight into the mechanisms by which dietary lipids influence the inflammatory response to cytokines.

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Effects of endotoxin on liver plasma membrane phospholipid class distribution in rats fed on maize and olive oils and butter. By H. T. BESLER and R. F. GRIMBLE, *Department of Human Nutrition, University of Southampton, Southampton SO16 7PX*

Dietary fats, both in quantity and type, have been shown to influence immune system functioning and the inflammatory response by altering the fatty acid composition of the cell membrane phospholipids, which in turn affects eicosanoid metabolism (Grimble, 1994). It has been suggested that the composition of liver plasma membrane polar lipids is not constant and may also vary with age, cell type and physiological state of the animal examined (McMurchie, 1988). However, the possible modulatory effect of dietary fat on phospholipid polar head groups at the membrane in animals followed by an inflammatory stimulus has received little attention. We have therefore examined the possible modulatory effect of dietary fats on polar phospholipid class distribution in liver plasma membrane in Wistar rats and the subsequent effects of endotoxin injection.

Male Wistar weanling rats were fed *ad lib* on olive oil, maize oil and butter at 50, 100 and 200 g fat/kg diet for 4 weeks. All synthetic diets were adequate in all nutrients. In all butter and olive-oil diets, 10 g maize oil/kg was added to prevent essential fatty acid deficiency. The study design also included the standard rat chow (27 g fat/kg) to see the response in rats fed on a habitual laboratory diet. At the end of the feeding period, rats were injected with endotoxin (END)(Difco strain,055:B9) at a dose of 0.8 mg/kg body weight or sterile non-pyrogenic saline (SAL). Saline-injected animals were pair-fed the intakes of the endotoxin animals over 24 h. After separation of plasma membrane from liver, the individual phospholipids were separated by thin-layer chromatography (Holub & Skeaff, 1987). The phospholipid assay was performed by the method of Barlett (1959). Cholesterol was also measured.

Dietary Fat..	Maize oil			Butter			Olive oil			Chow	Pooled
Fat (g/kg)	50	100	200	50	100	200	50	100	200	27	SEM
					μg P/mg protein						
PC (SAL)	43.8	40.9	39.4	40.9	45.1	41.7	42.5	42.2	40.3	48.1	
(END)	36.9*	29.9*	26.1*	41.0	46.1	34.7*	41.6	39.4	37.4	46.2	1.18
PS (SAL)	8.8	7.8	5.7	12.4	14.3	15.0	14.0	14.4	11.9	14.8	
(END)	11.6*	10.7*	8.7*	11.6	13.3	12.9*	13.7	13.9	13.6	8.6*	0.52
PI (SAL)	10.5	9.3	9.2	9.1	9.7	8.9	8.9	8.8	8.3	9.5	
(END)	9.5	6.9*	6.0*	9.2	9.3	7.7	8.9	8.4	7.8	7.9*	0.54
					CL:PL						
(SAL)	0.32	0.34	0.37	0.31	0.29	0.32	0.31	0.30	0.32	0.27	
(END)	0.41*	0.55*	0.64*	0.33	0.31	0.39*	0.30	0.31	0.36*	0.34*	0.05

Each observation represents the mean for four animals. * Significantly different from saline control (ANOVA): $P<0.05$. PC, phosphatidylcholine; PS, phosphatidylserine; PI, phoshatidylinositol; CL:PL, cholesterol : phospholipid ratio

Maize oil reduced the amount of PS in membranes and increased the CL:PL ratio in a dose-dependent manner. In chow-fed animals endotoxin decreased PS and PI in membranes and increased the CL:PL ratio indicating substitution of cholesterol for phospholipids in the membrane under the action of the inflammatory challenge. These changes did not occur in animals fed on olive oil, or butter at the two lowest levels of intake.

Alterations in dietary fat and inflammatory agents modify hepatic plasma membrane phospholipid class and cholesterol content. Consumption of olive oil or butter may exert an anti-inflammatory influence, and maize oil a proinflammatory influence, on response to endotoxin via changes in membrane architecture, by mechanisms yet to be elucidated.

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Prenatal nutritional adequacy and gender influence the ability of adult rats to produce interleukins 1 and 6 and tumour necrosis factor α. By P.S.TAPPIA, H.D.McCARTHY, S.C.LANGLEY-EVANS, A.A.JACKSON and R.F.GRIMBLE, *Institute of Human Nutrition, University of Southampton, SO16 7PX*

Recent epidemiological studies in human populations have demonstrated a link between low birth weight and poor early growth and non-communicable diseases in adulthood (Barker *et al.* 1993*a,b*). We have developed an animal model to investigate the mechanisms which underlie this phenomemon. In adulthood, rats whose mothers consumed a diet containing 90 as opposed to an 180 g casein/kg, exhibited blunted metabolic responses to endotoxin despite consuming standard laboratory chow from weaning (Langley *et al.* 1994). The ability to produce interleukins 1 and 6 (IL1 and 6) and tumour necrosis factor (TNF) are major determinants of the response to inflammatory agents.

We examined the ability of peritoneal macrophages derived from young adult male and female rats, with identical dietary treatment to those reported earlier, and cells from the adult offspring of mothers fed standard laboratory chow throughout pregnancy, to produce IL1, IL6 and TNF *in vitro*, in response to endotoxin (10 ng/ml). Macrophages were elicited with the use of an intraperitoneal injection of thioglycollate broth. Cytokine concentrations were measured by bioassay 24 h after endotoxin stimulation.

Maternal diet	TNF (pmol/l)		IL6 (pmol/l)		IL1 (pmol/l)	
	Mean	SD	Mean	SD	Mean	SD
Chow						
Females	106	19	148	52	11.1	3.9
Males	49	16	100	26	3.5	1.3
180 g Casein/kg						
Females	81	11	47	6	3.5	1.3
Males	64	15	190	99	5.9	1.9
90 g Casein/kg						
Females	94	46	51	14	4.4	1.7
Males	54	4	140	8	4.8	0.4

Each observation represents the mean for four animals per group.

These data provide the first evidence that altered maternal nutrition during pregnancy modifies the ability of offspring to produce IL1, IL6 and TNF in response to inflammatory stimuli in adulthood. There is a differential effect of low maternal dietary protein intake and the cytokine affected. However, differences in data between offspring of mothers fed on chow or the synthetic diets during pregnancy indicate that other nutrients may exert a modulatory influence *in utero*. Manifested within these changes is the influence of gender.

The ability of prenatal nutrition to alter cytokine biology in adulthood may have major implications for immune function and chronic inflammatory disease.

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Endotoxin-induced production of interleukin-6 (IL-6) is enhanced by vitamin E deficiency and reduced by dietary polyphenols. By A.M.T.AMARAKOON, P.S.TAPPIA and R.F.GRIMBLE, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

Inflammatory stimuli bring about cytokine and free-radical production by phagocytes and other immune cells (Farante *et al.* 1988). Free radicals enhance the production of cytokines *in vivo* and *in vitro* (Clark *et al.* 1989). *In vitro* studies indicate that synthetic antioxidants such as butylated hydroxy anisole (BHA) can prevent this phenomenon (Peristeris *et al.* 1992). It is not known however, whether vitamin E and other antioxidant substances in the diet will attenuate the actions of free radicals on cytokine production.

Forty-eight weanling male Wistar rats were randomly divided into four groups. Group 1 was fed on a synthetic diet containing casein (180 g/kg), methionine (3 g/kg), vitamin E-deficient vitamin and mineral mixture (50 g/kg), Maize oil (30 g/kg), cellulose (100 g/kg), sucrose (318 g/kg) and starch (318 g/kg). The second group was fed on a similar diet with the addition of a decaffeinated polyphenol-rich extract from black tea (5 g/kg diet). The third group was fed with a similar diet to that given to group 1 but which contained vitamin E (0.05 g/kg diet). The fourth group was fed with a diet which contained both vitamin E (0.05 g/kg diet) and tea extract (5 g/kg diet). All groups were fed *ad libitum* for 21 d. Each group was subdivided on day 21. One group was injected i.p. with bacterial lipopolysaccharide (LPS, 200 μg/kg body weight) and killed after 24 h. The other group was injected with sterile non-pyrogenic saline (9 g NaCl/L) on day 22, pair-fed for 24 h and killed. IL-6 and caeruloplasmin in plasma and liver glutathione were measured.

Dietary group	IL-6 (ng/ml)		Caeruloplasmin (U/ml)		Glutathione (mg/g liver)	
	LPS	Saline	LPS	Saline	LPS	Saline
1	2.05	1.49	0.23	0.17	1.11	0.98
2	1.68	1.47	0.23	0.17	1.15	0.89
3	1.69	1.50	0.25	0.16	1.20	0.86
4	1.65	1.53	0.20	0.13	1.27	0.81
SEM	0.03	0.02	0.01	0.005	0.07	0.04

The vitamin E-deficient diet enhanced IL-6 concentration after endotoxin injection. Addition of either vitamin E or tea extract or both produced similar reductions of IL-6 in plasma (17.6%, 18%, 19.5%, $P<0.05$). In animals fed with both vitamin E and tea extract significantly lower caeruloplasmin concentrations and increased liver glutathione concentrations were observed for both LPS and saline-injected animals (13% $P<0.05$, 25% $P<0.01$, 15% $P<0.05$, 17% $P<0.01$).These data indicate that both vitamin E and tea extract may improve poor antioxidant status *in vivo* thereby preventing exacerbation of cytokine production.

Thus the full range of antioxidant substances in the diet may contribute to the treatment of autoimmune/inflammatory diseases and cancer in which cytokines and free radicals interact to the detriment of the subject.

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The effect of fatty acids on the inflammatory response to cytokines by cultured rat hepatocytes.

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Dietary lipids can modulate the inflammatory response to tumour necrosis factor-α (TNFα; Mulrooney & Grimble, 1993), but not to interleukin-6 (IL-6; A. G. Clamp and R. F. Grimble, unpublished results). It is unclear whether the observed modulation is due to changes in cytokine production, altered target tissue responsiveness, or a combination of these factors. The aim of the present study was to assess the influence of exogenously added fatty acids on the acute-phase response of cultured rat hepatocytes to TNFα or IL-6.

Rat hepatocytes were incubated (CO_2-air; 5:95, v/v; 37°) in a medium containing 50ml foetal bovine serum/l, antibiotics, insulin and dexamethasone, supplemented with one of a variety of bovine serum albumin (BSA)-fatty acid complexes at a final concentration of 200 μM. After 24 h 1000 U TNFα or IL-6 in culture medium, or culture medium alone, was added to the hepatocytes, which were incubated for a further 24 h. Viability of 48-h-old cultures was >95%. Albumin concentrations in the cell lysates were determined, as a measure of the inflammatory response to the cytokines, using a quantitative enzyme-linked immunoassay.

Fatty Acid	14:0	16:0	18:0	18:1	18:2	18:3	20:4	20:5	22:6	BSA
Albumin concentration (% control)	86.0^{*b}	75.8^{*a}	86.2^{*bc}	90.0^{*bcd}	92.6^{*d}	92.1^{*cd}	91.6^{*bcd}	91.8^{*bcd}	95.1^{d}	90.7^{*bcd}

Figures represent the concentration of albumin in hepatocyte cultures treated with TNFα, as a percentage of untreated controls. * Significantly different from controls ($P<0.05$). a,b Values with unlike superscripts were significantly different ($P<0.05$). n 4 for each group.

Albumin concentrations in the cell lysates of hepatocytes treated with IL-6 were significantly lower than untreated controls, with the fatty acids having no observable effect on the response to this cytokine. However, certain fatty acids exerted a significant influence on the hepatocyte acute-phase response to TNFα. Palmitic acid (16:0) significantly enhanced the inflammatory response to TNFα, whereas supplementation with docosahexaenoic acid (DHA; 22:6*n*-3) resulted in a suppression of the response.

Feeding a diet rich in fish oil, which contains significant quantities of DHA, has been shown to result in an attenuated inflammatory response to TNFα *in vivo* (Mulrooney & Grimble, 1993). The results of the present *in vitro* study using DHA would therefore seem to agree with previous *in vivo* studies. However, the amplification of the acute-phase response to TNFα by palmitic acid observed in the present study has not been seen previously *in vivo*. Investigations of TNFα signal transduction in rat hepatocytes cultured with palmitic acid or DHA may provide information about the mechanisms underlying dietary lipid modulation of the inflammatory response to cytokines.

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Calprotectin in cystic fibrosis: a sensitive marker of the acute-phase response to infection. By P. A. CLOHESSY[1], M.K. FAGERHOL[4], J. FRIEND[3], D. GOUDIE[2], P.J. HELMS[1], M. OMRAN[2], G. RUSSELL[2] and B. E. GOLDEN[1], [1]Department of Child Health, University of Aberdeen, Aberdeen AB9 2ZD, [2]Royal Aberdeen Children's Hospital, Aberdeen AB9 2ZD, [3]Aberdeen Royal Infirmary, Aberdeen AB9 2ZD and [4]Department of Immunopathology, Ulleval Hospital, 0407 Oslo, Norway

Calprotectin is a calcium-binding protein of the S-100 family. It is expressed in neutrophils and monocytes (Fagerhol *et al.* 1990) and constitutes up to 60% of neutrophil cytosolic protein. It's release and detection in plasma provide a sensitive marker of neutrophil turnover. Plasma concentrations of calprotectin increase up to 130 times normal values during the acute-phase response to bacterial infection. Calprotectin is similar if not identical to a postulated marker of cystic fibrosis (CF), CFAg, also found in neutrophils and plasma (Andersson *et al.* 1988). However, CFAg has not proved a useful marker of CF because its specificity is too low: too many controls have high plasma concentrations (Hayward *et al.* 1987). Without effective antibiotic therapy, CF patients almost continuously harbour respiratory pathogens and cough up purulent sputum. However, evidence of an acute-phase response is typically lacking. We postulated that plasma calprotectin concentration, as a measure of granulocyte turnover, may be a useful index of the extent of infection in CF.

We compared plasma calprotectin concentration, measured by an ELISA, in forty-three CF patients and age and sex-matched controls, in relation to leucocyte count, neutrophil count and plasma C-reactive protein (CRP). Twenty-one CF patients produced purulent sputum samples which grew mainly *Staphloccus aureus* and/or *Pseudomonas aeruginosa*. Ten patients had raised leucocyte counts (mean 12.9 x 10^9/l, *n* 10); eight had raised neutrophil counts (mean 10.2 x 10^9/l, *n* 8); and eight had raised CRP (mean 2.1mg/dl, *n* 8). Twenty-six CF patients had calprotectin concentrations above the control range (185-1565 μg/l). The CF group as a whole had significantly raised plasma calprotectin levels, 7.62 (SE 0.15) *v.* 6.29 (SE 0.11) $\log_e$ μg/l, $P < 0.0001$). One CF child with sputum that grew *Haemophilus influenzae* had a plasma calprotectin of 25 850 μg/l.

Thus, calprotection appears to be a more sensitive marker of infection than leucocyte count, neutrophil count or CRP in CF. It shows a similar overlap to that reported for plasma CFAg concentration between CF patients and controls.

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The effect of dietary lipid manipulation on lymphocyte cell surface molecule expression. By P. SANDERSON, P. YAQOOB and P.C. CALDER, *Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU*

Immunomodulatory effects of dietary lipid manipulation have been previously described (Yaqoob *et al.*1994*a*,*b*). However, there is little information about the possible mechanisms by which dietary lipids can affect the immune response. It is possible that they influence the expression of key cell surface molecules. Therefore, the effect of dietary lipid manipulation on the expression of various lymphocyte cell-surface molecules involved in the immune response was investigated.

Adult Lewis rats were fed for 12 weeks on a low-fat diet (LF; 20 g/kg) or on high-fat diets containing 200 g/kg hydrogenated coconut oil (HCO), olive oil (OO), safflower oil (SO), evening primrose oil (EPO) or menhaden oil (MO). Spleen lymphocytes were prepared and stained for analysis by flow cytometry. Lymphocytes were stained using monoclonal antibodies against T-cell receptor (TCR), membrane-bound immunoglobulin (mIg), a non-polymorphic region of the major histocompatibility class II molecule (MHC II), inducer/helper T lymphocytes (CD4), suppressor/cytotoxic T lymphocytes (CD8), T lymphocyte specific adhesion and signalling molecule (CD2) and leucocyte functional antigen-1 (LFA-1). Lymphocytes were also double stained for CD2 and CD8 or CD4 and for LFA-1 and CD8 or CD4. Flow cytometry data are presented as mean fluorescence (MF) which represents the level of expression within a cell population.

	MF					
Diet	CD2/CD4	CD2/CD8	CD2	LFA-1/CD4	LFA-1/CD8	LFA-1
LF	337.34[a,b,d,e]	340.63[a]	408.86[a,d]	230.53 [a,d]	247.59 [a,d]	398.91 [a,d]
HCO	291.39	338.16[a]	383.57[a]	218.44 [a,d]	259.68 [a,d]	396.10 [a,d]
OO	307.06[a]	310.75	361.65	179.54	198.44	329.10
SO	326.71[a,c]	325.42[a]	392.79[a]	200.72	213.99	371.19 [a]
EPO	302.81[a]	314.38[a]	374.54[a]	203.65	234.12 [a]	377.31 [a]
MO	273.87	275.07	335.94	166.47	178.03	313.05
Pooled SD	19.61	26.48	26.61	22.96	25.95	35.21
Significance	P=0.001	P=0.01	P=0.006	P=0.003	P=0.001	P=0.004

Values are for five animals fed on each diet. Statistical analysis was by two way analysis of variance (second factor day) followed by least squared significance with a significance level of $P<0.05$ ([a] > MO, [b] > EPO, [c] > SO, [d] > OO, [e] > HCO).

No effect of dietary lipid manipulation on either mean fluorescence or percentage of cells staining positive for mIg, TCR, CD4, CD8 or MHC II was observed. This indicates that there was no difference in lymphocyte subsets between dietary groups. LFA-1 and CD2 (also known as LFA-2) are adhesion molecules important in the immune system for T-cell activation, endothelial adherence and consequent leucocyte migration, cytotoxic T-cell killing and antigen presentation. The present study found that the expression of these molecules is modulated by dietary lipid manipulation. Feeding the MO, EPO or OO diets consistently decreased the mean fluorescence. Feeding the MO diet had the most suppressive effect. There was no significant difference between dietary groups for the percentage of cells staining positive or the percentage of cells staining double positive for CD2 or LFA-1. Therefore, no change in the cell population size, as defined by these molecules, was observed, but the relative expression within the population was affected. Thus, this study provides evidence that the expression of adhesion molecules can be affected by dietary lipids. This could be one means by which lipids such as fish oils suppress immune function and limit leucocyte migration and so may partially explain the beneficial effects of these lipids in inflammatory diseases.

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The effect of dietary lipid manipulation on leucocyte proliferation in rat whole blood. By P. YAQOOB, E.A. NEWSHOLME and P.C. CALDER, *Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU*

Polyunsaturated fatty acids (PUFA) have been shown to suppress immune cell functions *in vitro* (see review by Yaqoob & Calder, 1993). Dietary studies in which animals have been fed with PUFA-containing oils before *in vitro* assessment of T-lymphocyte functions have yielded contradictory findings; such studies are difficult to compare since there are many variations in experimental protocols and culture conditions. For example, it has been demonstrated that although feeding oils rich in certain unsaturated fatty acids suppresses the proliferation of rat lymphocytes subsequently cultured in autologous serum, culturing the cells in fetal calf serum partially or totally masks these effects (Yaqoob *et al.* 1994). The present study investigated the effects of feeding a number of dietary lipids on rat leucocyte proliferation measured in whole-blood cultures. The use of whole-blood culture to test the effects of dietary lipids on leucocyte functions avoids the need for addition of serum, either autologous or from a different species, which may be lacking in the appropriate nutrients, growth factors, hormones and cytokines that the cells would normally be in contact with. Importantly, the technique has the advantage that the ratios between different cell types and the ratios between cells and serum components are the same as those *in vivo*.

Rats were fed for 10 weeks on a low-fat diet (LF; 20 g/kg) or on high fat diets containing 200 g/kg of hydrogenated coconut oil (HCO), olive oil (OO), safflower oil (SO), evening primrose oil (EPO) or menhaden (fish) oil (MO). Blood was collected into heparinized tubes immediately upon killing and diluted 1:10 with HEPES-buffered RPMI, supplemented with 2 mM glutamine and antibiotics. The diluted blood was cultured in the presence of either concanavalin A (Con A) or lipopolysaccharide (LPS) at concentrations between 1 and 50 μg/ml. Proliferation was assessed by incorporation of [^{3}H]thymidine over the final 18 h of a 66 h culture period and is expressed as stimulation index. Data are means with their standard errors of cell preparations from five animals fed on each diet.

	Stimulation index									
	Con A concentration (μg/ml)									
	1		5		10		25		50	
Diet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LF	37.88	4.74	86.78	9.98	207.58	56.05	363.18	79.82	133.60	43.27
HCO	5.58	2.44	45.50	6.52	134.10	11.38	371.54	53.83	303.70	32.48
OO	17.54	6.13	38.58	7.93	144.32	25.33	552.92	134.89	423.26	41.48
SO	49.34	9.82	107.16	15.29	244.86	55.46	461.75	128.50	371.30	59.40
EPO	52.18	13.57	114.34	19.33	246.50	52.31	652.38	109.53	450.58	85.71
MO	18.68	5.31	46.02	10.97	115.70	20.66	375.84	72.78	229.04	82.14

Compared with feeding the LF, SO or EPO diets, the HCO, OO and MO diets suppressed the proliferation of T-cells in whole blood at Con A concentrations of 1, 5 and 10 μg/ml. At a Con A concentration of 50 μg/ml, each of the high-fat diets enhanced T-cell proliferation compared with the LF diet (see Table). High fat feeding decreased the sensitivity of B-cells to LPS, but had no effect on the magnitude of the response to this mitogen (results not shown). These results suggest that dietary lipids have marked effects on leucocyte proliferation; the use of whole blood culture may be the most appropriate way of determining the effects of dietary lipids on immune function *ex vivo*.

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The effect of dietary fatty acids on lipoprotein composition in the rat. By N.M. JEFFERY[1], P. YAQOOB[1], P.C. CALDER[1], D. WIGGINS[2], G.F. GIBBONS[2] and E.A. NEWSHOLME[1], [1]*Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, and* [2]*Metabolic Research Laboratory, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE*

There is a correlation between elevated concentrations of cholesterol and triacylglycerol in the bloodstream and an increased risk of coronary heart disease (CHD). Dietary lipid manipulation has been shown to have a profound effect on serum lipid concentrations in laboratory animals (Yaqoob *et al.* 1993) and in man (Harris, 1989). Replacing dietary saturated fat, which tends to increase serum triacylglycerol and cholesterol concentrations, with unsaturated fatty acids, particularly long chain *n*-3 fatty acids, has been implicated as one way of decreasing the incidence of CHD.

In the present study, Weanling male Lewis rats were fed for 10 weeks on a low-fat diet (LF; 20 g/kg) or on high-fat diets containing 200 g/kg hydrogenated coconut oil (HCO), olive oil (OO), safflower oil (SO), evening primrose oil (EPO) or menhaden oil (MO). Chylomicrons (CM), very-low-density (VLDL), low-density (LDL) and high-density (HDL) lipoproteins were prepared from serum by density gradient ultracentrifugation. Total cholesterol and triacylglycerol concentrations were determined using standard procedures. The apolipoprotein B (Apo B) concentration of the VLDL fraction was determined using an enzyme-linked immunosorbant assay.

		DIET											
		LF		HCO		OO		SO		EPO		MO	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Cholesterol	CM	32	4	41	6	67	17	48	21	39	15	57	14
(μg/ml)	VLDL	76	30	81	18	72	15	45f	7	88	16	135d	32
	LDL	120	11	99	9	97	22	115	14	151	34	114	17
	HDL	612	90	745f	27	726f	50	685	91	704f	66	437bce	59
Triacylglycerol	CM	9bcf	1	65adef	10	74adef	4	11bc	3	17bc	7	18abc	1
(μg/ml)	VLDL	32	5	43	9	81abde	16	18cbf	1	31c	5	69d	20
Apo B (μg/ml)	VLDL	22	10	30	10	41	10	13	3	19	3	16	8

Values are the means and standard errors for three or four animals fed on each diet.
Statistical significance ($P<0.05$; analysis of variance) is indicated as: [a]v. LF, [b]v. HCO, [c]v. OO, [d]v. SO, [e]v. EPO, [f]v. MO

Feeding the HCO, OO or EPO diets increased the total serum cholesterol concentration compared with feeding the LF and MO diets. Feeding the MO diet produced a hypocholesterolaemic effect relative to feeding the other high-fat diets. This effect is mainly due to a decrease in the HDL-cholesterol concentration in animals fed on MO. The total serum triacylglycerol concentrations of animals fed on the LF, SO, EPO or MO diets were similar, but feeding the HCO or OO diets caused a hypertriacylglycerolaemic effect compared with the other diets. The elevation in triacylglycerol concentration is apparent in both CM and VLDL fractions of animals fed on the OO diet and in the CM fraction of animals fed on the HCO diet. There was little detectable triacylglycerol in the LDL and HDL fractions. VLDL from animals fed on the MO diet had a lower Apo B:triacylglycerol ratio than that from animals fed on the other diets. This may be indicative of an increase in VLDL particle size in animals fed on the MO diet.

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Dietary lipid manipulation influences phospholipid class distribution in the rat hepatocyte plasma membrane. By A. G. CLAMP and R. F. GRIMBLE, *Institute of Human Nutrition, University of Southampton, Southampton SO9 3TU*

Dietary lipids can modulate the hepatic acute-phase response to cytokines (Grimble, 1992). The mechanisms underlying this modulation are largely unknown, but may be related to the previously observed effects of dietary lipids on the fluidity and fatty acid composition of the rat hepatocyte plasma membrane (Clamp *et al.* 1993). However, changes in the relative quantities of the major phospholipid subgroups in the membrane may also influence the inflammatory response. We have therefore investigated the effect of dietary lipid manipulation on phospholipid class distribution in the rat hepatocyte plasma membrane.

Weanling male Wistar rats were fed for 5 weeks on a diet composed of either standard rat chow (23 g fat/kg diet) or one of four synthetic diets (100 g fat/kg diet) containing butter fat, coconut oil, maize oil or fish oil as the main lipid source. In all synthetic diets 100 g/kg of the fat was provided as maize oil to prevent essential fatty acid deficiency. At the end of the 5-week period, the animals were killed and hepatocyte plasma membranes isolated. Lipids were extracted from the plasma membrane fractions and the five major phospholipid classes were isolated and quantified.

Diet...	Chow		Butter		Coconut		Maize		Fish	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Sphingomyelin (SP)	10.5^{a}	2.7	20.1^{b}	1.3	16.7^{b}	0.7	18.3^{b}	0.6	12.2^{a}	1.0
Phosphatidylcholine (PC)	27.4^{a}	4.7	25.1^{a}	2.6	32.7^{ab}	1.4	35.7^{b}	1.0	37.6^{b}	1.6
Phosphatidylserine (PS)	13.0^{b}	2.0	14.1^{b}	0.8	12.3^{b}	0.1	7.5^{a}	0.4	13.6^{b}	1.1
Phosphatidylinositol (PI)	14.7^{b}	2.8	14.9^{b}	0.7	14.4^{b}	0.8	14.7^{b}	0.8	8.7^{a}	0.6
Phosphatidylethanolamine (PE)	34.4^{b}	2.6	25.8^{a}	1.1	23.9^{a}	0.9	23.8^{a}	1.1	27.9^{a}	0.7

Values for each phospholipid represent % by weight of the total mass of the five major phospholipid classes; a,b values in the same row with unlike superscripts were significantly different (ANOVA) $P<0.05$ (n 6 for each dietary group).

Hepatocyte plasma membranes from animals fed on the synthetic diets contained less PE and, with the exception of the fish-oil group, more SP than chow-fed controls. Membranes from maize-oil-fed animals contained more PC and less PS than controls. Feeding the fish-oil-based diet resulted in an increase in PC and a decrease in PI content of hepatocyte plasma membranes, relative to animals fed on the chow diet.

Both SP and PI are involved in signal transduction across the plasma membrane via generation of secondary messengers. Furthermore, the activities of several membrane proteins involved in cytokine signal transduction, such as phospholipases A_2, C and D, and protein kinases A and C, have been shown to be phospholipid dependent. The diet-induced changes in phospholipid headgroup distribution observed in the present study may therefore influence the hepatic acute-phase response to cytokines.

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Hepatic phosphatidylcholine biosynthesis in the rat: effects of pregnancy. By G.C. BURDGE and A.D. POSTLE, *Child Health, Level G, Centre Block, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD*

Late gestation in the rat is accompanied by a dramatic increase in *sn*-1 palmitoyl phosphatidylcholine (PC) molecular species concentration, in particular PC16:0/22:6, in both maternal liver and plasma (Hunt *et al.* 1991). This may serve to supply polyunsaturated essential fatty acids (EFA) to lipid stores which can be used during lactation, and as such, may play an important role in supporting growth and development of the neonate. In the present study we have investigated mechanisms underlying this pregnancy-associated change in hepatic and plasma PC composition. Rat liver PC is synthesized both *de novo*, from diacylglycerol (DAG) and CDP:choline, and by PE *N*-methylation pathways. Newly synthesized PC is composed of mainly *sn*-1 palmitoyl species which are subsequently partially remodelled to *sn*-1 stearoyl species by the actions of both phospholipases A_1 and A_2 (Tijburg *et al.* 1991). The effect of pregnancy upon each of these synthetic pathways was determined by following the incorporation of radiolabelled phospholipid head-group precursors into individual PC and phosphatidylethanolamine (PE) molecular species.

Adult rats, either non-pregnant or timed pregnant, were injected i.p. with 1.85MBq of either [^{14}C]choline or [^{14}C]methionine and sacrificed 6h later. Incorporation of radiolabelled head-group precursors into individual molecular species was analysed by reverse phase HPLC with on-line radiochemical detection (Burdge *et al.* 1993). Rates of PC synthesis were estimated from the specific radioactivities of phosphorylated choline and S-adenosyl methionine precursors. The composition of specific DAG pools for either PC or PE synthesis were determined from CDP:[^{14}C]choline and CDP:[^{14}C]ethanolamine incorporation into hepatic microsomal PC and PE molecular species *in vitro*.

Microsomal PC and PE synthesized *in vitro* at term were markedly enriched in 16:0/22:6, 1.8-fold and 2.0-fold respectively, compared with non-pregnant animals. Since hepatic microsomal PC and PE synthesized *in vitro* does not undergo acyl remodelling, these results indicate that the molecular species content of their specific DAG precursor pools are substantially modified during pregnancy. Similarly, incorporation [^{14}C]choline and [^{14}C]methionine into hepatic PC *in vivo* showed an increased content of 16:0/22:6-containing species at term compared with non-pregnant animals which suggests that although the extent of acyl remodelling appeared to be lower in term pregnant rats, its specificity was not significantly modified by pregnancy.

The rate of PC synthesis by the *de novo* pathway increased 2.7-fold (d21) during pregnancy and was accompanied by increased CDPcholine: 1,2-diacylglycerol cholinephosphotransferase (EC 2.7.8.2) activity (2.0-fold) at term. The rate of PE *N*-methylation was similar in both non-pregnant and term rat liver. These results were consistent with the increased proportion of plasma PC synthesized *de novo* compared with synthesis by *N*-methylation in term pregnant rats.

These results suggest that in the rat the adequacy of EFA supply to the neonate is dependent upon both maternal dietary intake and adaptations to maternal hepatic phospholipid metabolism.

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Pregnancy-associated adaptations to maternal hepatic and plasma phosphatidylcholine molecular species composition. By G.C. BURDGE and A.D. POSTLE, *Child Health, Level G, Centre Block, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD*

Optimal fetal growth and development require adequate supply of essential polyunsaturated fatty acids (EFA) from the mother at specific periods of gestation. Neuritogenesis in fetal brain is accompanied by substantial accumulation of docosahexaenoic acid (22:6*n*-3) into neural membrane phosphatidylethanolamine (PE). Failure to accumulate sufficient amounts of 22:6*n*-3 into brain PE during this critical developmental period impairs neurite formation and subsequent brain function (Innis 1991).

EFA are supplied to the fetus solely from the mother. One important mechanism of transport of EFA from maternal liver to the placenta is phosphatidylcholine (PC) on serum lipoproteins (Chen *et al.* 1992). Late pregnancy in the rat is accompanied by increased EFA concentration in maternal liver and plasma total phospholipids (Chen *et al.* 1992). This led us to speculate that one role of such adaptations to maternal hepatic and plasma phospholipid composition is to supply EFA to developing tissues at critical stages of maturation. In the present study we have investigated the effect of pregnancy upon the PC molecular species composition of human plasma, guinea pig liver and rat liver and plasma.

PC was isolated (Caesar *et al.* 1988) from chloroform-methanol extracts of sequential plasma samples from thirteen women between 16 weeks gestation and 6 months post-partum, rat liver and plasma and guinea pig liver from timed pregnant animals. Intact PC molecular species were resolved by reverse phase HPLC with post-column fluorescence detection (Postle 1987).

Human pregnancy was accompanied by increased plasma *sn*-1 palmitoyl PC molecular species concentration, specifically PC16:0/22:6 (46%), PC 16:0/20:4 (25%) and PC16:0/18:2 (37%) between gestational age 22 weeks and term, followed by a decrease over the first 6 months post-partum. This is coincident with rapid accumulation of 22:6*n*-3 into fetal human brain and the onset of neuritogenesis. There was no change in *sn*-1 stearoyl molecular species concentration.

In the fetal guinea pig the period of maximal neurite outgrowth and onset of electrical activity is preceded by rapid accumulation of 22:6*n*-3 into fetal guinea pig brain PE between gestational ages 25d and 40d (Burdge & Postle 1993). This was accompanied by increased maternal liver PC16:0/22:6 and 16:0/20:4 concentrations which were maximal at 35d gestation (1.8-($P<0.05$) and 2.8-($P<0.05$) fold compared with non-pregnant animals) and then decreased towards term (68d).

Late gestation in the rat (16 - 21d) was accompanied by increased PC16:0/22:6 concentration in liver (2.4-fold) and plasma (2.8-fold) compared with non-pregnant animals. Similarly, liver and plasma PC16:0/20:4 concentrations increased significantly in late gestation. Since accumulation of 22:6*n*-3 into developing rat brain is confined to the neonatal period, this adaptation to maternal hepatic and plasma PC composition may serve to supply EFA to lipid stores for use during lactation.

These results suggest supply of EFA to the developing fetus during critical periods in gestation is the result of specific adaptations to maternal hepatic PC metabolism rather than passive transfer of EFA from the maternal diet.

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The effects of adrenaline on fatty acid turnover and oxidation in lean and obese subjects.

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Metabolic abnormalities which are of importance in the aetiology and maintenance of the obese state have been extensively searched for. It does not appear that a low basal metabolic rate or a reduced metabolic response to thermogenic stimuli is responsible for most cases of obesity. Recently attention has centred on possible differences between lean and obese subjects in their ability to utilize fat as an energy source (Tremblay, 1992). The present study was designed to examine fat mobilization and oxidation in the basal state and in response to a catecholamine infusion in a group of lean and obese subjects. The thermogenic data for this study have already been reported as showing no difference between lean and obese groups (Webber *et al.* 1993).

Eleven healthy, obese subjects (mean fat-free mass 51.7 (SE 3.5) kg) and ten healthy, lean subjects (fat-free mass 51.4 (SE 3.1) kg) were recruited. Whilst resting supine a bolus of $Na^{13}CO_3$ was given to all the subjects followed by an infusion of [1-^{13}C]-palmitate in albumin and after 45 min infusion four arterialized venous blood samples and four expired air samples were obtained for later measurement of plasma palmitate concentration, [1-^{13}C]-palmitate enrichment and $^{13}CO_2$ enrichment by mass spectrometry. An adrenaline infusion was then commenced at a rate of 25 ng/min per kg ideal body weight for 90 min. The [1-^{13}C]-palmitate infusion was continued for the duration of the adrenaline infusion. Further blood samples were taken at intervals of 10 min during the study. Indirect calorimetry was carried out throughout the study.

Basal plasma palmitate turnover rates were similar in lean and obese subjects when expressed per unit fat-free mass (1.9 (SE 0.3) and 2.3 (SE 0.3) μmol/min per kg fat-free mass for lean and obese respectively), but were much lower in the obese when expressed per kg fat mass (2.3 (SE 0.2) vs 7.6 (SE 1.2) μmol/min per kg fat mass, $P<0.01$). Basal palmitate oxidation rates were higher in the obese (1.5 (SE 0.2) μmol/min per kg fat-free mass) than the lean (0.8 (SE 0.1) μmol/min per kg fat-free mass, $P<0.01$). In response to adrenaline plasma palmitate turnover increased by a similar amount in both groups, but plasma palmitate oxidation fell in the obese to 1.1 (SE 0.1) μmol/min per kg fat-free mass by the end of the adrenaline infusion ($P<0.05$), whilst oxidation was unchanged in the lean (0.8 (SE 0.1) μmol/min per kg fat-free mass).

In the basal state the obese subjects of this study do not appear to have a defect in fat oxidation, but their response to infused adrenaline may favour fat storage over fat oxidation.

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Impaired postprandial clearance of triacylglycerol-rich lipoproteins in adipose tissue in obesity.

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The increased risk of coronary heart disease in obesity is mediated in part through adverse changes in plasma lipoproteins, with elevated plasma triacylglycerol (TG) and decreased high-density-lipoprotein- (HDL)-cholesterol concentrations. Lipoprotein metabolism during the postprandial period may be an important determinant of plasma TG and HDL-cholesterol concentrations. We have investigated lipoprotein metabolism in subcutaneous adipose tissue *in vivo* in the postabsorptive and postprandial states in control and obese subjects.

Eleven healthy control subjects (body mass index, BMI 19 - 28 kg/m^2) and eight obese subjects (BMI 32 - 56 kg/m^2) were studied. Two of the obese subjects had been diagnosed as having non-insulin dependent diabetes, but were well controlled by diet at the time of study. Samples of the venous blood draining the abdominal subcutaneous depot were obtained as described previously (Coppack *et al.* 1992), and compared with arterial (or arterialized) blood. Adipose tissue blood flow was measured by ^{133}Xe washout. Samples were taken after overnight fast and then at intervals until 6 h after eating a meal providing 3.1 MJ, of which 41% were from fat. Lipoprotein fractions were separated by ultracentrifugation.

Arterial(ized) concentrations of plasma TG, very-low-density-lipoprotein- (VLDL)-TG and chylomicron-TG were higher in both the postabsorptive and postprandial states in the obese group, and for plasma and VLDL rose more after the meal. Clearance of TAG across the adipose tissue (assumed to reflect the action of lipoprotein lipase, *EC* 3.1.1.34) was greater before and after the meal in controls than in the obese (Table), as was the clearance of TG from the chylomicron fraction after the meal (Table). In the controls, postprandial chylomicron-TG removal was accompanied by a decrease in clearance from the VLDL fraction; in the obese, VLDL-TG clearance was lower initially and did not change after the meal (results not shown). The extraction of TG from TG-rich lipoproteins was accompanied by exchange of cholesterol and apolipoprotein A1 in the HDL fraction. In control subjects, exchange of HDL-cholesterol (during passage through the tissue) changed from net loss to net gain after the meal; in the obese, there was no such tendency (not shown).

TG clearance in adipose tissue before and for 3 - 6 h after a meal (μl/100 g per min)

	Plasma TG				Chylomicron-TG	
	Basal		Postprandial		Postprandial	
	Mean	SEM	Mean	SEM	Mean	SEM
Controls	310	70	370	90	910	210
Obese	70**	20	50**	50	370*	70

Significantly different from control: *$P<0.05$; **$P<0.01$.

We conclude that impaired clearance in adipose tissue of TG from the TG-rich lipoproteins in obese subjects is associated with impaired movement of cholesterol into the HDL fraction. Thus, metabolic events in adipose tissue in the postprandial period may underlie the unfavourable lipoprotein pattern of obesity.

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Determinants of carotenoid antioxidant activity in model membranes. By A.A. WOODALL[1], G. BRITTON[1] AND M.J. JACKSON[2], *Departments of [1]Biochemistry and [2]Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX*

Interest in the protective capacity of carotenoids as dietary antioxidants has meant that evaluation of the chemical and structural features which determine the efficacy is required. ß-Carotene has been shown to possess antioxidant activity in solution (Burton & Ingold, 1984), lipid bilayers (Kennedy & Liebler, 1992) and microsomal preparations (Palozza & Krinsky, 1992). Few other dietary carotenoids have been examined. In the present study we report on the chemical and biophysical determinants of the antioxidant activity of seven carotenoids in multilamellar liposome preparations.

Egg-yolk phosphatidylcholine liposomes (5 mM) were prepared with carotenoids (ß–carotene (ß-CAR), lycopene (LYC), ß-cryptoxanthin (ß-CRY), zeaxanthin (ZX), canthaxanthin (CTX), astaxanthin (ASTX), echinenone (ECH)) or α-tocopherol (VIT-E) at a final concentration of 1 mol. % and subjected to oxidative stress using 2,2'-azo*bis*(2-amidinopropane) hydrochloride (AAPH, 10 mM) or 2,2'-azo*bis*(2,4-dimethylvaleronitrile), (AMVN, 0.5 mM) as water-soluble and lipid-soluble free-radical initiators respectively. The samples were incubated at 50° and portions removed at intervals and assayed by HPLC for phospholipid hydroperoxide formation (Stocker *et al.* 1987).

Percentage oxidation of phosphatidylcholine (compared to control)

	VIT-E		ß-CAR		LYC		ZX		ß-CRY		CTX		ASTX		ECH	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
AAPH*	53.7	1.8	80.1	2.0	129.1	2.6	61.0	0.5	66.0	1.4	93.4	3.1	92.0	3.4	75.1	2.4
AMVN**	27.3	2.3	27.3	2.3	84.0	2.1	15.2	0.4	17.3	1.3	38.1	1.8	28.9	1.7	29.1	0.7

*Percentage oxidation *after 3h incubation; after ** 2h incubation.
Results represent means of 4 experiments.

The relative antioxidant activity of the carotenoids against lipid peroxidation in these two systems is strongly correlated (r 0.96; $P<0.01$). The most effective carotenoids in this model membrane system are ZX and ß-CRY, both of which possess a 3-hydroxyl group on the beta-ring; in both systems these carotenoids are better antioxidants than ß-CAR, which does not have a 3-hydroxyl group. Carotenoids with a conjugated keto-group (ASTX, CTX and ECH) are less effective antioxidants than those which have hydrogen atoms at positions allylic to the chromophore. Previous work (Woodall *et al.* 1993) has shown that the relative reactivity of the carotenoid with free-radicals in solution is dependent on the availability of hydrogen atoms at positions allylic to the chromophore. This also appears to be a determinant of carotenoid antioxidant activity in model membranes. In solution there was no significant difference between carotenoids which did or did not possess 3-hydroxyl groups. Hence, the increased efficacy of ZX and ß-CRY compared to ß–CAR in membranes appears to be due to a biophysical interaction between the 3-hydroxyl group and the bilayer. The results suggest that dietary carotenoids such as ZX and ß-CRY may also have antioxidant activities comparable with that of ß-carotene *in vivo*, and thus should also be considered when assessing micronutrient antioxidant status.

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Total antioxidant capacity and lipid peroxidation in newly diagnosed patients with non-insulin dependent (type II) diabetes. By H.F. GOODE[1], S.G. GILBEY[2], N.R. WEBSTER[1] and B.E. WALKER[1], [1]*Clinical Oxidant Research Group and* [2]*Diabetic Unit, St James's University Hospital, Leeds LS9 7TF.*

Inadequate antioxidant defences and increased free-radical production have been shown to be significantly involved in the development of atherosclerosis and heart disease. In patients with established diabetes antioxidant levels are reduced and poor diabetic control is associated with increased free-radical generation and development of diabetic complications such as hypertension and cardiovascular disease. Several studies have shown that accelerated non-enzymic glycosylation and glucose auto-oxidation may be the underlying mechanism for the development of diabetic complications. Non-enzymically glycosylated proteins have been shown to be a source of free radicals, including superoxide and hydrogen peroxide, and thus oxidative stress may be an important factor in the pathophysiology of diabetic complications. We have therefore undertaken a study of newly diagnosed patients with diabetes, to investigate antioxidant capacity and free radical damage prior to progressive protein glycosylation.

Seven newly diagnosed patients (two women, aged 38-79 years) with non-insulin dependent diabetes were recruited at their first visit to the diabetic clinic. Results were compared with those from twenty-two healthy subjects (ten women, aged 39-69 years). Plasma malondialdehyde and total lipid peroxides were measured using a specific commercially available kit assay and plasma total antioxidant capacity was measured by enhanced chemiluminescence (Whitehead *et al* 1992).

Both plasma malondialdehyde and plasma total lipid peroxides were significantly raised ($P<0.0001$ and $P<0.05$ respectively, Table). The total plasma antioxidant capacity was also raised compared to healthy subjects, $P<0.002$, Table).

	Healthy subjects		Diabetic patients		
	Mean	SD	Mean	SD	*P* value
Malondialdehyde μmol/l	0.19	0.20	0.68	0.22	< 0.0001
Total lipid peroxide μmol/l	0.90	0.70	1.70	0.64	< 0.05
Total antioxidant capacity μmol/l	743	238	1090	158	< 0.002

These data imply that newly diagnosed type II diabetic patients have increased evidence of free radical damage, but that either their antioxidant capability is being maintained at above normal capacity at this stage, or that possibly increased plasma glucose and/or uric acid are contributing to their apparent antioxidant capacity. We suggest that newly diagnosed diabetic patients have normal antioxidant potential, but that progressive non-enzymic protein glycosylation with release of free radicals gradually depletes antioxidant resources and reduces antioxidant recycling. The resulting combination of increased circulating free-radical levels and decreased free-radical scavenging may be an important factor in the greatly increased incidence of vascular disease in diabetic patients.

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Urea-appearance: critical determinant of urea-nitrogen salvage on a low-protein diet. By T.S. MEAKINS and A.A. JACKSON, *Department of Human Nutrition, University of Southampton, Southampton, SO16 7PX.*

Estimations of protein requirements should take into account the need for non-essential N and improved N balance, achieved by the addition of non-essential N to diets low in protein (Kies & Fox, 1978). In adult males, N balance, urea production and urea salvage is maintained on 35 g protein, but on 30 g protein/d balance becomes negative, urea production falls and salvage appears to fail (Danielsen & Jackson, 1992).

Six adult males were fed four diets containing 70 g protein/d (11.2 g N), 30 g protein/d (4.8 g N), 30 g protein/d +6.9 g urea (8.0 g N) and 30 g protein/d +13.7 g urea (11.2 g N) for a period of 5 d and urea kinetics were measured using the prime/intermittent oral dose method over the final 24 h (Danielsen & Jackson, 1992).

Urea...	Appearance (mgN/kgperd)		Production (mgN/kgperd)		Salvage (mgN/kgperd)		Excretion (mgN/kgerd)	
Intake	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
70 g protein	208[a]	27	208[a]	27	100[a]	20	109[a]	11
30 g protein	118[b]	8	118[b]	8	51[b]	6	67[b]	2
30 g protein, 6.9 g urea	165[c]	13	121[b]	13	53[b]	14	112[a]	10
30 g protein, 13.7 g urea	204[a]	13	115[b]	13	83[a]	10	121[a]	12

Mean values within a column with unlike superscripts were significantly different, $P < 0.05$ (Paired rank test).

N balance was negative (-45 mgN/kgperd) on 30 g protein/d, but became less negative (-20 mgN/kgperd) with the addition of 13.7 g urea. Urea production was not different on any of the 30 g protein diets, and the difference in the rate of urea appearance could be directly attributed to the oral intake of urea. Urea salvage was not related to the rate of urea production or the dietary protein, but did show a relationship with the rate of urea appearance, or total N intake. Urea salvage was not related to the plasma concentration of urea.

These results show that a diet which provides 30 g protein/d is not adequate, but the addition of large amounts of N in the form of urea can produce improved N balance, through the enhanced salvaging of urea N. As urea production appeared to be related to protein intake, but urea salvage appeared related to the rate of appearance of urea N, we would conclude that the availability of urea is an important determinant of urea salvage. The urea available for excretion in urine is that which is not salvaged, thus the active process appears to be urea salvage and hence the ability to maintain urea salvage appears to be a critical mechanism through which N balance is maintained.

Therefore, N from urea can be incorporated into the body N pool and may be particularly important when the dietary intake of N is restricted. At the boundary of successful adaptation, relatively large amounts of urea were required to improve N balance, suggesting that there may be other limiting factors of importance.

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The effect of amino acids upon the pathways of $^{15}NH_4Cl$ conversion to urea in isolated sheep hepatoctyes. By Q.J. LUO[1], S.A. MALTBY[2], G.E. LOBLEY[1], M.A. LOMAX[2] and A.G. CALDER[1]. [1]*Rowett Research Institute Aberdeen AB2 9SB and* [2]*Department of Biochemistry and Physiology University of Reading Reading RG6 2AJ.*

Ruminants absorb a large proportion of dietary N across the gut as ammonia N (Huntington, 1986) and this must be detoxified by the liver primarily by ureagenesis. Theoretically ammonia can provide N for the synthesis of urea either directly via the mitochondrial synthesis of carbamoyl phosphate or indirectly via the synthesis of glutamate for transamination to aspartate in the cytoplasm (Meijer *et al.* 1978). The relative distribution of ammonia between the cytoplasmic and mitochondrial pathways in ruminants has not yet been quantified.

The present study used isotopomer analysis of $^{15}NH_4Cl$ labelling of urea to elucidate the origin of the two N in urea synthesized by isolated ovine hepatocytes. Hepatocyte suspensions were prepared from four lambs (live weight 35 kg) that had been fasted for 48 h and were incubated (4 mg wet weight cells/ml) for 2.5 h with varying concentrations of $^{15}NH_4Cl$ in the absence and presence of a physiological mixture of amino acids. Rates of ammonia removal and urea synthesis (nmol/mg wet weight cells/h) were determined as were rates of incorporation of label as [$^{14}N^{15}N$]- or [$^{15}N^{15}N$] urea species.

	Metabolite flux (nmol/mg wet weight cells per h)								
	Absence of amino acids				Presence of amino acids				
[$^{15}NH_4Cl$] mmol/l	0.0	0.33	0.67	1.0	0.0	0.33	0.67	1.0	SED
Ammonia[a,b]	0.1	-22.5	-37.7	-44.6	1.1	-18.7	-30.8	-33.8	1.99
Total urea[a,b,d]	1.9	14.7	21.9	27.3	13.8	26.4	29.4	29.9	1.52
$^{14}N^{15}N$ Urea[b]	-	3.9	3.4	3.0	-	9.4	9.2	7.4	0.75
$^{15}N^{15}N$ Urea[a,b,c]	-	9.7	17.6	23.0	-	6.8	12.4	15.4	1.04

[a]Effect of ammonia $P<0.0001$, [b]effect of amino acids $P<0.0001$, [c]effect of interaction $P<0.0001$, [d]effect of interaction $P<0.05$

Rates of removal of ammonia and synthesis of urea by hepatocytes were linear throughout the incubation period and were increased with the addition of $^{15}NH_4Cl$. The presence of amino acids decreased ammonia removal and was associated with a plateau in the rate of urea production. Although both $^{14}N^{15}N$ urea and $^{15}N^{15}N$ urea were produced by the hepatocytes the predominant form of labelled urea was $^{15}N^{15}N$ urea. Increased $^{15}NH_4Cl$ concentration increased the synthesis of $^{15}N^{15}N$ urea but to a greater extent in the absence of amino acids. Increased $^{15}NH_4Cl$ addition had no effect on the production of $^{14}N^{15}N$ urea although in the presence of amino acids production of $^{14}N^{15}N$ urea increased. The difference between the sum of the labelled urea species and total urea synthesis must be attributed to endogenous N sources contributing to the synthesis of urea and this was greatest in the presence of amino acids ($P<0.0001$) and was largely accounted for by the removal of arginine and glutamine from the incubation media to urea.

Results demonstrate that ammonia can provide N for the synthesis of urea via both the mitochondrial and cytoplasmic routes of entry into the urea cycle in isolated ovine hepatocytes and continues to do so in the presence of a physiological mixture of amino acids. The data suggest that under these conditions hepatocytes preferentially utilize ammonia for N transfer to aspartate in the cytoplasm. This may be because the exogenous amino acids (except arginine and glutamine) supplied in the incubation media are unavailable to the hepatocytes for urea synthesis perhaps through regulation of transporters.

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The effects of potassium exclusion on resting energy expenditure and kidney protein synthesis in young rats. By R.D.HAILWOOD and S.A.WOOTTON, *Department of Human Nutrition, University of Southampton, Southampton SO16 7PX.*

The omission of a specific nutrient from an otherwise adequate diet is thought to result in an increased energy expenditure and decreased efficiency of energy utilization (Kleiber, 1945). Potassium exclusion has been shown to limit growth in young rats and increase resting energy expenditure (REE) after 7 d (Hailwood *et al.* 1993). These animals also show increases in kidney size associated with changes in protein metabolism (Gustafson *et al.* 1973).

The effects of K exclusion on both REE and fractional synthetic rate of protein synthesis (FSR) of the kidney have been studied in young male Wistar rats after 7 and 14 d K exclusion. K-adequate (KAD) rats were fed on a synthetic diet containing 110 mmol K^+/kg diet. K-excluded (KEX) rats were given the same diet *ad libitum*, but with K excluded from the mineral mix. Pair-fed (PF) rats were fed with the adequate diet to the intake of the KEX group. After 7 d of receiving their respective diets, measurements of REE were made by indirect calorimetry, then rats were immediately injected with tritiated phenylalanine and tissue FSR measured by the method of Jepson *et al.* (1986). Further groups were measured on day 14, and a TIME 0 group was also included (*n* 6 for each group).

		Day 7			Day 14		
Group ...	TIME 0	KAD	KEX	PF	KAD	KEX	PF
Weight (g)	108 9	134[a] 11	118[b] 12	127[a,b] 10	167[a] 15	129[b] 7	145[a,b] 9
REE (kJ/kg per d)	8.15 0.42	7.39[a] 0.94	7.86[a] 0.68	6.06[b] 0.29	6.63[a] 0.34	5.97[b] 0.33	5.65[b] 0.34
Kidney weight (g/kg)	0.089 0.007	0.086[b] 0.005	0.117[a] 0.009	0.087[b] 0.003	0.084[b] 0.002	0.132[a] 0.013	0.082[b] 0.002
Kidney FSR (%/d)	50.6 4.8	47.5[a] 5.1	48.2[a] 5.4	42.0[b] 3.1	48.6[a] 5.1	43.1[a,b] 5.1	38.8[b] 4.1
Kidney protein synthesis (mg/d)	63 10	81[b] 12	108[a] 27	70[b] 12	102[a] (12)	108[a] (25)	69[b] (5)

a,b Values with the same superscript measured on the same day are not different (P>0.05).

Relative REE was higher in the KEX rats than their pair-fed controls on day 7, despite their lower body weight, although this effect was not apparent by 14 d. Kidney wet weight, FSR and total protein synthesis were also increased in KEX rats at day 7. However, the increased costs associated with elevated renal protein synthesis may only explain a small part of the increase in REE.

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Selection of dietary tryptophan concentration by growing pigs. By R. A. C. FAIRLEY[1], S. P. ROSE[1] and M. F. FULLER[2], *[1]Harper Adams Agricultural College, Newport, Shropshire TF10 8NB [2]Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Growing pigs appear to be able to select an appropriate mixture of two feeds to meet their protein requirements (Kyriazakis *et al.* 1990). However, they do not have the same ability to select an appropriate mixture of two feeds with differing lysine or threonine concentrations (Fairley *et al.* 1993). Tryptophan is a precursor of 5-hydroxytryptamine, which has been implicated in the control of food intake (Fernstrom, 1985). The aim of the present experiment was to discover if growing pigs can select a diet that meets their tryptophan requirements from two feeds differing only in tryptophan concentration.

A diet selection experiment was carried out with forty-eight male growing pigs with an initial mean weight of 14.5 kg. A basal feed was formulated with 164 g digestible protein/kg feed, which was supplemented with synthetic tryptophan to form four feeds (L, ML, MH and H). The feeds were similar in all respects except tryptophan concentration which was 4.4, 7.2, 15.0 and 20.3 g digestible tryptophan/kg digestible protein respectively. Each pig was allocated at random to one of four dietary choice treatments each consisting of a high- and a low-tryptophan feed (H + L, H + ML, MH + L and MH + ML). A 14 d training period was given at the beginning of the experiment in which the pigs were allowed access to only one feed for alternate 24 h periods. The pigs were then given access to both feeds for a further 14 d. All the pigs were penned separately with continuous access to both feeds. The experiment was carried out over two replicates of twenty-four pigs. In addition a single feed experiment was carried out with a further twenty-four male growing pigs, with an initial mean weight 18.9 kg. Each pig was allocated to one of six treatments which consisted of the same four feeds as above with an additional two intermediate feeds (IL and IH) with tryptophan concentrations of 8.6 and 12.5 g digestible tryptophan/kg digestible protein. These pigs did not have a training period: they were 14 d older than the choice fed pigs.

The pigs clearly discriminated between the two feeds offered with 65% eating mostly (> 85%) one feed. Where the choice included feed L, most pigs (sixteen out of twenty-four) rejected this in favour of either feed H or MH. However, in the choice between H and ML, seven out of twelve pigs preferred feed ML, but two pigs ate mostly H and the remaining three pigs ate from both feeds. In the choice between MH and ML, pigs made a range of selections from eating all feed ML to eating all feed MH. The relationship between weight gain and tryptophan concentration in the single-fed pigs was non-linear, with weight gain increasing with increasing tryptophan concentration, and reached an asymptote at 8.2 g digestible tryptophan/kg digestible protein. There was no relationship between weight gain and tryptophan concentration in the choice-fed pigs, However only one pig selected a digestible tryptophan concentration below 7.2 g/kg digestible protein.

Thus, the pigs did select feeds on the basis of their tryptophan concentrations. However, as with lysine and threonine, few pigs selected a blend of feeds which would have provided a tryptophan concentration close to current estimates.

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Incorporation of [^{15}N]ammonia into urea and amino acids as influenced by fasting and feeding in man. By P. J. M. WEIJS, A. G. CALDER and G. E. LOBLEY, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB*

Ammonia-N can be used directly for ureagenesis, via carbamoyl phosphate (CP) synthesis, but can also be incorporated into glutamate and aspartate. The latter reactions allow a second route of entry for ammonia-N into urea. The quantitative importance of this pathway can be assessed by infusion of [^{15}N]ammonia and monitoring the relative productions of [^{14}N^{15}N]- and [^{15}N^{15}N]-urea. In fed sheep, [^{14}N^{15}N]urea was the predominant species (Lobley *et al.* 1995) whereas, in a recent study with one fasted human, significant quantities of [^{15}N^{15}N]urea were apparently synthesized (from Patterson *et al.* 1993). Possible explanations for this contrast include differences between species and nutritional status. In the present study the latter has been considered with humans, each of whom was monitored in both the fed and fasted state.

For 2 d the subjects (*n* 4) received a liquid diet (5 g protein and 630 kJ/100 ml, Fortisip, Cow & Gate Nutricia), providing 1 g protein/kg body weight per d. On day 3 [^{15}N]ammonium chloride (82 mg/20 min) was ingested orally for 6 h. Blood samples were obtained just before and at 3, 4, 5 and 6 h after the start of isotope intake. Each subject either continued the overnight fast or received 50 ml Fortisip/20 min, starting 1 h before isotope ingestion, in a balanced design. One week was allowed between treatments for each subject.

Blood and plasma were analysed for enrichment of urea species and certain amino acids by gas-chromatography mass spectrometry in electron impact mode with selected ion monitoring. Data for plasma are presented and analysed by Student's *t* test for paired samples.

Atom % excess enrichments

	[^{14}N^{15}N]urea		Amide-N-gln		Arginine	
	Mean	SE	Mean	SE	Mean	SE
Fed	5.58	0.47	1.38	0.08	4.03	0.45
Fasted	5.78	0.55	1.33	0.22	4.29	0.53

	[^{15}N^{15}N]urea		Amino-N-gln		Glutamate		Leucine		Valine		Isoleucine	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fed	0.29	0.02	0.55	0.07	1.48	0.14	0.21	0.02	0.19	0.02	0.24	0.02
Fasted	0.49*	0.07	1.08*	0.13	1.70*	0.12	0.32*	0.05	0.28*	0.05	0.41*	0.10

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

[^{14}N^{15}N]urea, amide-N-glutamine (gln) and arginine enrichments were unaffected by nutritional status. However, because the urea concentration in the fed state was 15% greater than during the fast, a larger proportion of the ^{15}N-ammonia was incorporated into urea. This, or the greater isotope dilution associated with higher concentrations, might explain the lower values for amino acid enrichment during feeding.

Elevated enrichments in the fasted condition for glutamate and amino-N-glutamine are compatible with the increase in [^{15}N^{15}N]urea. The overall effect is small, however, with the majority of ammonia detoxified through CP synthesis. The source of the additional N required for urea production is unknown but may have consequences for net protein synthesis.

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Postprandial utilization of wheat protein in normal adults. By A. FEREDAY[1], N. GIBSON[2], M. COX[2], D. HALLIDAY[3], P.J. PACY[2] and D. J. MILLWARD[1], [1]School of Biological Sciences, University of Surrey, Guildford GU2 5XH, [2]Nutrition Research Unit, St Pancras Hospital, London NW1 0PE, and [3]Nutrition Research Group, CRC Middlesex HA1 3UJ

The importance of protein quality in human nutrition is currently difficult to assess in the absence of widely acceptable requirement values for indispensable amino acids (Millward *et al.* 1993). The ability of wheat and other plant protein sources to support N balance in humans has been studied but the variability in such trials results in great uncertainty about the overall importance of protein quality in human nutrition (Millward *et al.* 1989). In order for wheat or any other dietary protein to maintain N balance the diet must allow sufficient protein deposition to replete postabsorptive losses in the postprandial state. This in turn requires not only the substrate provision for the net protein deposition but also the appropriate regulatory response to the dietary protein in terms of the inhibition of proteolysis and stimulation of protein synthesis. We have adopted a [^{13}C]leucine balance protocol to measure utilization of wheat protein in normal adults enabling measurement of both the extent and mechanisms of postprandial protein utilization (PPU) from a wheat-based food.

PPU is calculated from the slope of the leucine balance-intake relationship between two protein intake levels fed successively during a constant infusion of [^{13}C]leucine with leucine balance calculated as leucine intake minus leucine oxidation (Gibson *et al.* 1994).

The subjects were normal adults all in good health and previously studied with milk protein. We measured [^{13}C]leucine balance, during a single 9 h prime dose constant infusion of [1-^{13}C]leucine, initiated in the postabsorptive state 12 h after the last meal, and comprising three 3 h periods, (1) the postabsorbtive state (PA period), (2) low-protein feeding (LP period, 30 min feeding of 1/24 of daily energy needs and 2% protein energy) and (3) an isoenergetic high-protein feeding (HP period, 30 min feeding of approximately 14% protein energy). The protein source was stoneground wholemeal bread fed with margarine and potato starch, and with fat providing 30% and 18% energy in phases 2 and 3 respectively. Leucine balance was calculated as leucine intake minus leucine oxidation, calculated from $^{13}CO_2$ excretion and plasma α-ketoisocaproate enrichment, measured during the third hour of each 3 h phase.

The value for PPU calculated as the slope of the balance-intake relationship between the LP and HP periods was 0.91 (SD 0.117). Protein deposition was mediated through an inhibition of proteolysis and stimulation of protein synthesis as indicated by values for leucine endogenous appearance rates of 1.99 (SD 0.04), 1.70 (SD 0.24) and 1.37 (SD 0.29) μmoles/min per kg and leucine non-oxidative disposal rates of 1.68 (SD 0.12), 1.62 (SD 0.22) and 1.84 (SD 0.24) μmoles/min per kg for PA, LP and HP periods respectively. These responses were accompanied by lower plasma leucine concentrations in the LP compared with PA and HP periods and lower plasma lysine in both LP and HP compared with PA period.

Thus these results show that in adults adapted to normal mixed diets whole wheat protein is utilized with near maximum efficiency for postprandial protein deposition in the early phase of repletion of postabsorptive losses. Further studies will be required to assess the extent to which this is maintained throughout the entire postprandial period.

This work was supported by Research into Ageing

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Postprandial protein utilization in the elderly. By A. FEREDAY[1], N. GIBSON[2], M. COX[2], D. HALLIDAY[3], P.J. PACY[2] and D.J. MILLWARD[1], [1]School of Biological Sciences, University of Surrey, Guildford GU2 5XH, [2] Nutrition Research Unit, St Pancras Hospital, London NW1 0PE, and [3] Nutrition Research Group, CRC Middlesex HA1 3UJ

Nitrogen homeostasis in man requires that postabsorptive losses are repleted in the postprandial state. Thus any difficulty in maintenance of N homeostasis can ultimately be attributed to either excessive postabsorptive losses or inadequate postprandial repletion. In the elderly there is a loss of lean body mass, mainly skeletal muscle, which is associated with increasing immobility. The reason for this must reflect either inadequate (or inappropriate) food intake or defective mechanisms controlling dietary protein utilization. However studies to date have failed to identify any cause. We have described a stable isotope procedure for the assessment of the efficiency of postprandial protein utilization (PPU), (Gibson *et al.* 1994) based on assessment of leucine balance during a constant infusion of [^{13}C]leucine in subjects in the postabsorptive state and fed successively with low- and high-protein milk meals. PPU is calculated from the slope of the leucine balance-intake relationship between the two intake levels. We have used the protocol to evaluate the efficiency of PPU under standard conditions with milk protein in a group of elderly subjects in comparison with younger adults.

The subjects were all in good health and mobile, the elderly comprising four men and five women aged 67-91 years (*n* 9), and the younger group aged between 20-55 years (*n* 15, ten men, five women). We measured [^{13}C]leucine balance during a single 9 h prime dose constant infusion of [1-^{13}C]leucine with three 3 h phases, (1) the postabsorptive state, (2) low-protein feeding (30 min feeding of 1/24 of daily energy needs and 2% protein energy) and (3) high-protein feeding (30 min feeding of approximately 14% protein energy), the protein fed at the habitual intake level based on a dietary questionnaire and 24 h urinary N collections. The frequent small meals ensured a metabolic steady state. Leucine balance was calculated as leucine intake minus leucine oxidation, calculated from $^{13}CO_2$ excretion and plasma α-ketoisocaproate enrichment, measured during the third hour of each 3 h phase.

The values for PPU were 0.783 (SD 0.106) for the elderly and 0.774 (SD 0.115) in the younger age group, with no difference between the two groups. Thus under these standardized conditions this healthy elderly group exhibited no defect in their ability to deposit dietary protein. This suggests that there is no decline with age in this component of the homeostatic mechanisms of N balance. Furthermore there was no evidence of any increase in postabsorptive losses as measured in the present study since the mean value was lower in the elderly (13.8 (SD 3.9) μmol leucine/kg per h), compared with the younger age group (22.7 (SD 5.1) μmol leucine/kg per h). While this may reflect the altered body composition with a lower proportion of lean tissue in the elderly it may also indicate an adaptation to a lower habitual protein intake, although our dietary assessments did not indicate any difference.

In conclusion then we have not been able to identify any change with age in the efficiency of postprandial protein utilization of milk protein measured under standardized conditions.

This work was supported by research into Ageing

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Double-blind pilot trial, in elderly women with fractured femur, of ornithine α-ketoglutarate v. a defined formula peptide oral supplement. By N.BEAN[1], J.REDDEN[2], H.GOODE[3], G.GRIMBLE[4] and S.P.ALLISON[1], *[1]University Hospital, Nottingham NG7 2UH, [2]Royal Infirmary, Doncaster DN2 5LT, [3]St James's University Hospital, Leeds LS9 7TF and [4]Central Middlesex Hospital, London NW10 7NS.*

Fractured femur among elderly women is associated with a high incidence of malnutrition. Nutritional supplements (1046-4184 kJ) have been shown to improve outcome in hospital and up to 6 months of follow up (Bastow *et al.* 1983; Delmi *et al.* 1990). α-ketoglutarate, the ketoanalogue of glutamine, and ornithine α-ketoglutarate (Cetornan 20 g/d) have been shown, in various clinical settings, to enhance protein synthesis and outcome, not by total energy or N content but via specific biochemical effects or by stimulation of growth hormone and insulin secretion. The purpose of this three-centre study was to compare the effects of 20 g Cetornan/d with an isoenergetic (293 kJ) and isonitrogenous (2.73 g N) defined formula peptide supplement (Pro-up) in a group of fractured femur patients who where (1) 70-85 years old, (2) relatively undernourished (mean arm circumference <25 cm; triceps skinfold <18 mm), (3) not demented (Cape Score > 8/12), (4) had no other major medical disorder, (5) gave informed consent.

Patients (n 1146), admitted to Nottingham University Hospital over a 2.5 year period, were screened by anthropometrics, Cape Score and clinical assessment. Fifty patients from Nottingham (4.3%) and a further nine patients (six from Leeds, three from Doncaster) fulfilled the criteria for inclusion and were randomized in a double-blind fashion to receive twice daily supplements in unlabelled identical sachets for a period of 2 months. Patients were followed up at home for 6 months after the fracture. All patients were treated surgically by pinning, plating or hemiarthroplasty. Serial measurements included weight, (BMI) and anthropometrics, bioimpedance, food intake (3 d diaries), appetite (analogue score 1-10), fatigue (analogue score 1-10), Nottingham Activity of daily living and Barthel Score, hand dynamometry, biochemistry, haematology, side effects and complications (major and minor).

Anova 2 time factor was used for intragroup and between group analysis.

Thirty-five of the fifty-nine patients took the supplements for the full 2 months. Seventeen received Cetornan, eighteen Pro-up. Analysed by intention to treat, there was no significant difference between the two treatments (1) in duration of treatment or hospitalization, (2) initial characteristics, (3) mortality (Cetornan 12.5%, Pro-up 11.1%), (4) complications, although there was a significant delay in major complications in the Cetornan group until after the 2 months supplementation (Cetornan 96.2 (SD ± 55.5) d v. Pro-up 13.2 (SD ± 1.3) d, $P<0.03$), (5) the proportion completing 2 months' treatment. Among the thirty-five patients able to complete 2 months of treatment the benefits of Cetornan were significant in terms of (1) change in arm muscle circumference at 8 weeks ($P=0.041$), (2) increase in food intake at 6-8 weeks ($P=0.015$), (3) decrease in fatigue score up to 8 weeks ($P=0.001$). BMI and albumin remained stable.

Cetornan supplementation after fractured femur may have benefit compared with an isoenergetic isonitrogenous defined formula feed. These findings require confirmation in a larger and less highly selected population of fractured-femur patients. The small proportion of patients fulfilling the entry criteria illustrates the dilemma of all such trials, i.e. whether to include all admissions or a selected group without other complicating factors.

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Antioxidants in diabetes. By N. R. WILLIAMS[1], J. RAJPUT-WILLIAMS[1], S. V. NIGDIKAR[1], J. A. WEST[1], S. McGRATH[2], J. FOOTE[1], B. HENDERSON[3], A. N. HOWARD[1] and D. I. THURNHAM[2], *[1]COAG Laboratory, Papworth Hospital, Cambs. CB3 8RE, [2]Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA, and [3]Hinchingbrooke Hospital, Cambs. PE18 8NT*

An increasing amount of evidence in recent years has suggested a possible involvement of antioxidants in the development of diabetic complications. Low antioxidant micronutrient levels (Cu, Zn, Se), reduced activity of antioxidant enzymes (e.g. superoxide dismutase, SOD; *EC* 1.15.1.1) and increased free-radical-induced measures of lipid peroxidation (TBARS) have been reported in diabetics. In humans and rats, deficiencies of Cu and Zn have also been shown to induce glucose intolerance (Strain, 1991).

The exact nature and role of antioxidants in the development of diabetes and associated complications is unclear. We investigated the concentrations of various antioxidants such as Cu and Zn in leucocytes, Cu,Zn SOD in erythrocytes, and plasma carotenoids such as lutein, lycopene, α-carotene, ß-carotene, α-cryptoxanthin, β-cryptoxanthin, α-tocopherol, β-tocopherol and retinol in a group of age- and sex-matched diabetics compared with controls. In the present study Cu and Zn were measured in specific types of leucocytes such as granulocytes and mononuclear cells (lymphocytes and monocytes).

Blood was collected from eighty-eight diabetics (Type 1 and Type 2, aged 30-65 years) from Hinchingbrooke hospital and fifty-three age matched healthy controls from East Anglia. Leucocytes were separated by dextran sedimentation followed by further differentiation on Nycodenz (Nycoprep 1.077) on a subgroup of the patients. Cells were counted and haemoglobin measured on a haematology analyser. Leucocyte, erythrocyte, and plasma Cu and Zn were determined by graphite furnace atomic absorption spectrophotometry. SOD activity was measured spectrophotometrically using a kit (SOD-525, Bioxytech). Plasma carotenoids were determined by HPLC on the ten patients with the lowest mononuclear cell Cu concentrations. The data were log transformed before statistical analysis by *t* test. Results are shown in the Table.

	Controls		Diabetics		*P*	
	n	Mean (95% CI)	*n*	Mean (95% CI)		
Mono. Cu (nmol/10^9cells)	25	11.3 (9.4-13.7)	21	8.5 (7.3-9.9)	0.022	*
Mono. Zn (nmol/10^9cells)	25	158 (137-182)	22	176 (134-231)	0.464	NS
Gran. Cu (nmol/10^9cells)	25	4.7 (3.6-6.1)	20	3.4 (2.5-4.6)	0.099	NS
Gran. Zn (nmol/10^9cells)	25	109 (93-128)	22	100 (79-125)	0.481	NS
Plasma Cu (μmol/l)	26	15.6 (14.2-17.1)	88	13.0 (12.3-13.8)	0.003	**
Plasma Zn (μmol/l)	26	12.5 (11.9-13.1)	88	10.5 (10.1-10.9)	<0.001	***
RBC SOD (units/g Hb)	53	1319 (1224-1421)	51	1089 (1018-1166)	0.002	**
Ascorbate (μmol/l)	10	59.8 (44.9-79.7)	10	41.9 (29.1-60.2)	0.098	NS
α-Carotene(μmol/l)	10	0.106 (0.082-0.135)	10	0.049 (0.032-0.075)	0.002	**
ß-Carotene(μmol/l)	10	0.472 (0.316-0.705)	10	0.231 (0.140-0.382)	0.022	*
Lutein (μmol/l)	10	0.433 (0.336-0.558)	10	0.307 (0.243-0.389)	0.037	*
Lycopene(μmol/l)	10	0.707 (0.547-0.912)	10	0.369 (0.236-0.576)	0.010	*
Retinol (μmol/l)	10	2.61 (2.01-3.39)	10	2.05 (1.62-2.60)	0.137	NS
α-Tocopherol (μmol/l)	10	35.3 (29.3-42.4)	10	30.2 (22.1-41.3)	0.348	NS

NS, not significant; Mono., mononuclear cells; Gran., granulocytes; RBC, erythrocytes

There were no significant differences in height, weight, body mass index, α- or ß-cryptoxanthin, γ-tocopherol, or RBC Cu between the groups. The significant differences observed in the antioxidant carotenoids lutein, lycopene, α-carotene, and ß-carotene provides further support for a putative role of antioxidants in the pathology of diabetes. The reduced concentrations of plasma Cu, plasma Zn, and mononuclear cell Cu, may be related to the lower SOD activity.

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A randomized clinical trial of parenteral selenium supplementation in preterm infants. By L.A. DANIELS, R.A. GIBSON and K. SIMMER, *Department of Paediatrics and Child Health, Flinders Medical Centre, Adelaide, South Australia, Australia 5042*

Selenium (Se) levels decline rapidly to very low levels in preterm infants who receive unsupplemented parenteral nutrition (PN). It has been postulated that Se deficiency will reduce antioxidant defence thereby enhancing susceptibility to oxidant damage and chronic lung disease (CLD Lockitch *et al* 1989). Se supplementation of PN at 2 μg/kg per day has been recommended (Greene *et al* 1988) but is not routine practice in Australian neonatal intensive care units.

Forty-two preterm infants with mean gestational age (GA) 29 (SD 2) weeks and mean birth weight (BW) 1215 (SD 364) g were randomly assigned to receive unsupplemented PN (PN-Se, n 20) or PN supplemented with 3 μg/kg per day of selenious acid (PN+Se, n 22). The aim of the present study was to determine whether this level of supplementation would achieve a Se status in preterm infants similar to that of breast-fed infants. All infants received oxygen therapy for a mean of 45 (SD 38) and 93% were mechanically ventilated for 24 (SE 24). There was no difference in BW, GA, days of PN or time to full enteral feeding between the groups. Plasma, erythrocyte and urine Se and erythrocyte glutathione peroxidase (GSHPx;EC1.11.1.9) were measured at week 0 (2.9 (SD 1.5) of age), week 1 (8.2 (SD 2.2) and then weekly until discharge. Infants received total PN for 12.5 (SD 6) and greater then 75% of energy from PN for 17.5 (SD 8). However by week 3 less than 50% of the infants were still predominantly PN-fed. A reference group of thirty-two healthy term infants were assessed at week 0 (day 5) and week 6. Results for preterm infants receiving ≥ 75% energy from PN for each week are shown in the Table.

Group	Postnatal age (weeks)	Plasma Se (μg/l) Mean	SD	n	Erythrocyte Se (μg/g Hb) Mean	SD	n
PE-Se	0	29	15	20	40	33	19
	1	21	18	16	42	36	16
	2	18	14	13	38	38	12
	3	16	9	9	45	35	9
PN+Se	0	27	12	21	49	37	17
	1	28	11	17	55	33	14
	2	24	14	12	62	36	10
	3	29	16	5	68	14	14

Although none of the between-group differences are significant, the supplemented group had higher plasma and erythrocyte Se levels at every time point. Moreover repeated measures analysis of all infants in each group showed that plasma Se declined significantly (n 18, $P<0.05$) over the first 3 weeks in the PN-Se group but not in the PN+Se group. The unsupplemented group had 50% more plasma Se readings below detection (<10 μg/l) compared with the PN+Se group. Se urine, expressed per mmol creatinine, was two to three times higher ($P<0.001$) in the PN+Se group. The supplemented group had higher GSHPx activity than the unsupplemented group at week 1(1.19 (SD 0.29) v. 1.00 (SD 0.22) IU/gHb, $P<0.05$) and week 2 (1.29 (SD 0.32) v. 1.04 (SD 0.13) IU/gHb, $P<0.01$). There was no evidence of the increase with postnatal age in plasma Se seen in the healthy term reference group and at week 6 both preterm groups had plasma Se levels 50% ($P<0.05$) those of the term infants. The incidence of sepsis was higher in the PN-Se group (75% v. 46%, $P<0.05$) but the incidence of other clinical variables was similar.

These results suggest that PN supplementation with 3 μg Se/kg per day results in a marginal improvement in Se status of preterm infants but does not achieve a plasma Se pattern similar to term breast-fed infants. A significant proportion of the supplemented Se is excreted in the urine. Further studies are required to establish whether higher levels of PN supplementation can achieve a more substantial improvement in Se status and ultimately a reduction in the incidence of CLD.

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Response of selenium content of chicken flesh to increased selenium in feed. By M.N.I. BARCLAY and A. MacPHERSON, *Scottish Agricultural College, Auchincruive, Ayr KA6 5HW*

The recommended daily intake for Se in adults is 50-200 μg. Our previous work (Barclay & MacPherson, 1992) has shown that food eaten in SW Scotland may result in levels below this. Chicken has become a more popular dietary item and so it seemed a suitable vehicle for increasing the Se available to the human diet.

Twelve male chicks were reared on control diet (1) (0.313 mg Se/Kg dry matter) and another twelve on the same diet fortified with sodium selenite (0.66 mg Se/Kg dry matter). Four from each group were killed after 35 d, 45 d and 55 d respectively. The Se contents of breast and thigh muscles were measured by atomic absorption spectrometry via hydride generation following acid digestion. The relatively high levels of Se in the thigh muscles after 35 d caused the experiment to be repeated with a further twelve chickens, six male and six female. These were fed on a commercial food of Se content 0.419 mg/Kg dry matter (Control 2). Three male and three female were killed at 35 d, the remainder at 45 d.

		Se content (mg/100g fresh wt)					
		Control 1		Control 2		Added Selenium	
		Mean	SE	Mean	SE	Mean	SE
35 d							
	Breast	11.3	0.09	13.4	0.68	13.7	0.79
	Thigh	16.6	1.23	15.4	0.38	18.6	0.73
45 d							
	Breast	12.5	0.78	14.7	0.53	13.5	0.37
	Thigh	12.5	0.27	15.1	0.74	14.5	0.75
55 d							
	Breast	12.9	0.78			12.5	1.7
	Thigh	13.4	0.63			14.0	0.46

Table 1 summarizes the Se content of breast and thigh for each group at each age. Holland et al. (1991) quote the Se content of chicken as seven μg/100 g so all these tissues were markedly higher. Only a few birds were involved in the present study and no significant correlation was found between tissue and feed Se.

It is of interest that the thigh muscle incorporates Se to a greater extent than the breast muscle in the first 35 d. These birds are genetically selected to produce excess breast muscle as they are designed for the consumer's preference in table birds. Hence from around 35 d the bulk of increase in weight is in the breast. This may explain the sustained levels of breast Se while the thigh content comes into equilibrium.

While further work on Se incorporation is indicated, parallel work on lambs as yet unpublished will show that muscle and organ Se can be increased by increased levels in the animal feed.

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Effect of low-dose fish-oil supplementation with or without added vitamin E on plasma vitamins. By E. TURLEY, J.M.W. WALLACE, W.S. GILMORE and J.J. STRAIN, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

Epidemiological studies suggesting that fatty fish consumption is protective against coronary heart disease have led to interest in the use of fish oils as therapeutic agents. Concurrently workers have cautioned that increased consumption of the highly polyunsaturated *n*-3 fatty acids, eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), may result in increased oxidative stress *in vivo* (Meydani *et al.* 1991). Commercially available fish-oil supplements all contain vitamin E although the recommended figures vary considerably. Much of the work focusing on the effect of fish-oil on plasma tocopherol and lipid peroxidation has involved mega-dose supplementation and remains inconclusive.

Thirty healthy female volunteers (aged 18-28 years) completed the double-blind study. For 4 weeks subjects added 2.4 g encapsulated fish oil, with (FO+Vit E 3 mg/g) or without added vitamin E (FO) (kindly provided by Seven Seas) to their otherwise unchanged diets. Venous blood samples were taken before (baseline) and after the 4-week supplementation (post-sup) and again after a 9-week washout period (washout). Plasma ascorbic acid and the fat-soluble vitamin concentrations were determined by HPLC. Results are mean values and standard errors (SEM).

	α–Tocopherol (μmol/l)		Ascorbic acid (μmol/l)		β-Carotene (μmol/l)		Retinol (μmol/l)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
FO (*n* 14)								
Baseline	23.2	0.9	99.9	9.0	0.40	0.02	1.67	0.11
Post-sup	25.1*	1.0	79.9*	6.9	0.45	0.06	1.80	0.15
Washout	21.8	0.2	94.4	5.0	0.34	0.05	1.71	0.78
FO+vit E (*n* 16)								
Baseline	24.2	1.0	98.3	7.9	0.41	0.07	1.69	0.09
Post-sup	26.9**	1.4	105.8	11.0	0.43	0.06	1.92*	0.12
Washout	24.9	1.1	107.2	11.8	0.39	0.05	1.78	0.08

Values were significantly different from baseline (Student's *t* - test) *$P<0.05$ **$P<0.01$.

Plasma α-tocopherol concentrations were increased significantly in both groups. Plasma ascorbic acid was significantly decreased in the FO group. The addition of vitamin E significantly increased plasma retinol concentrations, an effect similar to that described by Nair *et al.* (1993).

Decreased ascorbic acid may reflect sparing of α-tocopherol in plasma of those subjects receiving fish oil only. These results suggest that low-dose commercial fish-oil supplementation has no adverse effect on plasma vitamin status in healthy individuals.

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High plasma vitamin C in premature babies may inhibit the antioxidant activity of caeruloplasmin By A. LOBAN[1], K.M.SILVERS[1], A.T.GIBSON[2] and H.J.POWERS[1]. [1]*University Department of Paediatrics and* [2]*The Jessop Hospital for Women, The University of Sheffield, S10 2TH.*

We have reported that reduced antioxidant activity in the plasma of premature infants at birth is strongly predictive of mortality and antioxidant activity is closely negatively correlated with the ratio of vitamin C : caeruloplasmin (Silvers *et al.* 1994). Ascorbic acid is an important extracellular antioxidant but reports suggest that at high concentrations it can inhibit the antioxidant activity of caeruloplasmin (Gutteridge, 1991).

We have measured the ferroxidase activity of caeruloplasmin *in vitro* in the presence of ascorbic acid over a range of concentrations observed in premature babies (0-300μmol/l). At a given concentration of caeruloplasmin, increasing concentrations of ascorbic acid were associated with a progressive reduction in ferroxidase activity ($p<0.001$). The degree of inhibition was dependent on the ascorbic acid : caeruloplasmin ratio. Concentrations of vitamin C observed in premature babies would be predicted to inhibit the ferroxidase activity of caeruloplasmin and compromise the plasma antioxidant activity *in vivo*.

We have measured the ferroxidase activity in fresh plasma collected from seventeen babies born between 24 and 36 weeks gestation. Blood samples were collected at times between birth and 20 postnatal days. Ferroxidase activity (OD units/minute) ranged from 0.056 to 0.238 with a mean of 0.139. These values were significantly lower than values measured in the plasma of fifteen healthy adults, which ranged from 0.164 to 0.466 with a mean of 0.290 ($p<0.001$).

Vitamin C and caeruloplasmin concentrations were also measured in the same plasma samples. Plasma ferroxidase activity was positively correlated with plasma caeruloplasmin concentration in both groups of subjects ($p<0.001$), but in the babies only, was also negatively correlated with the vitamin C : caeruloplasmin ratio ($p<0.05$). There was a progressive increase in ferroxidase activity from birth with increasing postnatal age ($p<0.01$) coinciding with an increase in caeruloplasmin concentration ($p<0.01$) and a fall in the vitamin C : caeruloplasmin ratio ($p<0.05$). The inhibition of plasma antioxidant activity by plasma vitamin C in premature babies is likely to be greatest in the period shortly after birth, when plasma caeruloplamin concentrations are at their lowest.

Gutteridge, J.M.C. (1991). *Clinical Science* **81**,413-417.

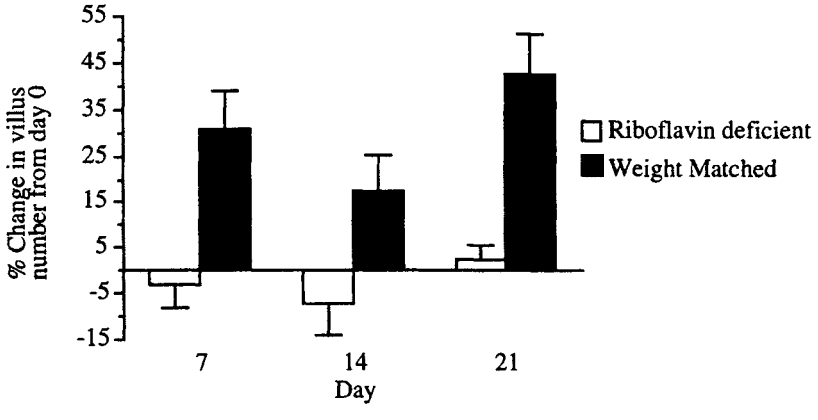
Silvers K.M., Gibson A.T. & Powers H.J. (1994). *Archives of Diseases of Childhood* **71**, F40-F44

Riboflavin depletion at weaning is associated with a failure to reach normal villus number in the rat duodenum. By E.A. WILLIAMS[1], H.J. POWERS[1] and R.D.E. RUMSEY[2], [1]University Department of Paediatrics, Sheffield Children's Hospital and [2]Department of Biomedical Science, University of Sheffield, S10 2TH

We have reported that riboflavin deficiency induced at weaning is associated with increased villus dimensions (Williams *et al.* 1993). We have also observed an increased cell number per villus column but an unaltered total mucosal DNA content in the small intestine in response to riboflavin deficiency (unpublished results). We investigated whether an effect of riboflavin deficiency on villus number could be responsible for this anomaly.

Twelve female weanling Wistar rats were fed *ad libitum* on a riboflavin-deficient diet and weight-matched to a control group fed on a complete diet. After 8 weeks the rats were killed, the small intestine removed and the total villus number estimated in a stained 1 cm length section of duodenum by counting the villi under a light microscope. There were 1780 (SEM 88.4) villi/cm length in the control group, compared with 1335 (SEM 115.4) in the riboflavin deficient group. These values were significantly different ($P<0.01$).

The alteration in villus number was investigated further in a second experiment, in which the villus number was counted at 0,7,14 and 21 d of feeding a riboflavin-deficient diet. There were significantly more villi in the sections of the small intestine from animals in the control group on days 7,14, and 21 compared with the number of villi on day 0 ($P<0.02$, $P<0.05$, and $P<0.01$ respectively). In contrast, in the riboflavin-deficient group there was no increase in the number of villi from day 0 over 21 d of feeding a riboflavin-deficient diet (Figure). There was no difference in the length of the small intestine; animals in the riboflavin-deficient group had a mean length of 56 (SEM 1.4) cm compared with a mean length of 60 (SEM 1.5) cm in animals in the weight-matched group.



Riboflavin deficiency impairs the increase in the number of villi in the upper part of the small intestine during post-weaning development. This is likely to have implications for nutrient absorption.

Williams, E.A., Rumsey, R.D.E., & Powers, H.J. (1993). Proceedings of the Nutrition Society **52**, 320 A.

The effects of copper deficiency in male Syrian hamsters fed on diets containing either maize or fish oil. By N.C. ARMSTRONG, J.M. ALLEN and J.J.STRAIN, *Department of Biological and Biomedical Sciences, University of Ulster, Cromore Road, Coleraine, Co. Londonderry, BT52 1SA*

Previous studies have indicated that either Cu-deficiency or fish-oil consumption can have prooxidant effects. The current study investigated the effects of Cu status and fish-oil consumption on tissue antioxidant enzymes in Syrian hamsters.

Male weanling Syrian hamsters were weighed, divided into groups (*n* 8) and fed *ad libitum* on diets either adequate or deficient in Cu containing either maize oil (MO) or fish-oil (FO). After a period of 14 weeks, a blood sample was obtained by cardiac puncture and liver and kidney were excised. Samples were stored at -70° until time of analysis. Significant results are presented in the Table.

	Cu-adequate				Cu-deficient				Statistical effects (ANOVA)		
	MO (*n* 7)		FO (*n* 7)		MO (*n* 8)		FO (*n* 8)				
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Cu	FO	CuxFO
Daily food intake (g)	7.5	0.3	5.8	0.4	7.0	0.2	5.6	0.2	NS	***	NS
Final Body weight (g)	107.2	5.0	80.9	4.3	79.8	5.2	67.8	4.5	***	***	NS
Relative liver weight (% body weight)	4.91	0.19	6.49	0.20	4.80	0.26	7.39	0.41	NS	***	NS
Hepatic Cu (µg/g dry weight)	14.3	1.4	15.8	1.2	7.8	0.7	4.8	0.3	***	NS	*
Hepatic Fe (µg/g dry weight)	1688	133	573	206	2650	315	1206	275	**	***	NS
Caeruloplasmin (U/l)	17.72	2.80	25.14	6.68	1.53	0.64	3.40	1.89	***	NS	NS
Plasma cholesterol (mmol/l)	3.81	0.28	10.20	0.62	3.87	0.66	7.83	1.36	NS	***	NS
Erythrocyte SOD (U/g Hb)	823.3	19.7	864.5	15.6	455.9	24.3	403.4	58.1	***	NS	NS
Hepatic SOD (U/mg protein)	19.2	0.6	14.1	0.9	7.9	0.9	5.9	0.7	***	***	*
Renal CCO (U/mg protein)	0.54	0.02	0.47	0.03	0.44	0.03	0.31	0.04	***	**	NS

Significance: **P*<0.05, ***P*<0.01, ****P*<0.001; NS, not significant; two-way ANOVA.

Results showed that a deficiency of dietary Cu in the Syrian hamster produced effects similar to those previously reported for other animal species : significant decreases in body weight, hepatic Cu, caeruloplasmin (EC 1.16.3.1; Cp) and other Cu-dependent enzymes (erythrocyte superoxide dismutase (EC 1.15.1.1; SOD), hepatic SOD and renal cytochrome c oxidase (EC 1.9.3.1; CCO)) with a significant increase in hepatic iron. Hamsters on the FO diet had significantly lower food intakes and body weights than those consuming MO diets. In addition FO consumption was found to decrease hepatic Fe levels whilst increasing relative liver weight and plasma cholesterol.

These data suggest that FO consumption lowers copper status and may further compromise enzymic antioxidant defences.

We thank Seven Seas for providing the fish-oil.

Size-related artifacts may lead to spurious relationships between calcium intake and bone mineral density measured by absorptiometry. By A. PRENTICE, T. J. PARSONS and T. J. COLE, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

Absorptiometric methods, such as dual-energy X-ray absorptiometry (DXA), are being used increasingly to study determinants of peak bone mass and osteoporosis risk. We have evidence that the failure to adjust absorptiometric data adequately for bone size can lead to spurious relationships with variables that contain size-related information, such as Ca intake.

In general, DXA results are expressed as areal densities (bone mineral density, BMD, g/cm^2), obtained by dividing measured bone mineral content (BMC) by scanned bone area (BA). Expressing results as BMD assumes that BMC and BA are directly proportional, i.e. that a change in BMC is matched by a proportionately equal change in BA. There is no *a priori* reason why this should be the case and, in practice, it rarely occurs. For example, Table 1 gives the power coefficients for the relationships between BMC and BA at different sites that were obtained in a recent DXA study of men and women aged 18-21 years (Parsons *et al.* 1993). A coefficient of 1 signifies direct proportion.

Table 1	Men (n 40)		Women (n 40)	
	Coefficient	SE	Coefficient	SE
Spine: lumbar 1-4	1.65***	0.16	1.28*	0.14
Hip: femoral neck	1.32	0.17	0.97	0.08
Hip: Wards region	1.30*	0.14	0.77	0.15
Hip: trochanter	1.51**	0.16	1.33*	0.13
Whole body	1.56***	0.08	1.22*	0.09

Table 1 gives coefficients obtained by linear regression of ln BMC on ln BA. The relationship at all sites was significant at $P<0.001$ (i.e. coefficient≠0). Significance of difference of coefficient from 1 : * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

At sites where the power coefficient is substantially different from 1, dividing BMC by BA does not completely remove the influence of BA. In these circumstances, BMD is related to BA, and, in its absence, to any variable that provides information about bone size. An example of the problems that can be caused by using BMD without regard for the possible residual relationship with BA is given in Table 2. In the study of young men and women (Parsons *et al.* 1993), a significant relationship was found between whole-body BMD and current Ca intake (model 1). However, when BA was added to the regression (model 2), the relationship disappeared, demonstrating that Ca intake was providing information about bone size that had not been corrected for adequately by using BMD.

Table 2	Coefficient	SE	t ratio	*P*
Regression model 1: Dependent variable BMD (g/cm^2)				
Constant	1.13	0.03	44.2	<0.0001
Ca intake (x10^{-3}; mg/d)	0.06	0.02	2.7	0.009
Regression model 2: Dependent variable BMD (g/cm^2)				
Constant	0.89	0.06	14.1	<0.0001
Ca intake (x10^{-3}; mg/d)	0.02	0.02	0.7	NS (0.47)
BA (x10^{-3}; cm^2)	0.13	0.03	4.2	<0.0001

We recommend that BA should be included in analyses of DXA data to avoid size-related artifacts, and that there should be a re-evaluation of published studies investigating bone mineral determinants.

Parsons, T.J., Prentice, A., Smith, E.S., Cole, T.J., Laskey, M.A. & Compston, J.E. (1993). *Proceedings of the Nutrition Society* **52**, 323A.

Ontogenic changes in peri-renal brown adipose tissue of newborn lambs. By J.A. BIRD, L. CLARKE, M.A. LOMAX and M.E. SYMONDS, *Department of Biochemistry and Physiology, School of Animal and Microbial Sciences, University of Reading, Whiteknights, PO Box 228, Reading RG6 2AJ*

The activity of the enzyme iodothyronine-5'-deiodinase (I5'D) may play an important role in the local generation of triiodothyronine (T_3) in brown adipose tissue (BAT), and therefore in determining metabolic rate in the newborn lamb (Symonds and Lomax 1992). The present study examines the extent to which the I5'D activities in BAT alter during the first 24 h of life.

Sixteen lambs born normally at term, were immediately removed from their ewes and housed at an ambient temperature of 3-8°. At 1 h 15 min of life eight lambs were fed 120 g of milk powder (Lamlac™, Volac) in a 600 ml volume during the first day of life, and humanely killed at 24 h of age to enable BAT sampling. Type I I5'D activity, thermogenic activity of BAT (guanosine diphosphate (GDP) binding), total protein and DNA contents of BAT were measured as described by Darby *et al.* (1994).

Age (h)	Type I I5'D activity (pmol/mg protein per h)		GDP-binding (pmol/mg mitochondrial protein)		Protein (g)		DNA (mg)	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM
1.25	527	31	233	38	3.91	0.81	130	19
24	398*	33	175	14	2.54	0.17	80*	6

* significantly different from 1.25 h $P<0.05$ (Student's t test).

Over the first 24 h of life an appreciable decrease in both BAT protein and DNA content was observed, in conjunction with a decline in I5'D activity. However, there was little change in the thermogenic activity of BAT to generate heat over the first 24 h of life may remain constant despite an apparent loss of adipocytes and decline in I5'D activity.

This work was funded by the Wellcome Trust and AFRC (J.A.B.) and MRC (L.C.) studentships.

Darby, C.J., Clarke, L., Lomax, M.A. and Symonds, M.E. (1994). *Journal of Developmental Physiology* (In the Press).

Symonds, M.E. and Lomax, M.A. (1992). *Proceedings of the Nutrition Society* **51**, 165-172.

Effect of fetal thyroidectomy on thermoregulation in newborn lambs. By S.J. LYKE, J.A. BIRD, M.A. LOMAX, D.A.L. SHEPHERD and M.E. SYMONDS. *Department of Biochemistry and Physiology, University of Reading, Whiteknights, Reading RG6 2AJ*

Thyroid hormones are known to have a primary role in the control of metabolism during postnatal development in lambs (Symonds *et al.*, 1989), but their importance in regulating heat production immediately after birth has not been fully established. The present study investigates the effect of fetal thyroidectomy on thermoregulation in newborn lambs.

Fourteen lambs from seven single and four twin pregnancies were entered into this study. At 127±0.3 d gestation seven fetuses (Tx) (four singles, plus one lamb from each of three sets of twins) were thyroidectomized, whilst maintained under halothane anaesthesia (25 ml/l O_2). All lambs were born normally at term (147 d), into a cool ambient temperature of 15°, and rapidly established continuous breathing with the exception of one Tx lamb, for which results are not included. They were fed 50 g artificial colostrum (VOLAC VOLOSTRUM, Royston, Herts), plus milk replacer *ad libitum* (VOLAC LAMLAC). Controls were pair-fed to intake of Tx group (28 g/kg body weight per d). Colonic temperature was monitored in all lambs and O_2 consumption was measured during non-rapid eye movement sleep (six control and five Tx lambs) between 6 and 45 h after birth.

		Body weight (kg)		Body temperature (°)		Oxygen consumption (ml/min per kg body weight)		Number of lambs shivering
		Mean	SEM	Mean	SEM	Mean	SEM	
Control	7	4.69	0.61	39.66	0.19	23.63	3.00	1/6
Tx	6	5.29	0.17	37.31*	1.24	15.46*	2.08	3/5

* Probability of difference from controls of $P=0.06$ (Student's t test)

Fetal thyroidectomy had no effect on birth weight but colonic temperature (Tb) was 2.35° lower in Tx than control lambs. In addition, Tx lambs exhibited a 35% lower rate of O_2 consumption and were more reliant on shivering thermogenesis in order to maintain heat production. The inability of Tx lambs to maintain a normal body temperature is emphasized by the observation of hypothermia (Tb < 35°) in two Tx lambs.

It is concluded that thyroidectomy of the late gestation fetus has no effect on fetal growth but greatly decreases the ability of the neonatal lamb to thermoregulate.

This work was funded by the Wellcome Trust and University of Reading (S.J.L.), and AFRC (J.A.B.) studentships.

Symonds, M.E., Andrews, D.C. & Johnson P. (1989). *Journal of Developmental Physiology* 11, 289-298.

Effects of nutrition and thyroxine administration on perirenal adipose tissue development in hand-reared postnatal lambs. By J.J. GATE[1], J.A. BIRD[1], M.J. BRYANT[2], L. CLARKE[1], M.A. LOMAX[1] and M.E.SYMONDS[1], *Departments of [1]Biochemistry and Physiology and [2]Agriculture, University of Reading, PO Box 228, Reading RG6 2AJ*

The postpartum surge in plasma concentrations of thyroid hormones is important in the initiation of non-shivering thermogenesis in brown adipose tissue (BAT; Symonds *et al.* 1994), but the role of thyroid hormones in regulating BAT development, particularly in response to changes in nutrient intake, has yet to be established. The present study investigates the effect of both nutritional status and thyroxine (T_4) administration on BAT development in artificially-reared lambs.

Twenty eight lambs of similar birth weights (3.74 (SE 0.14) kg), were removed from their ewes within 6 hours of birth, and individually housed at an ambient temperature of 10-15°. Fourteen lambs were fed on milk replacer at a high level of 200 g/d (H) and the remainder at a low level of 100 g/d (L). Seven lambs, from each nutrition group were also given a daily oral dose of T_4 (T; 15 mg/kg body weight). At 8 days of life the lambs were humanely killed and the perirenal adipose tissue was obtained for measurement of its thermogenic (guanosine diphosphate (GDP) binding) and type I iodothyronine 5'deiodinase (I5'D) activities (Clarke *et al.* 1994).

		Weight (g)		Lipid (g)		GDP-binding (pmol/mg protein)		Type I I5'D (pmol/mg protein per h)	
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
H	7	26.8	5.0	5.14	2.01	173	23	693	153
HT	6	28.5	4.4	4.66	1.31	142	18	604	204
L	7	10.4**	1.4	0.99**	0.57	186	47	386*	124
LT	6	12.5**	1.5	1.53**	0.43	93*	16	361*	145

Significantly different from corresponding H/HT group, (Students t-test): *$P<0.05$ **$P<0.001$.

H lambs deposited 3-5-fold more lipid in perirenal adipose tissue, and exhibited a higher I5'D activity than L-fed lambs, but there was no effect of nutrition on the thermogenic activity of BAT. T_4 treatment had no effect on BAT development in H-fed lambs, but caused a marked reduction in the thermogenic activity of BAT in L-fed lambs without any influence on I5'D activity.

It is concluded that hand-rearing lambs on a high level of nutrition promotes lipid deposition and I5'D activity. In contrast prolonged T_4 treatment has no effect on adipose tissue growth, but in conjunction with a low level of nutrition markedly decreases the thermogenic activity of BAT.

This work was funded by the Wellcome Trust and AFRC (JB), MLC (LC) and MAFF (JG) studentships.

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Symonds, M.E., Clarke, L. & Lomax, M.A. (1994) In *Early Fetal Growth and Development* pp. 407-419 [R.G.T. Ward, S.K. Smith & D. Donnai, editors]. London: RCOG.

Effects of supplementary protein in the dry period on milk production in the subsequent lactation.
By J.M. MOORBY,[1] R.J. DEWHURST[1] and S. MARSDEN,[2] *SAC Auchincruive, Ayr KA6 5HW and [2]Dalgety Agriculture Ltd, 180 Aztec West, Almondsbury, Bristol BS12 4TH. [1]Current Address: Ruminant Nutrition Department, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB*

Much work has investigated the effect of dry-period (DP) nutrition on body fat content and subsequent lactation performance in dairy cattle (Garnsworthy, 1988). Relatively little attention has, however, been paid to the residual effects of body protein content on milk production. The aim of the present study was to examine the residual effects of DP nutrition on milk production.

Two experiments were conducted to investigate the effect of protein supplementation during the DP on lactation performance during the subsequent lactation in dairy cows. In Expt 1, twenty-two multiparous animals were paired according to condition score and predicted calving date and allocated to two dietary treatments at the end of lactation. Control (C) animals received *ad libitum* access to a 3rd-cut grass silage. Supplemented (S) animals were allowed access to the same silage for a limited period (2-3 h) each day, and were given *ad libitum* access to barley straw together with 0.5 kg/d high-protein supplement (providing approximately 250 g/d digestible undegraded protein; DUP). In Expt 2, thirty-two animals were allocated in the same way to similar dietary DP treatments: *ad libitum* access to a 3:1 mix (on a fresh matter basis) of grass silage:distiller's grains or pressed beet pulp (C) or a restricted quantity of the same (4.1 kg DM/d) plus *ad libitum* access to barley straw and 0.5 kg/d protein supplement. Following parturition, animals from both groups in each experiment were offered the same diets without reference to DP treatment. These consisted of *ad libitum* access to a 3:1 mix of grass silage:distiller's grains or pressed beet pulp plus concentrates fed at a stepped flat rate. In both experiments, animals were condition scored (CS), and blood samples were taken for metabolic profile analyses at 7 d after being dried off and 7-10 d before calving.

Due to the repetition of measurements on animals, the preliminary analysis of variance used mean values for each animal across the period studied so that SED values presented are extremely cautious. Parallel curve analysis was used to extract more information from the data. Results are presented in order of C *v.* S. In Expt 1, mean DP blood albumin concentrations of the two groups (35.8 *v.* 38.3 g/l; SED 0.835) were significantly different (P=0.017). No difference in CS at calving was seen. Although there was no effect of DP treatment on mean milk yields to week 31 of lactation (27.2 *v.* 27.9 kg/d; SED 2.12), mean milk protein concentrations (28.9 *v.* 31.8 g/kg; SED 0.58) were significantly different (P=0.001), and the effect of parallelism was significant (P=0.003), i.e. milk protein concentrations declined more sharply during early lactation for C animals than for S. Mean milk fat concentrations (40.2 *v.* 42.7 g/kg; SED 2.40) tended to be higher (NS). In Expt 2, no differences in metabolic profiles were seen during the DP, although C animals calved at a small, but significantly greater CS (2.9 (SE 0.51) *v.* 2.5 (SE 0.39); P=0.027). No significant differences in mean protein and fat concentrations (31.0 *v.* 31.2 g/kg; SED 0.62; 45.1 *v.* 44.6 g/kg; SED 1.92) were seen to week 18 of lactation. However, milk yields were greater for group S (33.3 *v.* 35.4 kg/d; SED 1.66; P<0.001 for effect of shift displacement), resulting in higher protein yields (1031 *v.* 1104; SED 53.7; P<0.001 for effect of shift displacement).

Milk protein concentration was significantly increased by the provision of the protein supplement during the DP in Expt 1, in agreement with the effects observed by Van Saun *et al.* (1993). Increased milk yields in Expt 2, but not increased protein concentrations, indicate a difference between the two experiments which may have been due either to the initial protein status of the animals, or the energy background of the control diet. Significantly reduced blood albumin concentrations in C animals during Expt 1 indicate much reduced true protein intakes by these animals due to the poor quality of the diet offered, suggesting that maternal body protein may have been needed to support fetal growth . However, the possibility of a low protein status of these animals at the end of the previous lactation cannot be dismissed, and further work is therefore needed to elucidate the effects.

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Energy intake in the follicular and luteal phases of the menstrual cycle in Mexican women. By S.A. BONNER[1], C.P. SANCHEZ-CASTILLO[2], M. DE L. SOLANO[2], N. LOPEZ[2], L. DAVIDSON[1] and G. McNEILL[1,3], [1]*Human Nutrition Unit, Rowett Research Institute, Aberdeen AB2 9SB,* [2] *Instituto Nacional de la Nutricion Salvador Zubirán, Dept. de Fisiología de la Nutrición, México, D.F. and* [3] *Department of Medicine and Therapeutics, University Medical School, Aberdeen AB9 2ZD*

That energy intake (EI) varies in women according to the phase of the menstrual cycle is a possibility which has been investigated by several authors with varying results. Many have found an apparent increase in EI in the luteal phase compared with the follicular phase (Dalvit, 1981; Webb, 1986; Gong *et al.* 1989) while others have concluded that the menstrual cycle has no effect on energy intake in women (Fong & Kretsch, 1993). The present study compares the reported EI by 7 d weighed intake (validated by the urinary nitrogen (N) method) between two phases of the menstrual cycle in Mexican women. Completeness of urine collections was assessed using the PABACheck method (Bingham & Cummings, 1983). The timing of each phase was estimated by comparison with individual subjects previous cycles and was confirmed by determining the concentration of luteinizing hormone (LH) in the first sample of the morning and defining ovulation as the day after the surge in LH. Thirty-five healthy urban Mexican women took part in the study (age 27 (SD 6.9) years; weight 58.8 (SD 8.23) kg). None of the women were taking the oral contraceptive pill. These women were selected from staff at the Institute of Nutrition in Mexico City and students from a local college. Of the thirty-five women, twenty-two had a urinary excretion ratio (urinary N/dietary N) of >91% in one or both weeks and were therefore rejected as under-reporters. Reported EI in the follicular and luteal phases were compared in the remaining thirteen women of whom eight started the study in the follicular and five in the luteal phase. Changes in body weight (BW) and urinary N excretion were also compared between the two phases. The Table below shows mean EI, body weight and urinary N in both phases.

	Follicular (n 13)	Luteal (n 13)
	Mean SD	Mean SD
EI (MJ/d)	8.41 2.67	9.18 3.09
BW Change (g/week)	+230.8 370.6	+330.8 555.9
Urinary N (g/d)	7.74 1.24	7.95 1.44

EI was slightly greater in the luteal phase but not significantly so (P=0.279). Values were within the range of suggested requirement values for light and moderate activity of 8.4 - 9.2 MJ/d (WHO, 1985). Body weight tended to increase during both recording periods and this was significantly different from zero in the follicular phase (P<0.05). The degree to which the women gained weight was not significantly different between the two phases. No significant difference was observed in urinary N between phases (P=0.477). The implications of these results are that, if cycle-related differences in intake exist they are modest and insufficient to warrant consideration in the design of dietary studies in this population.

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Thermic effect of continuous feeding in young and elderly healthy adults. By N.GIBSON[1], A.FEREDAY[2], M.COX[1], D.J. MILLWARD[2] AND P.J. PACY[1],[1] *Nutrition Research Unit, St Pancras Hospital, 4 St Pancras Way, London NW1 2PE and* [2]*School of Biological Sciences, Surrey University, Guilford GU2 5XH*

It is now well established that ageing is associated with a reduction of resting energy expenditure which may well reflect the accompanying decline in fat-free mass. The influence of the ageing process on diet-induced thermogenesis (DIT) has not only been much less studied but available data are conflicting; some investigators reporting no effect (Fukagawa *et al.* 1991) while others that DIT is reduced (Schwartz *et al.* 1990) in comparison with young adults. The aims of the present study were to examine whether the DIT response was influenced by age and level of dietary protein. Healthy young and elderly adults were studied for 9 h; for the first 3 h the protein content of the diet was low (LP) at 2% energy while for the latter 3 h it represented habitual (HP) intake (14% energy). The carbohydrate component of both diets was held constant at 50% energy. Indirect calorimetry via a ventilated hood was measured for at least 1 h during each 3 h phase. The physical characteristics of the young adults (YA) were: 5M, 5F, age 24 (SD 4) years, weight 62 (SD 6) kg, body mass index 21 (SD 1) kg/m^2, while those for the elderly adults (EA) were: age 75 (SD 7) years, weight 66 (SD 9) kg, body mass index 24 (SD 2) kg/m^2. Student's *t* tests were used to determine levels of significance. The results, expressed in absolute terms, are below. EE represents energy expenditure derived from Weir's formula.

		PA		LP		HP	
		Mean	SD	Mean	SD	Mean	SD
YA	O_2 (ml/min)	233	25	252	24	253	26
	CO_2 (ml/min)	188	20	225	24	226	26
	EE (kJ/d)	6630	884	7383	699	7437	774
EA	O_2 (ml/min)	190**	28	199**	30	198**	28
	CO_2 (ml/min)	155**	24	171**	27	171**	27
	EE (kJ/d)	5471**	824	5793**	891	5793**	837

** $p < 0.01$ compared with YA.

The increase, above PA values, of CO_2 production (20 (SD 10)% *v.* 10 (SD 9)%, $P< 0.05$) and EE (12 (SD 9)% *v.* 6 (SD 3)%, $P< 0.05$) during LP feeding was higher in young adults than the elderly; almost identical responses were observed during the HP period. This study demonstrates that DIT is higher in healthy young adults than their weight-matched elderly counterparts. However there was no suggestion that the level of dietary protein intake influenced the DIT response. In addition these data confirm that the REE is significantly lower in the elderly than in weight-matched young adults.

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Effects of diet composition on 24h energy expenditure during energy restriction. By J.M. WHITEHEAD[1], G. M[c]NEILL[2] and J.S. SMITH[1], [1]Human Nutrition Unit, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB and [2]Department of Medicine and Therapeutics, University Medical School, Aberdeen AB9 2ZD

To investigate the effects of macronutrient composition of the diet on energy expenditure during energy restriction, eight adults (two men, six women; mean age 48.5 years; mean body mass index 30.7) ate diets containing 4.2 MJ/d, one of which was high in protein (HP; 36% protein, 32% carbohydrate (CHO), 32% fat) and two which were low in protein but differed in the amount of fat and CHO (HC: 53% fat, 32% CHO, 15% protein; HF: 53% CHO, 32% fat, 15% protein). These diets were fed in a random order for periods of 7 d with a washout period of 1 week in-between dietary periods when subjects ate their normal diet. A standard diet based on the subjects' calculated energy requirements and providing 45% CHO, 40% fat and 15% protein was given on the day before each 7 d period (day 0). All subjects spent days 0 and 7 of each week in a whole-room indirect calorimeter. Twenty-four hour urine collections were made for each week of each diet and body weight was measured at the beginning and end of each week. Results are presented in the Table below:

	HP		HC		HF		HP*v.* HC+HF	HC*v.* HF
	Mean	SD	Mean	SD	Mean	SD	*P*	*P*
ΔWt (kg/week)	-2.06	0.92	-2.33	0.72	-1.90	0.73	0.866	0.277
Δ24hEE/kg(%)	-0.50	2.4	-3.40	6.1	-4.60	3.1	0.040	0.483
ΔSMR/kg(%)	-2.00	5.2	-5.60	5.3	-6.00	6.7	0.009	0.774
UN as % intake	85.4	21.3	112.6	19.0	110.0	24.2	0.001	0.847

Body weight decreased in each subject in each of the three diets. There was no significant difference in the weight lost on each diet.

Twenty-four hour energy expenditure, when expressed per kg body weight, decreased in all subjects on each dietary period. The decrease was significantly lower on the HP diet than on the two low-protein diets ($P = 0.040$). There was no significant difference between the HC and HF diets ($P = 0.483$). The same effects were seen in sleeping metabolic rate (SMR) where the HP was significantly different from the other two ($P = 0.009$).

When urinary N (UN) was expressed as a percentage of dietary N, and compared with the expected range of 81 (SD 10)%, it was found that those on the HP diet were close to N balance but that the other diets showed subjects to be in negative N balance.

These results suggest that maintaining protein intake on an energy-restricted diet is beneficial in preventing a fall in metabolic rate and in retaining lean body mass. This would allow a greater loss of adipose tissue over time while consuming the same amount of energy.

Changes in energy and macronutrient intakes after smoking cessation. By R.L. THOMPSON[1], E.A. SCOTT[2], B.M. MARGETTS[1] and D.A. WOOD[2], *[1]Institute of Human Nutrition, University of Southampton, SO9 3TU and [2]Clinical Epidemiology, National Heart and Lung Institute, University of London, SW3 6LY*

There are data from cross-sectional studies showing that people who smoke consume different diets to people who do not smoke (Thompson *et al.* 1992). Ex-smokers have lower intakes of energy and total fat and a higher intake of protein than smokers, but similar dietary habits to never smokers. We have carried out a prospective dietary study to determine whether the diets of smokers change towards those of never smokers after smoking cessation.

Diet was measured using a 10 d weighed record in a population sample of 301 male and female cigarette smokers, invited to join a smoking cessation programme and followed up at 4 months and 1 year. Full attendance and dietary assessment were completed by 101 (thirty-six men) subjects. Fifteen (five men) subjects had quit smoking between baseline and first follow-up appointment (confirmed by breath carbon monoxide and serum cotinine measurements). The mean lengths of time since quitting were 13 (range 4-26) and 46 (range 39-52) weeks for the 4 month follow-up and 1 year follow-up respectively. Dietary intakes did not differ at baseline between smokers who continued to smoke and those who went on to quit. Daily mean intakes for the total sample at baseline were 8.4 (SE 0.2) MJ for energy, 73.4 (SE 1.8) g for protein, 86.1 (SE 2.5) g for fat and 230.8 (SE 6.7) g for carbohydrate.

	4-month follow-up					1-year follow-up				
	Smokers (*n* 86)		Quitters (*n* 15)			Smokers (*n* 86)		Quitters (*n* 15)		
	MD	SE	MD	SE	AD	MD	SE	MD	SE	AD
Energy (MJ)	-0.5	0.1	0.5	0.4	1.1*	-0.3	0.2	0.1	0.3	0.4
Protein (g)	-1.3	1.4	3.8	3.4	5.2	-0.7	1.5	6.9	3.2	7.2*
Fat (g)	-7.6	2.0	6.9	4.9	13.0*	-5.2	2.3	-0.2	5.2	3.5
Cho (g)	-8.6	4.2	9.0	11.0	26.5	-4.2	6.5	-1.2	7.8	2.2

MD, mean difference; AD, difference attributable to smoking cessation (quitters - smokers) after adjustment for age, occupation, baseline intake and length between appointments; cho, carbohydrate. * $P < 0.05$, ANOVA.

After an average of 13 weeks smoking cessation statistically significant increases were detected for energy and fat intakes. By an average of 46 weeks after quitting there were no statistically significant differences in energy and fat intakes but protein intake was statistically significantly higher than at baseline. Changes in dietary habits in the short term (around 13 weeks) after smoking cessation are not consistent with the differences observed in cross-sectional studies. At the 1 year follow-up the changes in protein intake seem to agree with the cross-sectional observations that ex-smokers consume more protein than smokers. Cross-sectional studies of ex-smokers usually reflect dietary habits in people who have stopped smoking for a number of years. It is possible that dietary habits may take in excess of 1 year before diets have completely reverted to those of never smokers (Bolton-Smith *et al.* 1993).

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Influence of short-term cessation of endurance training on postprandial lipaemia in man. By N.D. NELSON, N.V. TSETSONIS and A.E. HARDMAN. Department of Physical Education, Sports Science and Recreation Management, Loughborough University, Leics LE11 3TU

Endurance-trained individuals exhibit a low lipaemic response following challenge with a fatty meal (Cohen *et al.* 1989). However, the residual effects of the last training session (Aldred *et al.* 1994) may contribute to the difference between endurance-trained and sedentary individuals in this regard. The purpose of the present study was therefore to examine the effect of cessation of training for 1 week on postprandial lipaemia in endurance-trained young adults.

Seven (six male, one female) normolipidaemic subjects aged 24.1 (SEM 1.1) years participated. Concentrations of serum lipids in venous blood samples obtained in the fasted state were: triacylglycerol (TAG) 1.18 (SEM 0.19) mmol/l, total cholesterol 4.11 (SEM 0.28) mmol/l and high-density-lipoprotein-cholesterol 1.21 (SEM 0.13) mmol/l. Subjects consumed a high-fat test meal on each of three occasions, i.e. 12 h, 2 d and 7 d after the last training session. Food intake was weighed and recorded during the day before the first trial. The same food intake was consumed on the day before the second and third trials. Subjects reported to the laboratory in the morning, after an overnight fast. After a baseline capillary blood sample had been obtained from a pre-warmed hand the subjects ingested the meal. This consisted of cereal, fruit, nuts, chocolate and cream: the weight of the meal given provided 1.2 g dietary fat and 71 kJ/kg subject's body mass. Further capillary blood samples were obtained at 1, 2, 3, 4, 5 and 6 h after the meal. Serum was separated and stored at -20° until analysed for TAG by an enzymic method (Boehringer). Three indices of postprandial lipaemia were adopted: (1) peak TAG concentration, (2) the total lipaemic response (area under the TAG *v.* time curve) and (3) the maximal TAG increase (average of two highest TAG values minus fasting value). Fasting TAG concentrations increased after 7 d without training (Table). Therefore, to allow meaningful between-trial comparisons (paired *t*- test), the area under the TAG *v.* time curve for each trial was normalized to the zero hour level for the 12 h trial. No significant between-trial differences were found in indices of lipaemia. However, after 2 and 7 d without training the mean TAG area had increased by 69% and 89% respectively, compared with values obtained 12 h after training. The other indices of lipaemia showed similar trends.

Time after last training session	Fasting TAG (mmol/l)		TAG area (mmol/l.h)		Max TAG increase (mmol/l)		TAG Peak (mmol/l)	
N 7	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
12 h	0.89	0.12	2.69	0.78	0.71	0.20	1.70	0.27
2 d	0.93	0.13	4.55	1.27	1.05	0.30	2.01	0.32
7 d	1.23*	0.14	5.09	1.17	1.18	0.20	2.11	0.23

* Significantly different from 12 h and from 2 d, $P<0.05$.

The results of this pilot study suggest that the low levels of postprandial lipaemia characteristically shown by endurance-trained individuals increase rapidly when training is interrupted. Frequent exercise may well be needed to maintain a high metabolic capacity for TAG.

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The postprandial response to high-fat, low-carbohydrate and low-fat, high-carbohydrate meals providing the same amounts of energy in subjects at risk of heart disease. By D.L. FRAPE[1], N.R. WILLIAMS[1], J. PICKERSGILL[1], R. MURRILLS[1], C. PALMER[2], and R.J. FLETCHER[3], [1]*Pathology Department Papworth Hospital, Papworth Everard, Cambs CB3 8RE,* [2]*University of Cambridge, Department of Community Medicine, Institute of Public Health, Cambridge CB2 2SR and* [3]*The Kellogg Company of Great Britain Ltd, The Kellogg Building, Manchester M16 0PU*

Previously we showed that plasma insulin response to a fatty breakfast was greater than that to a fatty lunch. Elevated plasma free fatty acid (FFA) concentrations impair glucose metabolism and insulin binding, leading to insulin resistance (Griffiths *et al.* 1994; Smith, 1994). The purpose of the present work was to determine whether differences occur in insulin response, fat clearance and plasma FFA between morning and afternoon with both high- and low-fat meals of equal energy content.

Male and female non-smoking subjects (average age 55 years, body mass index 26.7 kg/m^2, fasting plasma triacylglycerols 1.5 mmol/l and total cholesterol 6.5 mmol/l) participated. In one study eight subjects received low-fat cereal meals at breakfast and lunch (1.24 MJ/meal), followed 110 min after the start of each meal by an i.v. bolus infusion of homogenized soya-bean oil in saline (0.1 g oil/kg BW). In a second study of a 4 x 4 latin square design twelve subjects received a high-fat potato and egg omelette (H) or a low-fat cereal (L) meal providing 2.2 MJ/meal at breakfast and lunch, or a fast (F) at breakfast and an H lunch (HH, LL, FH, LH). Responses to three treatments are reported here. All subjects received a meal of the same composition at 18.10 hours and were accommodated similarly overnight.

In the first study plasma glucose responses did not differ between morning and afternoon: mean glucose, am *v.* pm 6.34 *v.* 6.26 (SE 0.166) mmol/l. A difference of 20 % in fat clearance rate was not significant: am 0.00335 *v.* pm 0.00401 (SE 0.000855), measured as % decrease per min in light absorbance of plasma at 340 nm). The fat was 80 % cleared in 40 min.

In the second study the area under the curve (AUC) insulin response to LL was greater than that to HH in the morning and in the afternoon (P<0.0001). Within treatment the differences, am-pm, in peak insulin response were 27.8 μU/ml for LL (P,0.0005) and 15.8 μU/ml for HH (P,0.001), and differences, am-pm, in AUC of insulin were for LL 2645 (P,0.028) and for HH 576 (P,0.004), μU/ml x min; but AUC for plasma FFA and glycerol tended to be greater pm (P, 0.060 & P, 0.066 respectively). AUC for plasma FFA and glycerol were greater for HH than for LL with probabilities respectively of 0.0005 an 0.001. The AUC values are given below:

		F		M		
		am	pm	am	pm	SE
FFA	LL	59.5	51.0	68.7	70.8	
(μmol/l)(min)	HH	115.6	127.8	98.5	137.7	8.18
Glycerol	LL	9.70	8.69	10.31	13.22	
(mmol/l)(min)	HH	14.60	16.81	13.57	21.67	2.27

In treatment LH the FFA response to the high-fat meal at lunch was decreased (cf HH) by the cereal breakfast (HH-LH, difference during the afternoon in FFA AUC was 27.9, SE 7.01 μmol/l x min, P,0.0005). A cereal breakfast reduces the plasma concentration of the risk factor FFA; but the diurnal change demonstrated in insulin response to both high-fat and high-carbohydrate meals is unrelated to changes in plasma FFA concentration.

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Measurement of gross body fat of rats using dual energy X-ray absorptiometry. By SUSAN A. JEBB, S.W. GARLAND, G. JENNINGS AND M. ELIA. *MRC Dunn Clinical Nutrition Centre. Hills Road. Cambridge. CB2 2DH.*

The Hologic QDR-1000W dual energy X-ray absorptiometer (DXA) rat whole-body module is designed to provide whole-body bone mineral and soft-tissue composition measurements in rats weighing between 200 and 750 g. We have assessed the accuracy of this system for the measurement of fat mass by comparison with direct chemical analysis.

Three adult rats from the Dunn Norwegian strain were matched as closely as possible for total body weight and body dimensions yet with a range of anticipated fatness (normal male, normal female and post-partum female). Animals weighed between 330 and 350 g in weight, were 150 - 200 mm in length and had a measured maximum thickness of 50 mm. In addition two genetically obese fa/fa rats were measured weighing 360 and 481 g respectively. All the animals were killed, scanned freshly using the DXA, according to the manufacturer's operating instructions, and analysed using software Version 5.61P. Each animal was scanned in triplicate. The animals were then homogenized, freeze-dried to measure the water content and analysed for fat (petroleum ether extraction) protein (Kjeldahl N x 6.25) and ash (combustion at 600 ^{o}C). Five samples were taken from each animal for analysis.

The sum of the components for each animal measured by direct analysis was within 1.5% of body weight. The DXA-measured body weight and the true mass of each animal agreed to within 6 g. However there was a highly significant overestimate of fat mass measured using DXA v. direct analysis in all animals as shown in the Table. The percentage error was remarkably constant, showing a strong relationship between the true fat mass and the error made by DXA in the measurement of total fat ($R^2 = 0.92$, $P < 0.01$).

	Weight (g)	DXA (g)		Direct (g)		Difference (g)	% Error
		Mean	SD	Mean	SD		
Male	347.0	64.5	1.04	49.6	1.35	14.9***	30.0
Female	333.1	76.6	1.67	57.9	1.30	18.7***	32.3
Post-partum	333.8	144.9	1.00	108.7	2.17	36.2***	33.3
fa/fa	360.1	165.6	0.78	119.1	3.56	46.5***	39.0
fa/fa	481.0	306.4	0.72	231.9	1.44	74.5***	32.1

*** $p < 0.001$ (paired t-test)

This systematic overestimation of fat mass by DXA suggests that, with this system and the current software, this method is an inappropriate substitute for carcass analysis for the measurement of fat mass in rats.

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The effect of an elemental diet on stool output in irritable bowel syndrome. By A.J. COSTELLO[1], J.L. MURPHY[1], S.A. WOOTTON[1], J.T. RUCKER[2], G.A. KIRBY[2] and J.O. HUNTER[2], *[1]Institute of Human Nutrition, Southampton SO16 7PX and [2]Gastrointestinal Research Unit, Addenbrookes Hospital, Cambridge CB2 2QQ*

Irritable bowel syndrome (IBS) is a common condition in which abdominal pain and abnormal bowel habit occur in the absence of any detectable pathology. Many cases can be controlled by dietary management (Hunter & Alun Jones, 1985). Colonic bacteria ferment dietary carbohydrate, non-starch polysaccharide (NSP), resistant starch (RS) and other fermentable substrates delivered to the colon such as endogenous losses including mucin and sloughed intestinal epithelia. Endogenous losses may provide 6-9 g of a total of 10-60 g carbohydrate delivered to the colon each day as substrate available for fermentation (Cummings & Macfarlane, 1991). Elemental diets may be used clinically to limit the amount of dietary residue reaching the colon in conditions such as IBS such that the only fermentable substrate available is endogenous material (Hunter & Alun Jones, 1985). The aim of the present study was to examine the changes in bowel habit and stool output in a group of IBS patients following administration of an elemental diet for 14 d.

Eight female patients (aged 29-57 years) with IBS completed the study. Stools were collected over three 3 d study periods: immediately before commencing the elemental diet on their habitual diet and over days 3-5 and 12-14 whilst exclusively consuming an elemental diet (E028 EXTRA, Scientific Hospital Supplies, UK). All stools passed over each 3 d collection period were pooled, homogenized and freeze-dried. The results expressed as medians and ranges are summarized in the Table.

Diet	SF	SWW (g/d)	SDW (g/d)	Water (%)	BM (g/d)
Pre-E028 EXTRA	1.0 (0.7-1.0)	115.3 (45.8-238.3)	30.3 (12.6-52.4)	77.0 (64.4-80.6)	3.5 (1.1-11.3)
Days 3-5	1.0 (0.7-1.0)	52.0* (23.6-12.7)	7.6* (2.7-33.2)	85.8 (61.7-91.9)	1.4 (0.3-3.3)
Days 12-14	0.7* (0.3-1.0)	44.0* (16.3-58.3)	5.4+ (0.04-14.2)	83.2* (72.5-99.9)	1.2 (0.003-2.1)

SF, daily stool frequency; SWW, stool wet weight; SDW, stool dry weight; BM, bacterial mass. *Significantly different from pre-E028 EXTRA, +significantly different from days 3-5 (Wilcoxon Rank Sum): $P<0.05$.

These results indicate that stool weights in this group of patients with IBS are within the range seen in normal healthy subjects (100-150 g/d; Murphy, 1991). Consumption of an elemental diet resulted in significant improvements in patients symptoms with reference to a simple scoring system, from 0 to 8, based on presenting symptoms (Pre-E028 EXTRA, median: 4; days 12-14, median: 1; $P<0.05$). A decrease in the frequency of stools passed over the 14 d period was observed. There was also an immediate significant reduction in stool weights following consumption of the elemental diet by days 3-5, after which time daily stool output remained unchanged. The appearance of the stool was markedly different by days 3-5 with an increased water content and a bright green coloration indicative of increased biliverdin in the stool reflecting reduced bacterial activity within the colon. These results suggest that exclusion of dietary residue results in a rapid reduction in bacterial fermentation although endogenous losses were sufficient to sustain excretion of faecal bacteria in this group of IBS patients.

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The effect of the addition of raw potato starch to habitual diet on stool output.

By A.J. COSTELLO and S.A. WOOTTON, *Institute of Human Nutrition, Southampton SO16 7PX*

Colonic bacteria ferment dietary carbohydrate, non-starch polysaccharide (NSP), resistant starch (RS) and other fermentable substrates delivered to the colon, stimulating colonic bacterial growth with the excretion of a softer stool. The potential physiological significance of RS in the diet and the effect of RS on bowel habit are poorly understood (Muir *et al.* 1993). There is no information on the effect of supplementing the habitual diet with RS on the frequency, amount and composition of stools passed. The aim of the present study was to examine the effect of the addition of raw potato starch, as an isolated form of RS to the habitual diet on stool output.

Six normal healthy males (aged 22-41 years) completed a weighed food intake record for 19 d whilst consuming a habitual diet. The diet was supplemented with 25 g raw potato starch (Fisons Scientific Equipment, UK), approximately 75% RS (Englyst *et al.* 1992), in the form of a milkshake, for the final 14 d of the study. Over the study period the habitual diet provided a daily average 16 g NSP (range 11-22 g) and 1.2 g RS (range 0.7-1.5 g). Stools were collected over the study between carmine markers to denote the period of RS supplementation. Subjects also recorded the number of daily episodes of flatulence. Subjects were also required to record stool type according to Heaton *et al.* (1992). Stools were pooled into 3 d collection periods and homogenized. Faecal bacteria were isolated from freeze-dried stool (Stephen & Cummings, 1980). The results expressed as medians and ranges are summarized in the Table.

Diet	SWW (g/d)	SDW (g/d)	BM (g/d)
Habitual	117.6 (89.3-190.0)	37.7 (28.0-46.0)	4.6 (2.2-7.0)
Days 1-3	169.6 (71.3-212.3)	39.3 (18.0-62.0)	3.6 (1.1-6.2)
Days 10-12	123.7 (83.5-221.0)	33.7 (15.5-59.2)	3.2 (0.8-4.8)

SWW, stool wet weight; SDW, stool dry weight; BM, bacterial mass.

There were no significant differences between days 1-3, days 10-12 and habitual diet and days 10-12 and days 1-3 (Wilcoxon Rank Sum: $P>0.05$). The percentage water content of stool remained constant. The number of daily episodes of flatulence increased significantly from 5 to 11 (medians) during the supplementation period. Stool type was found to be consistent throughout the study period. These results do not support the assumption that the addition of RS in the form of raw potato starch to the habitual diet will result in a sustained increase in stool output or in the bacterial mass excreted within the stool. This would imply that further studies are required before any recommendation is made to increase the RS content of the diet in order to increase stool output.

A.C. is a recipient of an MRC studentship.

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Excretion of ^{13}C within stool and breath following oral and intravenous administration of ^{13}C-labelled sodium bicarbonate. By A.E. JONES, J.L. MURPHY and S.A. WOOTTON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

Breath tests utilizing stable isotopes of carbon (^{13}C) as well as studies of energy expenditure and substrate oxidation assume that the rate at which carbon dioxide (CO_2) is excreted on breath directly reflects the rate of CO_2 production. The extent to which CO_2 is retained within body pools over the study period, the factors that influence CO_2 retention and alternative routes of elimination (i.e. stool and urine) are not well understood (Keles *et al.* 1990). The present study examined the metabolic fate of ^{13}C-labelled $NaHCO_3$ introduced orally and intravenously following a standardized protocol.

Following an overnight fast six healthy women (22-28 years) consumed a test meal (1667 kJ) of low natural ^{13}C abundance followed by $NaH^{13}CO_3$ (10 mg/kg) administered orally (oral) and intravenously as a bolus injection (I.V.). Breath samples were collected before and at 0, 5, 10, 20 and 30 min after label administration then at half-hour intervals until 3 h, hourly until 10 h and again at 15 and 24 h. Whole-body breath CO_2 excretion was measured before and at hourly intervals for 10 h after label administration by indirect calorimetry (Deltatrac, Datex Instrumentarium Corp., Helsinki, Finland). A baseline stool sample and all stools passed over a 5 d period between carmine taken with the test meal were collected. ^{13}C-enrichment was analyzed by isotope ratio mass spectrometry (ANCA and ABCA system, Europa Scientific Ltd., Crewe). Oral administration always preceded I.V. which was repeated at the same time in the menstrual cycle and was matched for food consumption. The results for ^{13}C excretion as a percentage of administered label (% admin) are shown in the Table.

	Stool 5 d (% admin)		Breath 24 h (% admin)		Breath 3 h (% admin)	
	Oral	I.V.	Oral	I.V.	Oral	I.V.
Median	0.08	0.15	64.8	69.2	56.7	64.1*
Minimum	0.00	0.03	50.5	62.6	46.2	57.6
Maximum	0.61	0.50	68.0	78.5	60.0	68.7

* Significantly different from oral (Wilcoxon paired rank sum test); $P<0.05$.

Stool ^{13}C excretion was negligible and comparable to baseline variability throughout the 5 d in both trials suggesting that ^{13}C excretion within stool following ^{13}C-labelled substrates is unlikely to be derived from $^{13}CO_2$. Breath ^{13}C excretion occurred immediately label was administered with the majority excreted within 3 h and was greater following I.V. rather than oral administration. Over 24 h 69% of the intravenously administered label was excreted within breath; from 8 to 24 h excretion was minimal and similar to baseline variability. These values would suggest that with this protocol breath $^{13}CO_2$ excretion and thus substrate oxidation maybe underestimated by 31%. The undetected label may be excreted in urine (Elia *et al.* 1992), via skin, delayed within the body's bicarbonate pools (Irving *et al.* 1983) or retained trapped by metabolic processes.

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Increasing luminal viscosity stimulates crypt-cell proliferation throughout the gut. By M.E. LYNN, J.C. MATHERS and D.S.PARKER, *Department of Biological and Nutritional Sciences, University of Newcastle Upon Tyne, Newcastle Upon Tyne NE1 7RU*

Integrity of the gut epithelial barrier is maintained by crypt-cell proliferation (CCP) matching luminal cell loss. CCP is often increased when viscous or gelling non-starch polysaccharides (NSP) are included in the diet possibly as a consequence of increased short-chain fatty acid (SCFA) production from fermentation in the large bowel (LB; Sakata, 1987). An alternative hypothesis is that luminal viscosity *per se* stimulates CCP (Johnson & Gee, 1986) by an unknown mechanism. The present study was a direct test of the latter hypothesis.

Twenty male Wistar rats (initial weight 163 g) were offered 15 g/d of one of four diets, viz: basal (semi-purified diet containing 50 g cellulose/kg as the sole NSP source) and three diets containing 100 g guar gum (GG)/kg at the expense of maize starch. The guar gum was modified to produce three different viscosities namely 30 (low), 600 (medium) and 3700 (high) mPa/sec when measured under standard conditions. After 14 d feeding, CCP was measured by the metaphase arrest, crypt microdissection method (Mathers *et al.* 1993).

Crypt cell production (arrested cells/crypt per 2 h).

Diet. . .	Basal	Low viscosity	Medium viscosity	High viscosity	SEM	*P* value
Small intestine (% distance from pylorus)						
10	34.1	46.6	68.5	90.5	6.3	<0.01
50	28.5	37.4	58.4	69.1	6.1	<0.05
Caecum	15.6	22.2	29.1	34.0	3.2	<0.05
Colon (% distance from caeco-colonic junction)						
10	5.8	8.7	7.8	17.1	2.7	<0.05
90	8.8	9.8	12.3	22.0	1.8	<0.01

As expected, CCP declined from the duodenum to the colon and at all the intestinal sites, GG feeding increased CCP. In addition there was a consistent substantially greater CCP with increasing viscosity. Since GG is readily fermented in the LB, all GG diets were expected to result in similar SCFA production and these results suggest that gut luminal viscosity has a major effect on CCP independent from, and perhaps in addition to, any stimulatory effect of SCFA.

We thank Meyhall, Sonnenwiesentstr, 18,CH-8280 Kreulingen Switzerland for providing the guar-gum samples.

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Guar-gum feeding reduces *Lactobacillus fermentum* adherence to enterocytes. By M.E. LYNN, J.C. MATHERS and D.S. PARKER, *Department of Biological and Nutritional Sciences, University of Newcastle Upon Tyne, Newcastle Upon Tyne NE1 7RU*

Lactobacilli are significant members of the human gut commensal flora. They are believed to promote intestinal health possibly by out-competing pathogenic bacteria or by production of beneficial metabolites. Exclusion of pathogenic bacteria may result from adherence of the lactobacilli to the apical membrane of enterocytes thus sterically preventing access of pathogens to the gut mucosa (Bernet *et al.* 1994). The nature of adherence of lactobacilli has not been determined and the present study was designed to determine whether expression of receptor binding sites was influenced by the maturity of the enterocyte.

Ileal enterocytes were obtained from male Wistar rats fed on a basal diet or diets containing modified guar gums (100 g/kg diet) designed to produce different gut luminal viscosities (Lynn *et al.* 1994). These diets resulted in widely differing states of crypt-cell proliferation (CCP). Within 2 h of harvest, enterocytes were incubated with radiolabelled *L.fermentum* (a strain previously isolated from rats) for 30 min at 37° to quantify binding (Phillips & Parker, 1993).

Diet. . .	Basal	Low viscosity	Medium viscosity	High viscosity	SEM	*P* value
Terminal ileal CCP (arrested cells/crypt per 2 h)	25.6	26.7	43.0	56.0	6.01	<0.05
L. fermentum adherence (bacterial cells/enterocyte)	74.4	66.7	33.7	13.0	2.2	<0.001

CCP in the terminal ileum more than doubled from the basal diet to that containing the highest viscosity guar gum and was accompanied by a marked reduction in binding by lactobacilli. There was a significant ($P < 0.001$) negative linear relationship between the logarithm of diet viscosity and the number of *L. fermentum* adhering ($Y = -21.7x + 93.3$; r^2 0.76).

Since rapid CCP results in reduced maturity of villus enterocytes as illustrated by expression of mucosal hydrolases (Ferguson *et al.* 1980), it seems probable that expression of *Lactobacillus* binding sites is a late event in the differentiation of these cells. If *Lactobacillus* adhesion to the gut mucosa is an initial step in preventing pathogen colonization, then diets which increase CCP may limit their efficacy.

Bernet, M.F., Brassart, D., Nesser, J.R. & Servin, A.L. (1994). *Gut* **35** 483-489.

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Phillips, A. & Parker, D.S. (1993). *Animal Production* **53**, 459-460.

GIP and GLP-1(7-36)amide secretion in response to intraduodenal nutrient infusions in pigs. BY J.M.E. KNAPPER[1], L.M. MORGAN[1], J.M. FLETCHER[2] and V. MARKS[1], [1]*Nutritional Metabolism Research Group, School of Biological Sciences, University of Surrey, Guildford GU2 5XH and* [2]*Biosciences, Nutrition and Safety, URL Vlaardingen, The Netherlands*

Two gut hormones GIP (glucose-dependent insulinotropic polypeptide) and GLP-1(7-36)amide (the active truncated form of glucagon-like peptide-1) have attracted particular interest as components of the entero-insular axis. Both these hormones facilitate glucose-induced insulin secretion and both may have direct insulin-like actions on adipose tissue. GIP is stimulated by glucose and fat ingestion but the nature of the stimuli for GLP-1(7-36)amide secretion have not been determined. Study of the effects of mixed nutrients on gut hormone secretion is made difficult by the differential effects on gastric emptying exerted by different nutrients. We overcame this problem by direct infusion into the duodenum.

Three female pigs of approximately 20 kg were cannulated in the duodenum and catheterized in the jugular vein. Infusions of nutrients were carried out in fasted pigs at a constant rate over a period of 30 min using a peristaltic infusion pump. The infusates consisted of two concentrations of saline (0.85 g and 1.7 g in 100 ml); glucose (20 g and 80 g in 100 ml); fat -soya bean oil emulsion- (30 g and 60 g in 500 ml) and glucose+fat (20 g+30 g and 80 g+60 g respectively in 1000 ml). Two fasting blood samples were collected (-10 and 0 min) and following the infusion blood was sampled at 30, 45, 60, 90,180, 120, 150, 180, 240 and 300 min. Incremental areas under the curve are shown in the Table.

Infusate	Glucose response (mmol/l.min)		Insulin response (ng/ml.min)		GIP response (pmol/l.min)		GLP-1 response (pmol/l.min)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Saline (*n* 3)	-177	41	55	57	-242†	1099	-798	702
Glucose (*n* 4)	235*	299	66	59	15060*	11191	255*	1223
Fat (*n* 4)	-71	136	11	38	2098*†	4303	1443*	1061
Glucose+fat (*n* 5)	225	286	117	178	30373*	21972	1800*	1462

* Significantly different from saline $P<0.05$; † significantly different from glucose+fat $P<0.05$.

There was no effect of nutrient or saline infusate concentration on hormone secretion and data for each macronutrient were therefore pooled. Plasma glucose, GIP and GLP-1(7-36)amide concentrations fell below initial levels following saline infusion, producing negative incremental areas under the curve. Plasma glucose levels were elevated as expected by a glucose or glucose+fat infusion but plasma glucose profiles were not affected by the presence of fat in the infusate. Plasma TAG levels rose following fat and glucose+fat infusions. Mean plasma insulin levels were higher following glucose or glucose+fat infusions than following saline, but differences failed to reach statistical significance due to large individual differences. The greatest stimulus for GIP secretion was glucose+fat. Fat alone was a poor stimulus for GIP secretion but glucose was a potent stimulus. GLP-1(7-36)amide, in contrast, was moderately stimulated by glucose, and more markedly stimulated by fat or glucose+fat. We conclude that in pigs, dual nutrient infusion of glucose+fat is a strong stimulus for both GIP and GLP-1(7-36)amide secretion. Normal dietary intake consists of a mixture of macronutrients in both pigs and man. The potent stimulation of GIP and GLP-1(7-36amide) under these conditions implies a role for these hormones in the postprandial metabolism of both carbohydrate and fat following consumption of mixed meals.

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Role of the renin-angiotensin system in the maintenance of maternal-diet-induced hypertension in the rat. By S.C. LANGLEY-EVANS[1], J.J. MORTON[2] and A.A. JACKSON[1]. [1]*Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX and* [2]*MRC Blood Pressure Unit, Western Infirmary, Glasgow, G11 6NT.*

Recent studies have demonstrated that feeding low-protein diets to rats prior to conception and during pregnancy, induces hypertension in their offspring (Langley & Jackson, 1994). The hypertensive state is characterized by elevated systolic blood pressure, which is first noted at the age of weaning (4 weeks). The high blood pressure, which appears to be a lifelong phenomenon, arises independently of changes in maternal blood pressure during pregnancy, and is associated with lower birthweight (Langley-Evans *et al.* 1994). Preliminary studies with this model of hypertension programmed *in utero* have noted that the hypertensive state is associated with elevated pulmonary angiotensin converting enzyme (ACE) activity. This may suggest a role for the renin-angiotensin system in the development and/or maintenance of maternal-diet-induced hypertension.

Twelve female rats exposed, *in utero*, to either 180g casein/kg (control) or 90g casein/kg (low protein) maternal diets were used in the study. All rats had been reared on standard laboratory chow and differed only in terms of prenatal dietary experience. At 13 weeks of age systolic blood pressure was determined and the animals killed by decapitation. Pulmonary and plasma ACE activities were measured by the method of Hayakiri *et al* (1987). Assessments of plasma renin activity and angiotensin II (AII) concentrations were also obtained.

Maternal diet	Plasma ACE (units/ml)		Lung ACE (units)		Plasma renin (units)		Plasma AII (pg/ml)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	755	64	4.41	0.60	0.93	0.26	46	5
Low-protein	1768*	14	4.61	0.60	0.90	0.33	60	11

* significantly different from control, $P<0.05$. All observations are for n 6 observations. Units for ACE are μmol hippuric acid formed/hr per mg protein. Units for renin are ng AII formed/hr per ml plasma.

Systolic blood pressures were significantly higher in the low-protein group relative to control rats (low-protein 153 (SE 7) mm Hg, control 125 (SE 9) mm Hg, $P<0.05$). Lung ACE activity and plasma renin activity were unaltered by prenatal dietary experience. Plasma ACE activity was 134% higher in the low-protein exposed animals than in controls. Associated with this higher activity, plasma AII concentrations also tended to be elevated in the hypertensive rats.

The results support the hypothesis that altered renin-angiotensin status, and in particular plasma ACE, is involved in the maintenance of maternal-diet-induced hypertension in the rat.

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Hormonal effects of lignans in a group of men. By R. HUGHES[1], A. CASSIDY[2] and S. BINGHAM[2], *[1]Department of Biological and Biomedical Science, University of Ulster, Coleraine, BT52 1SA and [2]Dunn Clinicial Nutrition Centre, Hills Road, Cambridge CB2 2DH*

Some plant foods contain diphenolic compounds which are stereochemically similar to the mammalian oestrogen, oestradiol. These plant oestrogens compete for binding sites at the oestrogen receptor and recently Cassidy *et al.* (1994) showed that one group of plant oestrogens, isoflavones, suppressed the mid-cycle surges of the gonadotrophins and exerted significant effects on the physiological regulation of the menstrual cycle in a group of premenopausal women.

Linseed is a rich source of another group of plant oestrogens, lignans, and the present study examined the biological effects of a daily 40 g supplement of linseed (milled and incorporated into breadrolls) in a group of six elderly men (age 60 to 63) over a 4-week period. Volunteer compliance was monitored by incorporating para-amino benzoic acid (PABA) into the bread rolls and quantifying recovery in urine. Four-day diet diaries were completed each fortnight to determine nutrient intake. Mean non-starch polysaccharide (NSP) intake increased significantly on the linseed diet (control *v.* week 2 $P<0.01$, control *v.* week 4 $P<0.02$), and total dietary fat intake was significantly increased over the same periods ($P<0.05$, $P<0.02$ respectively).

Two 10 ml fasting blood samples and one 24 h urine collection were taken each week throughout the 7 week study period (including 2 weeks post diet). Resulting serum values for luteinizing hormone (LH), follicle stimulating hormone (FSH), cholesterol (Chol), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) are shown in the Table below.

	Control		Diet		Diet 2		Diet 3		Diet 4		Post 1		Post 2	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
LH (mU/ml)	4.9	2.0	3.6	1.6	4.7	2.4	4.6	2.4	4.4	1.9	4.4	2.0	4.6	2.2
FSH (mU/ml)	6.0	4.1	5.6	4.0	5.7	4.0	5.3	4.0	5.1	3.9	5.4	4.6	5.8	4.5
Chol (mmol/l)	7.3	1.0	7.5	0.9	6.6	0.6	7.0	0.8	6.8	0.5	6.8	0.4	6.9	0.4
LDL (mmol/l)	4.8	1.3	5.1	1.1	4.0	1.0	4.4	1.0	3.9	0.9	4.3	0.7	4.3	0.5
HDL (mmol/l)	1.3	0.3	1.4	0.3	1.3	0.3	1.4	0.3	1.6	0.3	1.4	0.3	1.5	0.4

Total cholesterol and LDL levels were significantly reduced during the diet period (F 5.3 $P<0.03$ and F 9.84 $P<0.003$ respectively), due either to the effect of an increase in n-6 fatty acids or NSP with the linseeds, or to increased lignan consumption. There was a trend towards a decrease in LH levels but this was not statistically significant. However, analysis of variance showed that FSH levels were significantly reduced (F 36.8, $P<0.0001$) on the linseed diet. As with our previous study (Cassidy *et al.* 1994) this effect may be due to plant oestrogen suppression of gonadotrophin levels.

Cassidy, A., Bingham, S. & Setchell, K. (1994). *American Journal of Clinicial Nutrition* (In the Press).

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Calibration of a food frequency questionnaire to estimate food intake in relation to artificial sweetener consumption. By P. J. HALFORD[1], H. WARWICK[2] and B. MARGETTS[3], *Institutes of [1]Human Nutrition and [3]Public Health Medicine, University of Southampton, Southampton SO9 3TU and [2]Department of Nutrition and Dietetics, Southampton General Hospital, Southampton SO9 4XY.*

Few studies have calibrated and used a food frequency questionnaire (FFQ) to measure food intake and added substances such as artificial sweeteners. We have developed a FFQ to estimate food intake in relation to artificial sweetener intake in a group of diabetic patients. The relative validity of the FFQ was assessed by comparison with a 7 d weighed food record (WR) in forty-eight diabetic patients aged between 6 and 80 years, resident in the Southampton area. The FFQ listed 107 foods, thirty-seven of which were related to artificial sweetener consumption.

Method	WR		FFQ				
	Mean	SD	Mean	SD	Mean Difference*	SD	Spearman Rank+
Artificial sweetener in tablet form (no. tabs/d)	1.4	2.3	2.2	3.6	0.7	1.9	0.88
Diet Squash (ml/d)	8.8	23.9	25.6	45.2	16.8	38.2	0.61
Diet carbonated drinks (ml/d)	92.8	219.0	89.9	175.8	-2.9	151.0	0.70

*mean daily difference between individuals FFQ - WR, Bland & Altman (1986).
+Rank order correlation coefficient FFQ *v* WR, $P<0.001$ for all results shown.

The three most commonly consumed items containing artificial sweetener were artificial sweetener in tablet form, diet carbonated drinks and diet squash. Table 1 shows that when calibrated against the WR, the FFQ appeared to overestimate the consumption of diet squash and artificial sweetener in tablet form. The Spearman Rank order correlation coefficient was statistically significant for all three food items.

For the consumption of artificial sweetener in tablet form and for diet squash, the difference between methods was plotted against the average of the two methods (Bland & Altman, 1986). For artificial sweetener in tablet form the difference was not consistent across the range of intakes. There appeared to be a trend which suggested that as the intake of artificial sweetener in tablet form increased the mean difference also increased. For diet squash intake the FFQ overestimated consumption across the range of intakes. These results suggest that the FFQ is a useful tool for assessing food intake in relation to artificial sweetener consumption for groups of the population. However, care should be taken when large intakes of artificial sweetener in tablet form are consumed.

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Bland, J.M. & Altman, D.G. (1986). *Lancet* **i**, 307-310.

Dietary fat: grams or percentage of energy? Analysis of the Dietary and Nutritional Survey of British Adults and the Leeds High Fat Study. By J.I. MACDIARMID[1], J.E. CADE[2] and J.E. BLUNDELL[1], [1]Biopsychology Group, Department of Psychology and [2]Nuffield Institute for Health, University of Leeds, Leeds LS2 9JT

In the White Paper 'The Health of the Nation' recommendations were to reduce the average percentage of food energy from total fat by at least 12%, to about 35% of food energy. It is not clear whether it is the percentage of food energy from fat or the total amount of fat consumed which is most important in terms of health and health education.

The Dietary and Nutritional Survey of British Adults (Gregory *et al*, 1990) and the Leeds High Fat Study have been analysed to compare high and low fat consumers. High- and low-fat groups have been classified in two ways. In the first, the high fat consumers ate > 45% of their food energy from fat, and low fat consumers ate ≤ 35% of food energy from fat (FATPCT). In the second, subjects were divided into high- and low-fat groups by the absolute amount of fat in the diet (FATG) to give the same number of subjects as in the groups defined by the percentage energy from fat. The national study included 2197 adults who had completed a 7 d weighed intake. Subjects who were slimming, ill or who had an energy intake: basal metabolic rate ratio of < 1.2 were excluded. Of the subjects in the national study, 11% were in the low-fat group (seventy-six men and fifty-eight women) and 15% were in the high-fat group (ninety-three men and ninety-eight women). Results for men from the national study are shown in the table (similar patterns of intake were seen in the women).

	FATPCT		FATG	
	Low fat (*n* 76)	High fat (*n* 93)	Low fat (*n* 76)	High fat (*n* 93)
Age (years)	38	38	42	35**
Energy (MJ)	10.9	11.4	9.4	14.2**
Fat g	88	129**	76	154**
CHO g	341	250**	251	370**
Protein g	92	93	74	111**

CHO, carbohydrate

** Significantly different from low fat group (*t* test) $P<0.01$

There was no difference in energy intake between the high- and low-fat groups in the FATPCT analysis. However, when total grams of fat were used the high-fat group consumed more energy than the low-fat group. People who eat a large amount of fat seem to be 'big eaters' in general. Similar results were seen in the Leeds High Fat Study. Body mass index was only higher in the high-fat group for the FATG analysis. The distribution of BMI showed more obese subjects in the high-fat compared with low-fat groups; this was more pronounced in the FATG group. There were more smokers and heavy drinkers in the high-fat (FATPCT) group, this was not seen in the FATG analysis. People who are 'big eaters' may have a different set of risks from those who eat a high proportion of fat in the diet.

Gregory, J., Foster, K., Tyler H. & Wiseman M. (1990), The Dietary and Nutritional Survey of British Adults, London : HMSO.

Estimation of food portion size using household measures and food photographs: a comparison. By P.J. ROBSON, M.B.E. LIVINGSTONE and P.G. McKENNA, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

Inaccurate estimation of food portion size is a major issue in retrospective assessment of actual or habitual diet (Guthrie, 1984). Subjects may be helped to quantify foods using household measures or food photographs. Use of household measures is probably still more prevalent but photographs are used increasingly to provide direct visual representation of a wide range of foods. However relatively little work has been carried out to determine which, if either, method is likely to be more accurate.

Twenty-eight adult volunteers (thirteen male, fifteen female) aged 19-33 years were asked to select their usual portions of seventeen foods with different visual characteristics. These were subsequently weighed when the subjects left the room. Twenty-four hours later the subjects were asked to estimate how much of each food they had selected, using both household measures and single portion size colour photographs. The estimates were converted to weights and compared with the amounts weighed on the previous day. Differences were expressed as percentages of the weighed portions.

The percentages of subjects making portion size estimation errors of 0% to ±20%, ±20% to ±50% and greater than ±50% are shown in the Table. At group level more foods were overestimated (*n* 12) than underestimated (*n* 5) using both household measures (HM) and photographs (P).

Error category ...		0% to ±20%		±20% to ±50%		> ±50%	
	n	P	HM	P	HM	P	HM
Orange juice	28	71.5	46.4	10.7	50.0	17.8	3.6
Soup	28	71.4	28.6	25.0	39.3	3.6	32.1
Baked beans	28	67.8	42.8	28.6	53.6	3.6	3.6
Pasta shapes	28	64.3	42.8	32.1	39.3	3.6	17.9
Rice Krispies	28	64.3	28.6	21.4	28.6	14.3	42.8
Cornflakes	28	57.2	25.0	25.0	39.3	17.8	35.7
Apple pie	27	55.5	55.5	33.3	37.0	11.2	7.5
Mashed potato	28	53.5	32.1	35.7	39.3	10.8	28.6
Garden peas	27	51.8	29.6	40.7	29.6	7.5	40.8
Milk on cereal	28	50.0	50.0	42.9	32.1	7.1	17.9
Cheddar cheese (grated)	28	46.4	17.9	32.1	53.6	21.5	28.5
Jelly	28	46.4	39.3	50.0	53.6	3.6	7.1
Bolognese sauce	27	40.7	29.6	40.7	51.9	18.6	18.5
Broccoli	28	35.7	53.5	46.4	42.9	17.9	3.6
Tuna fish (flakes)	26	34.6	42.3	53.8	38.5	11.6	19.2
Margarine on bread	27	33.3	44.4	59.2	18.5	7.5	37.1
Cheddar cheese (sliced)	28	3.6	10.7	21.4	25.0	75.0	64.3

Large errors are likely to be incurred using either of these methods but photographs may offer the better alternative for this population. However portion size estimation in recall methods still remains a major source of measurement error.

Guthrie, H.A. (1984). *Journal of the American Dietetic Association* **84**, 1440-1444.

Collection of food intakes, food composition and food portion sizes in Central Cameroon, West Africa, to develop a food frequency questionnaire in rural and urban population samples, as part of an International comparative study. By S. SHARMA[1], J.C. MBANYA[2], J.E. CADE[1], S. GWANGWA'A[2] and J.K. CRUICKSHANK[1], [1]*Clinical Epidemiology Unit, University of Manchester Medical School, Oxford Road, Manchester M13 9PT and* [2]*Diabetes and Nutrition Research Centre, University of Yaounde, Cameroon*

Collection of reliable nutritional intake data in Cameroon presents a major challenge. Food tables are limited and no data are available on food recipes, weights and portion sizes. The present study aimed to determine recipe information and food and nutrient intake, in order to design a food frequency question (FFQ) for use in Cameroon. These data will be used as part of a larger study to determine the nutritional influences on the emergence of diabetes and hypertension in three international sites (Cameroon, Jamaica and their migrants to the UK).

In Cameroon, one urban and one rural site were selected, both previously censused, the former Cite Verte, part of the capital Yaounde, and rurally Evadoula, 70 km south west of Yaounde, made up of fifteen villages, from which three were randomly selected. Dietary intake was assessed by two methods; 2 d food diaries and 24 h recalls. Recipes were taken for each composite dish from a number of different households and a standard recipe was calculated from the average ingredients. Portion sizes recorded in the diaries and recalls were quantified by weighing duplicate portions, after careful observer training.

Despite frequent logistic and transport difficulties, the diary was completed by sixty urban and sixty-two rural subjects, response rates of 79% and 97% respectively. Recalls were obtained from fifteen people in Cite Verte and eighteen in Evadoula. The nutritional composition of recipes has been calculated for all forty-five recipes collected from selected households using values from the UK food tables (McCance & Widdowson, 1992) or where these were unavailable from the Cameroon food tables (Ngo Som & Abondo, 1989). Food portion sizes have been established for all eighty-five foods recorded.

Food habits differed substantially between the sites. A wider variety of foods were consumed in the urban site due to greater availability. In Evadoula dishes were mainly based on leafy vegetables. The composition of the same dish varied greatly between the sites as shown below for ndole a typical leafy dish.

Ingredients	Evadoula	Cite Verte
Leaves	46%	28%
Groundnuts	20%	22%
Palm Oil	19%	13%
Tomatoes	7%	3.7%
Melon Seeds	-	6%
Dried Fish	5%	-
Onions	2.3%	3%
Herbs	0.7%	-
Meat/Fresh Fish	-	24%
Dried Prawns	-	0.3%
Analysis per 100 g		
Energy	996 kJ	1063 kJ
Protein	6 g	9 g
Fat	23 g	23 g

The recipe in the village contains a much greater percentage of leaves and palm oil, while the urban recipe contains greater quantities of fresh meat and fish and hence more protein per 100 g.

This work has enabled the development of a single seventy-six item FFQ, which has been piloted and amended, and is now in use in both sites.

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Who are the 'low energy reporters' in the Dietary and Nutritional Survey of British Adults? By J. A. PRYER, M. VRIJHEID, R. NICHOLS and P. ELLIOTT, *Department of Public Health and Policy, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

The 7-day weighed dietary intake method has traditionally been viewed as a "gold standard" among dietary assessment methods. Until recently, most studies using this method have made the tacit assumption that either a valid measure of "habitual" diet has been obtained, or that bias, if present, operates equally across all study participants and thus within-study comparisons remain valid. However, validation studies of the weighed intake method have indicated that under-reporting bias is evident, and that it is more prevalent and severe at the lower end of the reported energy intake distribution (Livingstone *et al.* 1990). In population based dietary surveys, little is known about whether (and how many) people change their diets when surveyed, or under-report habitual intake, whether change or under-reporting is food and nutrient neutral, or whether there is a higher degree of change or under-reporting for specific foods and nutrients. The aim of this study was to identify the characteristics of men and women participants in the Dietary and Nutritional Survey of British Adults (DNSBA; Gregory *et al.* 1990), who stated that they were neither slimming nor ill, and who reported an average energy intake over a 7-d period below 1.2 times estimated basal metabolic rate (termed here "low energy reporters", LER); an intake considered incompatible with long-term energy balance (WHO, 1985). BMR was estimated using predictive equations based upon age, sex and body weight (WHO, 1985).

In the DNSBA, the LER population comprised 344 (39.1%) women and 264 (26.9%) men, and the non-LER population 529 women and 719 men. Compared with non-LER, LER had significantly ($P<0.05$) higher mean ratios of urinary urea nitrogen to dietary nitrogen, and urinary potassium to dietary potassium, indicating that as a group, LER were under-reporting at least for protein and potassium intakes. Compared with non-LER, LER were significantly over-represented among smokers ($P<0.05$), self-reported alcohol non-drinkers ($P<0.001$), among men, the manual social classes ($P<0.01$) and among women, those receiving state benefits ($P<0.01$). LER were also significantly heavier than non-LER ($P<0.001$). Men LER in the non-manual social classes reported significantly lower intakes compared to non-LER ($P<0.05$) of 11 of 28 food/drink groups, including a high proportion of foods with a negative "health image", whereas manual social class men LER reported significantly lower ($P<0.05$) intakes of 21 of 28 food/drink groups compared with manual non-LER. There were also differences ($P<0.05$) between LER and non-LER in both macronutrient and micronutrient expressed in terms of energy density.

These results lend support to the view that LER may not be randomly distributed within a population, but may be over-represented within specific sub-groups, and that under-reporting bias may not be food and nutrient neutral. If this is correct, this has implications for the design, analysis and interpretation of dietary surveys.

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Who complies with dietary recommendations for the prevention of CHD and what do they eat? Findings from the Dietary and Nutritional Survey of British Adults. By J.A. PRYER[1], E. BRUNNER[2], P. ELLIOTT[1], R. NICHOLS[1], H. DIMOND[3] and M. MARMOT[2]. *[1] Department of Public Health and Policy, London School of Hygiene and Tropical Medicine WC1E 7HT, [2] Department of Epidemiology and Public Health, University College, London WC1E 6EA, and [3] Centre for Human Nutrition, Sheffield University S5 7AU.*

The Committee on Medical Aspects of Food Policy (COMA) recommended, almost a decade ago, reductions in total dietary fat to yield between 31-35% food energy, and in saturated and trans fat to 15% food energy; it also recommended an increase in the P/S ratio to 0.23-0.45, depending upon total fat intake (DHSS, 1984). The Dietary and Nutritional Survey of British Adults (DNSBA; Gregory *et al.* 1990), was undertaken 2-3 years after the publication of the COMA 1984 recommendations. Only 5.2% men and 7.4% women, termed here 'compliers', appeared to meet all three COMA 1984 recommendations, reducing to 3.8% men and 4.7% women when 'low energy reporters' (LER) were excluded (ie. reported energy intake <1.2 estimated basal metabolic rate). With the exclusion of LER, compared to 'non-compliers', a significantly higher proportion of men 'compliers' were from the non-manual social classes ($P<0.05$) from the Northern, Central, Southwest and Welsh regions ($P<0.05$) and among women were Black/Ethnic ($P<0.001$). Compared with 'non-compliers', fewer men 'compliers' smoked ($P<0.05$), were 'heavy drinkers' ($\geq$300ml/week; $P<0.05$) or used salt at the table ($P<0.001$) and more took food supplements ($P<0.01$). 'Compliers' substituted low-fat dairy products for full-fat dairy products, low-fat and polyunsaturated fat spreads for butter, poultry for meats and meat products, reported higher intakes of high fibre cereals, and among men a lower beer/cider consumption and higher pudding consumption ($P<0.05$ to $P<0.001$). Compared with 'non-compliers', the diets of 'compliers' had a significantly higher density of carbohydrate (men 'compliers' (C): 50.8% (SE 1.0); 'non-compliers' (NC): 41.6% (SE 0.23) $P<0.001$), starch (men C: 78.0g/1,000 kcal (SE 2.73); NC: 62.3g/1,000 kcal (SE 0.46) $P<0.001$), sugar (men C: 56.7g/1,000 kcal (SE 2.99); NC: 47.9g/1,000 kcal (SE 0.53) $P<0.01$), and fibre (men C: 14.3g/1,000 kcal (SE 1.12); NC: 9.95g/1,000 kcal (SE 0.10) $P<0.001$), and among men a lower alcohol density (men C: 3.82% (SE 1.07), NC 7.4% (SE 0.30) $P<0.01$), and higher protein density (men C: 15.3% (SE 0.46); NC 13.5% (SE 0.08) $P<0.001$). The Health of the Nation (DH, 1992) targets include a reduction in total and saturated fat to 35% and 11% of food energy respectively. National Food Survey data indicate little change in the total fat density of the national diet since 1986/7 (MAFF, 1992). These results indicate the extent and direction of the shift required in the composition of the British diet if national dietary targets for the reduction of coronary heart disease are to be achieved.

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The effect of meals of varying fat and carbohydrate content on an electrophysiological measurement of sleepiness. By A. S. WELLS[1], N. W. READ[1] J. JONES[2] and C. IDZIKOWSKI[3], *[1] Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Herries Road, Sheffield S5 7AU, [2] NCE Brain Waves, PO Box 84, WF4 2XD and [3] Department of Therapeutics and Pharmacology, Queen's University, Belfast BT9 7BL*

Meals have frequently been reported to cause increased feelings of lethargy and inferior performance in tasks requiring sustained attention (Smith *et al.* 1988). Recently we observed that following ingestion of a high-fat mid-morning meal, subjects felt significantly more dreamy, feeble and fatigued than after a lower fat, high-carbohydrate (CHO) meal (Wells & Read, 1994). As accidents and errors are more likely to occur during periods of drowsiness, we sought to quantify these subjective feelings. In the Multiple Sleep Latency Test, the sleep latency (the time between a person trying to go to sleep and the point when electroencephalogical patterns of sleep first develop) is recorded five times throughout the day at two-hourly intervals. It is a direct measure of the functional consequences of sleepiness, namely falling asleep, and has been fully validated to be effective in detecting different degrees of sleepiness (Thorpy, 1992).

The aim of the present study was to investigate the effect on sleep latency of two isoenergetic meals of similar appearance and protein content but differing fat and CHO content eaten on two days 28 d apart. Eight male and eight female subjects were randomly divided into four groups (A to D) each consisting of two males and two females, and on each day subjects ate both the high-fat (fat : CHO energy ratio 54:41) and low-fat (fat : CHO energy 7:88) test meals in a balanced crossover design. Groups A and B fasted before the first meal and groups C and D ate similar low fat breakfasts at 08.00 hours. Test meals were eaten by group A at 09.00 hours and 13.00 hours, by group B at 10.00 hours and 14.00 hours, group C at 11.00 hours and 15.00 hours and group D at 12.00 hours and 16.00 hours. Sleep latency tests were conducted every 2 h starting at 08.00 hours for groups A and C and 09.00 hours for groups B and D.

Analysis of scores 30 min before and 1.5 h after each meal revealed a significant difference in sleep latency over time ($P<0.05$). Sleep latency decreased after both the morning and the afternoon meals (Fig), and after the morning meal this was highly significant ($P<0.000$). Meal composition, gender and time of ingestion did not significantly affect the results.

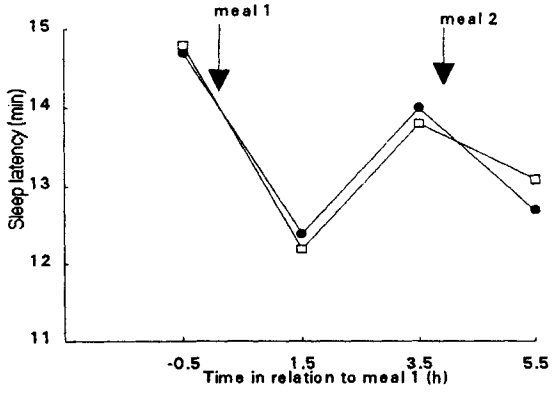

Fig. Sleep latency before and after
meal 1 high fat, meal 2 high CHO.
meal 1 high CHO, meal 2 high fat.

In conclusion, subjects were significantly sleepier after both meals than before eating, but this electrophysiological method of measuring sleepiness did not reveal any differences in postprandial sleepiness between high-fat and lower fat meals.

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Mothers' perceptions of food for their children. By M. M. RAATS, R. SHEPHERD and P. SPARKS, *Institute of Food Research, Earley Gate, Whiteknights Road, Reading RG6 2EF*

The amounts of additives found in foods are of concern to the public. Certain additives have been publicized in a negative light, some particularly in relation to children. As mothers have influence on the food their children eat through purchase and preparation, mothers' concerns may have an important influence on children's consumption of food.

One hundred and seventy-two mothers of children aged 5-11 years completed a questionnaire on their perceptions of four groups of foods, two concerning additives (food with artificial sweeteners and food with synthetic colourings) and two concerning nutritional issues (high-fat food and high-sugar food). The questionnaire was designed according to the theory of planned behaviour (e.g. Ajzen, 1988, 1991) and a number of its proposed extensions (i.e. moral obligation and negative affect), along with the influence of children's preference. Moral obligation is measured as the sum of the responses to three questions (e.g. "It would be wrong..."; "I would feel guilty..."; "It goes against my principles..." and negative affect is also measured as the sum of the responses to three questions (e.g. "How worried would you feel ..."; "How concerned would you feel..."; "How much regret would you feel...").

The groups of foods studied were perceived differently by the mothers (see Table 1). Levels of worry and concern (negative affect) were significantly higher for the nutritional issues. Children's preferences were very important in predicting intention for all four groups of foods, moral obligation was significant for three groups of foods and, for high-fat food, negative affect was also significant (see Table 2). These results would argue for the extension of the theory of planned behaviour to include moral obligation.

Table 1. *Mean scores and standard errors for intention (to present children with), attitude, subjective norm, children's preference, perceived control, moral obligation and negative affect*

	Food with artificial sweeteners		Food with synthetic colourings		High-fat food		High-sugar food	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Intention (range 1 to 7)	3.5^{a}	0.1	3.2^{b}	0.1	2.9^{c}	0.1	2.8^{c}	0.1
Attitude (range -12 to +12)	-2.6^{a}	0.3	-4.0^{b}	0.3	-6.1^{c}	0.3	-6.3^{c}	0.2
Subjective norm (range 2 to 14)	6.8^{a}	0.2	6.3^{b}	0.1	5.8^{c}	0.2	5.4^{d}	0.2
Children's preference (range 1 to 7)	4.0	0.1	3.8	0.1	3.7	0.1	4.1	0.1
Perceived control (range 2 to 14)	8.7^{a}	0.2	8.1^{b}	0.2	9.6^{c}	0.2	9.4^{c}	0.2
Moral obligation (range 3 to 21)	13.5^{a}	0.3	14.2^{b}	0.3	15.7^{c}	0.3	16.3^{d}	0.2
Negative affect (range 3 to 21)	10.0^{a}	0.4	11.0^{b}	0.3	13.2^{c}	0.3	14.2^{d}	0.3

^{abc}Values with same superscript in same row do not differ from each other ($P<0.05$; paired t test).

Table 2. *Multiple regressions of intentions to present children with different foods (each column refers to a separate multiple regression for that particular group of foods), on attitude, subjective norm, children's preference, perceived control, moral obligation and negative affect (significance levels refer to final β-coefficients)*

	Food with artificial sweeteners	Food with synthetic colourings	High-fat food	High-sugar food
Attitude	***	NS	*	*
Subjective norm	NS	*	NS	NS
Children's preference	***	***	***	***
Perceived control	**	**	*	**
Moral obligation	NS	***	*	***
Negative affect	NS	NS	*	NS

*$P<0.05$; ** = $P<0.01$; ***$P<0.001$; NS, not significant.

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Acute effects of breakfasts of differing fat and carbohydrate content on morning mood and cognitive performance. By H. M. LLOYD and P. J. ROGERS, *Institute of Food Research, Whiteknights Road, Earley Gate, Reading, RG6 2EF.*

There has been considerable interest in recent years regarding dietary influences on mood and cognitive performance, including the effects of meal size, timing, and macronutrient composition. Most studies on meal composition have concentrated on the effects of high carbohydrate *v.* high protein content, although recently it has been reported that the fat : carbohydrate ratio of a meal can have significant effects on postprandial mood and performance (Lloyd *et al.* 1992, 1994; Wells & Read, 1994). Comparing the effects of three levels of fat and carbohydrate in isoenergetic meals eaten at lunchtime, it was found that mood and performance were optimal following the medium-fat, medium-carbohydrate meal, which was closest in macronutrient content to the subjects' usual choice at lunch.

The aim of the present study was to investigate the effects on morning mood and cognitive performance of breakfasts differing in fat and carbohydrate content, and to compare this manipulation with the effects of missing breakfast. Sixteen young healthy subjects (mean age 26.1 years) consumed low-fat/high-carbohydrate (LFHC: 27% energy from fat, 62% energy from carbohydrate), medium-fat/medium-carbohydrate (MFMC: 44%, 47%), high-fat/low-carbohydrate (HFLC: 56%, 34%) isoenergetic breakfasts (2.52 MJ (603 kcal)), and no breakfast (NB) in a counterbalanced order on four separate days.The LFHC breakfast was similar in macronutrient composition to the habitual breakfast intake of the subjects. Subjects did not detect any differences in sensory qualities between the three test breakfasts which consisted of bread rolls and a milkshake. A battery of cognitive performance tasks together with mood and appetite ratings (made on a 10 cm line scale) were carried out before and during the 2.5 h following breakfast.

The Table shows the mood ratings (expressed as change from baseline ratings) made 30 minutes after breakfast. At this time subjects felt less muddled, dejected and drowsy, and more energetic after the LFHC breakfast compared with the other meals and also NB. A similar pattern of results was evident at 90 min after breakfast, while the effects on drowsiness and dejectedness were apparent as early as 15 min after finishing the meals. Results for the measures of cognitive performance showed no substantial differences, although there was a tendency for simple reaction time and immediate memory to be better following the LFHC breakfast. Missing breakfast did not produce any effects different from those seen following MFMC or HFLC breakfasts.

	LFHC		MFMC		HFLC		NB	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Drowsy	–1.10a	0.40	–0.25b	0.48	–0.36b	0.37	–0.13b	0.37
Muddled	–0.65a	0.54	0.02b	0.30	0.23b	0.20	0.04b	0.21)
Dejected	–0.52a	0.31	0.08b	0.07	–0.24b	0.14	–0.11b	0.25
Energetic	0.75a	0.42	–0.54b	0.61	0.53a	0.41	–0.15	0.43

a,b means within a row denoted by different letters were significantly different ($P<0.05$).

These findings show that the macronutrient content of breakfast, independent of differences in energy value and oro-sensory qualities, can exert subtle but possibly important effects on subsequent mood. Furthermore, this outcome, in line with previous results, indicates that deviation from subjects' usual meal composition can produce a relative decline in mood state. This in turn suggests a link between the mood effects of food and food choice, whereby choices producing positive (desired) effects are reinforced and consequently are more likely to be repeated on future occasions.

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Does short-term food deprivation affect cognitive function? By M. W. GREEN, N. A. ELLIMAN and P. J. ROGERS. Institute of Food Research, Whiteknights Road, Earley Gate, Reading, RG6 2EF

In recent years, there has been increasing interest in the effects of food deprivation on the efficiency of cognitive function. This work has ranged from investigations of the effects of missing meals (e.g. Smith & Miles, 1986), to the effects of spontaneous dieting behaviour (e.g. Green *et al.* 1994) and chronic food restriction found in eating disorders (e.g. Hamsher *et al.* 1981). Although it is a replicable finding that spontaneous dieting is associated with a range of task impairments consistent with an attentional deficit, the effects of individual meals on cognitive function are obscured by interactions between circadian variation, type of cognitive measure and practice or fatigue effects. The present study investigated the hypothesis that the impairments in cognitive function found among dieters is a function of food restriction, by assessing the effects of mild food deprivation on a task battery previously found to reveal differences in performance between dieters and non dieters.

The cognitive function of twenty-one, healthy, female subjects aged 18-25 years was assessed on five separate occasions, during test sessions 1 week apart. After completing the first, practice session, each subject was tested after four levels of food deprivation (order counterbalanced across subjects). These comprised 24 h food deprivation, missing two meals (either evening meal and breakfast, or breakfast and lunch), missing one meal (either breakfast or lunch), and a control condition which involved subjects eating normally during the week before to testing. Measurements of hunger, heart rate, affective state and personality were also made.

	24 h deprived		Miss 2 meals		Miss 1 meal		Non-deprived	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Simple reaction time (ms)	355	43	353	37	353	37	358	43
Immediate memory (words recalled)	9.2	2.9	9.8	2.4	9.5	2.1	8.7	2.8
Tapping rate (taps/s)	7.4	1.6	7.8	1.8	7.7	1.9	7.8	2.0
Eriksen effect*	16.5	36.6	14.4	37.5	21.7	40.5	18.5	40.2
Vigilance task (% correct hits)	57.7	16.2	61.4	17.8	45.3	13.4	58.4	19.4
Heart rate (pulses/min)	70.9	4.2	71.5	4.8	71.1	3.3	75.1	3.7

* see Eriksen & Eriksen (1974).

There were no significant effects ($P > 0.05$, Analysis of Variance) of the degree of food restriction on performance of any of the tasks (Table), and cognitive performance did not covary significantly with the self-report measures of affective state or personality. Heart rate, however, was significantly higher when the subjects were tested in the non-deprived state, $F(3,57) = 6.80$, $P < 0.001$. These results show that, contrary to what has often been assumed, missing one or even more meals in a single day does not substantially impair cognitive function. Furthermore, they support the suggestion that the impairments observed in dieting are due to eating-related psychological variables, rather than nutritional variables or differences in affective state (Green *et al.* 1994).

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Established behaviour influences public perception of nutrition education messages. By A. WISE, L. FARMER and A. MCLEISH, *The Robert Gordon University, Queen's Road, Aberdeen AB9 2PG*

McRobbie *et al.* (1993) wrote eight messages about foods in eight different styles and asked people to rank them for 'persuasiveness'. They identified differences in rankings between the styles and between the messages themselves, but did not show what aspects of the messages caused people to respond differently to them. In the present study, the same sixty-four messages were presented to people at the same location, a shopping-centre cafe in Aberdeen. There were eight questionnaires, each with eight messages about different foods; by using equal numbers of questionnaires, all eight styles were represented. Messages written as commands with negative ideas followed by positive ones were:

1. Avoid thin cut chips and use thick cut types instead. Thin cut chips absorb a lot of fat when fried.
2. Avoid white bread and eat wholemeal instead. White bread is low in fibre.
3. Don't use canned soups; make your own instead. This can help lower the amount of salt in your diet.
4. Don't use standard soft drinks, try 'diet' drinks instead. This will help lower the amount of sugar you consume.
5. Avoid whole milk and use semi-skimmed instead. Whole milk contains a lot of fat.
6. Avoid sweet breakfast cereals and eat high bran types instead. Sweet cereals tend to be low in fibre.
7. Don't use butter, try polyunsaturated spreads instead. This will help reduce the saturates in your diet.
8. Avoid adding sugar to tea and coffee and try an artificial sweetner instead. This way you'll reduce your calorie intake.

At the top of the questionnaire were defined the words 'reasonable' as 'the idea behind the message is logical and consistent with current knowledge', 'practical' as 'is possible to include in current lifestyles', and 'compelling' as 'makes one want to change this eating habit'. Below each message were placed the three words with a scale from low to high (1-7) and the subjects (196 males and 204 females) were asked to circle a number and whether they already behaved as indicated by the message (do/don't).

		Reasonable		Practical		Compelling	
	%do	do	don't	do	don't	do	don't
chips	26	6.49	5.04	6.19	3.70	6.29	4.99
bread	71	6.27	5.47	6.27	4.39	5.36	3.82
soup	22	6.09	4.35	5.70	2.85	5.36	3.31
drink	55	6.42	5.18	6.28	4.80	5.87	4.16
milk	74	6.30	4.97	6.34	4.93	5.70	3.93
cereal	62	6.37	5.44	6.19	4.05	5.68	3.81
spread	71	6.43	5.39	6.28	4.63	5.72	4.15
sweetner	46	6.45	5.60	6.43	5.41	6.10	4.40

Three-way ANOVA using mean values as input showed that all factors affected the scores (do/don't $F_{1,14}$ 495; type of rating $F_{2,14}$ 48.4; but least for food $F_{7,14}$ 10.4; all at $P<0.001$) and there was an interaction ($F_{2,14}$ 8.6; $P=0.004$) between the scores given for the type of rating depending on do/don't. The scores were 23% less for people who did not perform the message (don't) for 'reasonable', 30% less for 'practical' and 29% less for 'compelling'. Scores for 'reasonable' were greater (5.73) than for 'practical' (5.21) and 'compelling' (4.92). Scores for the three types of rating were correlated (Spearman's) between 0.62 and 0.71. Stepwise analysis suggested that the scores for 'compelling' were predicted best from 'reasonable' (r^2 34.0%), though r^2 increased to 40.6% by adding 'practical'. 'Practical' scores best predicted whether subjects already performed the message (r^2 31.6%) and 'reasonable' increased r^2 only to 32.9%. Social class only significantly predicted behaviour for messages about bread ($P=0.004$) and cereal ($P<0.001$); for both messages the proportion who claimed to 'do' the message fell with social class from I to V (bread 87% to 59% and cereal 89% to 41%). Sex predicted behaviour for all messages, except for sweetner, with females more frequently claiming to 'do' the message. Older people were more likely to 'do' the message for chips, soup and cereal ($P<0.001$). It was concluded that established behaviour influenced perception of reasonableness, practicality and the compellingness associated with nutrition education messages.

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McRobbie, L., Wise, A. & McLeish, A. (1993). *Proceedings of the Nutrition Society* **52**, 357A.

Type of message construction affects public perception. By L. FARMER, A. WISE and A. MCLEISH, *The Robert Gordon University, Queen's Road, Aberdeen AB9 2PG*

McRobbie *et al.* (1993) wrote eight messages about foods in eight different styles and asked people to rank them for 'persuasiveness'. They identified differences in rankings between the styles and between the messages themselves, but did not show what aspects of the messages caused people to respond differently to them. In the present study, the same sixty-four messages were presented to people at the same location, a shopping-centre cafe in Aberdeen. There were eight questionnaires, each with eight messages about different foods; by using equal numbers of questionnaires, all eight styles were represented. The messages about the eight foods have been given earlier (Wise *et al.* 1994). Messages about chips have been chosen to illustrate how different types of construction have been incorporated:

1. Use thick cut chips. They don't absorb a great deal of fat when fried.
2. Avoid thin cut chips. They absorb a lot of fat when fried.
3. You could use thick cut chips. They don't absorb a great deal of fat when fried.
4. It's sensible to avoid thin cut chips. They absorb a lot of fat when fried.
5. Use thick cut chips and avoid thin cut types. Thin cut chips absorb a lot of fat when fried.
6. Avoid thin cut chips and use thick cut types instead. Thin cut chips absorb a lot of fat when fried.
7. It's sensible to use thick cut chips instead of thin cut. Thin cut chips absorb more fat when fried.
8. It's sensible to avoid thin cut chips and use thick cut instead. Thin chips absorb a lot of fat when fried.

At the top of the questionnaire were defined the words 'reasonable' as 'the idea behind the message is logical and consistent with current knowledge', 'practical' as 'is possible to include in current lifestyles', and 'compelling' as 'makes one want to change this eating habit'. Below each message were placed the three words with a scale from low to high (1-7) and the subjects (196 males and 204 females) were asked to circle a number and whether they already behaved as indicated by the message (do/don't). Mean scores for each type of message construction about each food were calculated for all those who do or don't perform the message and subjected to three-way ANOVA (*n* variable depending on the numbers who claim to perform each message). Scores are tabulated as the means for the eight foods.

		Reasonable	Practical	Compelling
1. Positive command		5.61	5.27	4.67
2. Negative command		5.43	4.89	4.49
3. Positive suggestion		5.45	4.95	4.06
4. Negative suggestion		5.44	4.87	4.37
5. Positive then negative command		5.91	5.41	5.36
6. Negative then positive command		6.11	5.51	5.67
7. Positive then negative suggestion		6.06	5.69	5.55
8. Negative then positive suggestion		6.14	5.60	5.60
Foods	$F_{7,49}$	7.77***	24.13***	11.80***
Types	$F_{7,49}$	8.22***	5.35***	20.54***
Do/don't	$F_{1,49}$	290.55***	582.17***	390.36***
Foods X types	$F_{49,49}$	2.23**	1.92*	2.44***
Foods X do/don't	$F_{7,49}$	2.93*	8.61***	1.02
Types X do/don't	$F_{7,49}$	1.21	1.42	0.78

Significance: * $P<0.005$, ** $P<0.01$, *** $P<0.001$ (ANOVA)

All factors affected the scores and there were also significant interactions. Message types containing positive and negative aspects were favoured over those with single aspects (shown by separate ANOVA at $P<0.001$). Differences in score between foods and for people who already perform the message (do) compared with those who don't have been reported elsewhere (Wise *et al.* 1994). Practicality was more influenced by the actual message (foods) than the type, the reasonableness was almost equally influenced by the message type and the food, but the score for compellingness was more influenced by the way in which the message was written.

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Interpretation of abnormal biochemical indicators of nutritional status during malaria infection. By B.S. DAS[1], D.I. THURNHAM[2] and D.B. DAS[1], [1]*Ispat General Hospital, Rourkela-769002, Orissa, India and* [2]*Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

Blood biochemical indices as sensitive indicators of nutritional status are frequently used to assess nutritional status before the deficiency becomes clinically evident. However nutritional status may not be the sole determinant of blood nutrient levels (Thurnham & Singkamani, 1991). To determine the effect of malaria on some representative nutritional indices, we measured albumin, cholesterol, transferrin, retinol, tocopherol, Fe and haemoglobin in children with severe malaria, mild malaria and normal healthy controls (*n* 40; male 18, female 22 in each group) and the results are shown in the Table.

Biochemical index	Severe malaria (*n* 40)		Mild malaria (*n* 40)		Control (*n* 40)		ANOVA*
	Mean	SD	Mean	SD	Mean	SD	*P*<
Age (years)	7.69	2.81	7.61	2.59	7.71	2.93	NS
% Ideal Weight	74.49	8.41	78.16	8.21	77.04	8.27	NS
Haemoglobin (g/l)	71.5	18.3	95.7	22.2	124.3	17.3	0.001
Albumin (g/l)	32.5	7.26	37.4	7.48	45.3	3.84	0.001
Transferrin (g/l)	2.01	0.84	2.51	0.91	3.10	0.69	0.001
Iron (μmol/l)	13.97	6.27	14.68	5.87	12.89	5.17	NS
Cholesterol (mmol/l)	1.91	0.61	2.50	0.62	3.52	0.52	0.001
Retinol (μmol/l)	0.47	0.24	0.75	0.39	1.15	0.55	0.001
Tocopherol (μmol/l)	8.29	3.78	11.70	4.73	17.59	6.68	0.001
Caeruloplasmin (IU/l)	310.92	67.63	273.42	56.92	196.50	43.78	0.000

NS, not significant.
* Intergroup differences for all groups is significant (Newman Keul test $P<0.05$) for relevant variables.

All measurements in malaria patients, even in mild malaria, were significantly lower than those in the controls. The factors responsible for the differences are not fully understood although all the indices may be affected by the infection. The fall in haemoglobin is partly explained by erythrocyte lysis at schizogony. Albumin and transferrin are negative acute-phase proteins and reflect disease severity (Koj, 1985). In spite of the fall in transferrin, Fe levels are maintained in the diseased patients. Low cholesterol and tocopherol levels may be partly explained by increased capillary permeability and extravasation of lipoproteins (Nilsson-Ehle & Nilsson-Ehle, 1990). Retinol too may be low as a consequence of the acute-phase response and the inhibition of retinol-binding-protein synthesis (Thurnham & Singkamani, 1991). As the acute- phase response is common to all diseases and plays a significant role in the aetiology of the above changes, the above results indicate that biochemical indices of nutrition should be interpreted with caution when there is concurrent disease. This may be particularly important in malaria-endemic areas where subclinical disease is a common occurrence.

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The effects of heat exposure on sodium balance at three levels of dietary sodium intake. By A.J.ALLSOPP[1], S.A.WOOTTON[2] and R.M.SUTHERLAND[2], [1]*Institute of Naval Medicine, Gosport PO12 2RD and* [2]*Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX*

Dietary restriction of sodium is known to initiate increased aldosterone secretion which promotes reabsorption of Na by the kidneys and the sweat glands (Collins, 1963), essential to the recovery of Na balance in the heat acclimatization process. It has been suggested previously that restriction of dietary Na intake before heat exposure may augment the recovery of Na balance (Taylor *et al.* 1943; Collins & Weiner, 1968). In the present study the effects of heat exposure on Na balance were investigated in unacclimatized men consuming either a low (LNa), moderate (MNa) or high (HNa) Na intake.

Unacclimatized men were confined to an environmental chamber at a temperature of 25° C, 40% relative humidity) for a period of 3 d (control) and then a further 5 d in the heat (40° C, 40% relative humidity) during 08.00-18.00 hours daily. Subjects consumed the LNa, MNa or HNa diet (<2 g, 4 g and 8 g Na respectively) for the entire 8 d. Dietary energy requirements were estimated as 1.4 x basal metabolic rate (Schofield, 1985) allowing for 1 h of light activity each day. Urinary Na concentrations form 24 h collections and sweat concentrations from 12 h washdown periods (daytime and overnight at the start and end of heat phase) were analyzed by ICP spectrophotometry and the amounts of Na lost via these routes was estimated.

	Day 3 (control)			Day 4 (1st hot day)			Day 8 (last hot day)		
Na mg/d	Sweat	Urine	Total	Sweat	Urine	Total	Sweat	Urine	Total
LNa (*n* 9)									
Mean	225	1678	1902	1342	618	1960	737	750	1487
sem	36	143	142	199	110	200	62	158	118
MNa (*n* 9)									
Mean	352	3374	3727	1643	1528	3171	1078	2132	3210
sem	40	166	156	189	128	154	109	257	215
HNa (*n* 7)									
Mean	670	6580	7250	2135	4717	6852	1525	5822	7347
sem	135	273	256	267	467	356	127	497	450

Analyses of the differences between paired means confirmed that, irrespective of diet, Na sweat losses rose significantly on day 4 ($P<0.01$) then fell significantly by day 8 ($P<0.01$) presumably as a result of heat adaptation. The decline in sweat Na excretion over days 4-8 were similar in all three dietary conditions. Renal Na losses in all three conditions decreased on day 4 ($P<0.01$) with no significant changes thereafter. Total Na excretion on day 8 was significantly lower compared to control for the low Na condition ($P<0.05$), but not for the moderate of high groups. No effect of low sodium intake upon the rate of Na recovery was apparent from these preliminary results.

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The relationship between dietary iodine intake during pregnancy and birth outcome.

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Iodine is an essential element for normal growth and development in animals and man. Some evidence shows an effect of I deficiency on fetal growth and development (Hetzel,1989). However, the relationship between the level of dietary I intake during pregnancy and birth outcome has not been determined. The objective of the present study was to investigate whether there is a positive relationship between I intake during pregnancy and birth outcome.

The study was done at Chiang Mai province in Thailand where 361 Thai pregnant women were recruited for the study. Women were recruited before or at 24 weeks of gestation and followed up throughout pregnancy up to delivery. Casual urine samples were collected at each clinic visit (maximum of five collections). Samples were collected in the morning (between 09.00 and 12.00 hours) and analysed for I and creatinine using modifications of standard techniques (Henry, 1964; Wayne *et al.* 1964). The CV for the estimate of I excretion and creatinine excretion were 4.49 % and 3.57 % respectively. Urinary I excretion (μg/g creatinine) was used to estimate I intake. Birth outcome included birth weight (BWT), head circumference (HC), length, and chest circumference (CC) of the infants measured by the standard methods. The ratio between HC and length (RHL, as a percentage of length) was calculated. The other factors which may affect birth outcome were also recorded (socio-economic status, food behaviour, maternal status, and gestational age at birth).

Overall there was no significant correlation between urinary I excretion and birth outcome. There was, however, an inverse relationship between I excretion during the gestational period of >10-27 weeks and the ratio of head circumference to length (RHL) as shown in the Table.

Urinary iodine excretion (I/Cr; log form)	*n*	RHL Mean	RHL SD	95% Confidence intervals
≤ 1.730	99	68.0[a]	3.1	67.4-68.6
1.731-1.980	96	66.9[b]	2.9	66.4-67.5
≥ 1.981	95	67.1[b]	3.2	66.5-67.8

Cr, creatinine a,b Significant by different, $P<0.05$.

These results support the hypothesis that there may be a time when the availability of I intake critically influences fetal development.

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Cation pumps in skeletal muscle undergo dramatic up-regulation in the perinatal period.

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The fetal and early postnatal periods are critical stages during which the long-term development of the individual can be affected. However, despite the marked changes in muscle function which occur at birth, knowledge of perinatal muscle development at the cellular level is limited. Two cation pumps play a key role in muscle function: the sarcolemma Na^+,K^+-ATPase (*EC* 3.6.1.37; Na^+,K^+-pump), which is essential for the maintenance of excitability in muscle, and the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (*EC* 3.6.1.38; Ca^{2+}-pump), which in conjunction with the SR Ca^{2+}-pool, determines the rates of contraction and relaxation. The aim of the present study was to determine whether the neonatal period is characterized by particularly dynamic changes in the concentration of cation pumps in skeletal muscle.

Six litters each of four piglets were killed humanely at 0, 2, 5 or 14 d after birth, for sampling of *longissimus dorsi*. This long back muscle has important locomotor and thermogenic functions, and during the first 2 weeks of life comprises approximately 5-10% slow-twitch and 80-100% oxidative fibres. The concentration of Na^+,K^+-pumps was determined in muscle slices by the vanadate-facilitated ouabain-binding technique, and Ca^{2+}-pump concentration was measured in crude muscle homogenates using Ca^{2+}-dependent steady state phosphorylation from ^{32}P-ATP. Measurements of myofibre cross-sectional area were made to enable results for the Na^+,K^+-pump to be related to both muscle weight (pmol ouabain/g) and sarcolemma area (pump sites/μm^2).

Postnatal age (d) ...	0		2		5		14	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body weight (kg)	1.4	0.1	1.6	0.1	2.1*	0.1	4.3***	0.3
Myofibre size (μm^2)	98	12	149*	16	173	7	320***	16
Na^+,K^+-ATPase (pmol/g)	384	20	574***	26	577	35	471*	21
Na^+,K^+-ATPase (pump sites/μm^2)	884	56	1423**	132	1481	113	1618	118
Ca^{2+}-ATPase (nmol/g)	1.7	0.2	3.3*	0.4	6.0***	0.4	10.2***	0.4

Significantly different from previous age (Student's paired *t* test); * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Results indicate that the concentration of the Na^+,K^+-pump, which was already substantial at birth, showed a dramatic up-regulation during the first 2 d postnatally, with no subsequent increase. By contrast, the Ca^{2+}-pump concentration, which was low at birth, increased 4-fold during the first 2 weeks postnatally. Regulating factors involved in the development of cation pumps probably include the marked changes in endocrine status (Berthon *et al.* 1993; Silver & Fowden, 1989) and motor activity which occur during the perinatal period. Taken together with findings on cation pump concentrations in 2-month-old pigs (Harrison *et al.* 1994), it is concluded that (1) during the life-time of the individual, the greatest increase in myofibre cation pump concentration is likely to occur during late gestation and early postnatal development, and (2) during the perinatal period, muscle development will be particularly susceptible to modification by nutritional, environmental and endocrine status.

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Zinc-reversible candidastatic activity of leucocyte protein, calprotectin. By P. A. CLOHESSY[1], M.K. FAGERHOL[2], P.J. HELMS[1], H. MACKENZIE[3] and B.E. GOLDEN, [1]Department of Child Health, University of Aberdeen, Aberdeen AB9 2ZD, [2]Department of Immunology, Ulleval Hospital, 0407 Oslo, Norway and [3]Department of Medical Microbiology, University of Aberdeen, Aberdeen AB9 2ZD

Zinc is essential for all living cells. Yeasts and fungi generally require more than bacteria but most micro-organisms do not seem to produce Zn retrieval agents analogous to Fe siderophores (Sugarman, 1983). Consequently many pathogens may be more susceptible to Zn, than to Fe deprivation.

Calprotectin is a Ca^{2+}-binding protein expressed in neutrophils and monocytes, comprising up to 60% of neutrophil cytosolic protein (Fagerhol *et al.* 1990). It is released into biological fluids during neutrophil turnover and is found at particularly high concentrations in abscess fluid. Calprotectin has been shown to have growth inhibitory properties *in vitro*, fungi showing greater sensitivity than bacteria (Steinbakk *et al.* 1990). It has been reported that this fungistatic effect is Zn-reversible. However, although calprotectin can bind Zn *in vitro*, similar to other proteins in the S-100 family, the exact mechanism of calprotectin's fungistatic activity has not been determined (Sohnle *et al.* 1991).

We investigated the potential of Zn to reverse the fungistatic activity of purified calprotectin in *C. albicans* cultures obtained from a clinical isolate, and whether this activity was mediated by alterations to the culture medium (i.e. Sabarauds broth). *C. albicans* growth was assessed after 24 hr growth at 37° using total viable counts (TVC).

Calprotectin at concentrations above 1 μg/ml significantly inhibited the growth of *C. albicans*. Optimal growth inhibitory activity was achieved when calprotectin was incubated with Sabarauds broth (Sab) at 37° for 24 h before inoculation. When this calprotectin was removed by ultrafiltration, just before inoculation, growth of C. albicans was still inhibited. The growth inhibitory activity of calprotectin was completely reversed by adding 10 μM-$ZnSO_4$ to Sab

	Sab (control)	Sab + calprotectin	Sab + calprotectin 24 h incub	Sab + calprotectin ultrafiltered	Sab + $ZnSO_4$ + calprotectin
Mean (n=3) increase TVC ($\log_{10}$)/100 μl	5.5	4.2*	2.7**	4.6*	5.3

Significantly different than control: *$P<0.05$; **$P<0.001$.

Calprotectin has potent candidastatic activity, at concentrations found in many biological fluids during an acute-phase response. Zn completely reversed this activity, which appeared to be mediated by alterations to the culture medium, suggesting Zn chelation as calprotectin's mechanism of action.

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The effect of supplementation with groundnut (*Arachis hypogaea*) and/or milk on the viscosity and energy density of maize porridges as weaning foods in developing countries. By J. K. KIKAFUNDA[1] and A. F. WALKER[2], [1,2]*Department of Food Science & Technology, The University of Reading, Whiteknights, Reading RG6 2AP and* [1]*Department of Food Science & Technology, Makerere University, Box 7062, Kampala, Uganda*

The energy density of traditional starch-based weaning foods used in many developing countries, is a major constraint in ensuring adequate energy and nutrient intake for infants and young children. Cereal porridges, particularly maize, millet, sorghum and rice are among the most common traditional weaning foods in developing countries. When heated with water, the starch of cereal flours gelatinizes and becomes viscous at low concentrations. The resulting porridge has high dietary bulk and low energy density. As a consequence, large quantities of the porridge, much more than the capacity of the child's stomach, would be needed in order to satisfy the child's daily energy and nutrient requirements. This problem has been linked to the aetiology of protein-energy malnutrition (PEM) (Ljungqvist *et al.*, 1981).

Supplementation of the basic cereal flour with energy- and nutrient-rich foods is one of the practical methods recommended to overcome the dietary bulk problem of cereal porridges (Moussa *et al.*, 1992). However, previous work has concentrated on single nutrient foods such as oil and sugar which, in addition to being too expensive for most rural families in developing countries, can jeopardize the intake of other nutrients especially protein and the micro nutrients. In addition, other factors that can interact with the added ingredients to affect the viscosity and energy density of the porridges have not been fully investigated. The purpose of this Study was therefore, to investigate the effect of adding easily accessible energy- and nutrient-rich foods (groundnut and milk) on the viscosity and energy density of maize porridges under different influencing factors.

In the present study, the traditional method of porridge preparation used in many developing countries was adapted to laboratory conditions using the method of Walker and Pavitt (1989). Maize flour of two particle sizes (coarse and fine) at two concentrations (6% and 8%) were supplemented with 250 g groundnut paste /kg and/or 100 ml fresh cow's milk /kg. The porridges were cooked for 5 or 10 min and the viscosity measured using a Haake RV 3 viscometer at a temperature of 30^{o} or 40^{o} using a shear speed of 256 or 512 rpm and a shearing time of 20 or 60 s. The experiment was designed as a 2^{8} fractional factorial and carried out using the 1/4 replicate method.

The addition of groundnut paste and fresh milk, separately or together, significantly ($P \leq 0.001$) reduced the viscosity of the maize porridges. Overall, groundnut alone reduced the viscosity of the maize porridges by 61.3%, milk alone by 29.2% and groundnut plus milk by 69.8%. The influence of the addition of groundnut on the viscosity of the maize porridges was significantly ($P \leq 0.001$) influenced by concentration and shear speed with groundnut reducing the viscosity more at higher concentrations and at lower shear speeds. The addition of groundnut significantly interacted with cooking time ($P \leq 0.025$) and flour particle size ($P \leq 0.05$) with groundnut reducing the viscosity of the porridges more at higher cooking times and with fine particle-size flour. The influence of milk on the viscosity was significantly influenced by concentration ($P \leq 0.001$) and flour particle size ($P \leq 0.05$).

The energy density of the porridges was increased by the addition of maize and/or groundnut. Averaging the two concentrations, the energy density of the maize porridges was increased by 17.0, 27.0 and 44.5% by the addition of groundnut alone, milk alone and groundnut plus milk respectively.

In conclusion, the results of the present study show that adding groundnut and/or milk reduced the viscosity and increased the energy density of maize porridges. As energy deficit has been found to be the most significant factor in the aetiology of PEM, these results have important implications in the prevention and treatment of PEM in children in developing countries.

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Nutritional composition (by chemical analysis) of sweet home-prepared weaning foods for infants. 2. Sugars. By ALISON M. REDFERN, JANE B. MORGAN and B. J. STORDY, *School of Biological Sciences, University of Surrey, Guildford. GU2 5XH.*

We have previously reported on the nutritional content by chemical analysis of 108 savoury weaning food samples and the macronutrient content of sweet weaning foods (Morgan *et al.* 1993; Stordy *et al.* 1994). Here we report on the results of the sugars analysis of the sweet samples.

A study of weaning of 1004 infants in England was conducted between June and October 1992. A sub-sample of infants was identified as receiving a home-prepared meal at least once daily. Each mother or carer collected a duplicate sample (approximately 150 g) of the home-prepared sweet foods. The food samples were analysed for total sugars, sucrose, glucose, fructose and lactose (by National Measurement Accreditation Service accredited methodology) at Campden Food and Drink Association (Chipping Campden, Glos). Maltose was analysed by enzymatic reaction monitored by U.V absorption. We present the results of our analyses for sugars of 96 sweet food samples classified according to the age of the infant. The total sugars content is compared with the mean total sugars content of ready-to-use baby desserts and puddings from the range of two major manufacturers in the Table.

Age (months)	4-6		7-9		10-12		Manufactured infant foods	
n	28		45		23		63	
Nutrient (g/kg)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total sugars	89	47	89	47	77	54	102	28
Sucrose	15	22	24	32	18	31	*	
Glucose	23	20	19	19	14	18	*	
Fructose	39	29	27	25	21	22	*	
Lactose	7	11	14	16	21	15	*	
Maltose	6	15	4	10	3	4	*	

* Information not available from the manufacturers.

These data reveal that the mean sugar content of home-prepared meals is at least 12% lower than that of manufactured infant foods, and this lower sugar content is reflected in the low energy density previously reported (Stordy *et al.* 1993). The variation in total sugar content of the sweet home-prepared samples was far greater than that of the manufactured desserts. In some cases the sweet foods were presented as the main meal to the infant. The fructose content of the meals for the 4-6 month age group was higher than that of the other age groups, reflecting the high proportion of fruit in the meals. There is concern that fructose may not be absorbed well by young infants and may lead to diarrhoea and reduced energy availability (Hoekstra *et al.* 1993). In cases of persistent infantile diarrhoea particularly when associated with slow growth it may be worthwhile enquiring into the quantity of fruit and fruit juices given.

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Nutritional composition (by chemical analysis) of sweet home-prepared weaning foods for infants. 3. Sodium, zinc, iron, iodine. By B.J. STORDY, ALISON M. REDFERN, and JANE B. MORGAN. *School of Biological Sciences, University of Surrey, Guildford. GU2 5XH.*

We have previously reported on the nutritional content by chemical analysis of 108 savoury weaning food samples and the macronutrient content of sweet weaning foods (Morgan *et al.* 1993, Stordy *et al.* 1994). Here we report on the results of the minerals analysis of the sweet samples.

A study of weaning of 1004 infants in England was conducted between June and October 1992. A sub-sample of infants was identified as receiving a home-prepared meal at least once daily. Each mother or carer collected a duplicate sample into trace element free containers (approximately 150g) of the home-prepared sweet foods. The sweet food samples were analysed (by National Measurement Accreditation Service accredited methodology) for Na, Zn and Fe at Campden Food and Drink Association (Chipping Campden, Glos). I was determined by catalytic colorimetric determination. We present the results of our analyses for Na, Zn, Fe and I of 96 sweet food samples classified according to the age of the infant. The mineral content is compared with the mean mineral content of ready-to-use baby desserts from the range of two major manufacturers in the Table.

Age (months)	4-6		7-9		10-12		Manufactured infant foods	
n	28		45		23		63	
Nutrient (g/kg)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sodium	290	400	490	550	500	460	150	140
Zinc	3	3	4	3	4	4	1	1
Iron	6	9	4	5	4	3	3	1
Iodine	86	77	167	290	173	171	57*	76

* Compositional data for manufactured infant foods was incomplete for this nutrient.

These data reveal that the mean Na content of sweet home-prepared baby food is higher than that of sweet manufactured baby food in each age group. The mean Fe and Zn content at each age group was higher in the home-prepared foods than in manufactured baby food. For all nutrients the variation was far greater in the home-prepared samples than in the manufactured desserts. I content was determined because of the detrimental effect of long-term poor I status which may be compounded by exposure to the day-to-day industrial pollutants such as disulphides used in fumigation and present in some drugs and phenyl derivatives present in plastics and insulating materials. There is little published compositional data on chemically analysed I content of foods and food tables are incomplete (Holland *et al.* 1991).

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The contribution of breakfast to daily micronutrient intakes of adults in Great Britain. By H. McNULTY, J. EATON - EVANS, G. WOULAHAN and J.J. STRAIN, *Human Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

Breakfast is generally considered to be a nutritionally important meal but this has been examined by relatively few studies. In the current study the contribution of breakfast to daily micronutrient intakes was examined in adults aged 16-64 years (1085 males, 1108 females) by analysing the data collected in the Dietary and Nutritional Survey of British Adults conducted in 1986 and 1987 (Gregory et al. 1990).

Subjects (excluding shiftworkers, 9% of sample) were classified into consumers or skippers on the basis of their predominant breakfast pattern (four or more occasions during the 7 d recording period between 04.30 and 08.59 hours on weekdays and between 04.30 and 11.44 hours on weekends). Breakfast skippers were defined as those who consumed <4% of the reference nutrient intake (RNI) for protein (2.2 g males; 1.8 g females). Consumers of breakfast were further divided into those who included a breakfast cereal during the above time periods, and those who did not (Other breakfast). Means and standard deviations are given in the Table. Data were transformed as appropriate before analysis (one-way analysis of variance).

	Breakfast cereal		Other breakfast		Breakfast skipper		*P* Value
	Mean	SD	Mean	SD	Mean	SD	
Males							
n	257		382		318		
Body Weight (kg)	74.5^{b}	9.6	77.1^{a}	11.7	74.9^{b}	12.7	<0.007
Height (m)	1.76^{a}	0.07	1.74^{b}	0.07	1.74^{b}	0.07	<0.029
Energy (MJ/d)	10.80^{a}	2.17	10.44^{a}	2.37	9.61^{b}	2.63	<0.001
Thiamin (mg/d)	1.09^{a}	4.12	0.92^{b}	7.58	0.38^{c}	0.51	<0.001
Riboflavin (mg/d)	1.06^{a}	1.72	0.73^{b}	4.51	0.35^{c}	0.6	<0.001
Niacin (mg/d)	47.5^{a}	19.7	41.6^{b}	11.6	38.3^{c}	13.1	<0.001
Vitamin B_6 (mg/d)	0.95^{a}	1.75	0.72^{b}	4.31	0.37^{c}	1.34	<0.001
Folate (µg/d)	319^{a}	94	318^{a}	94	299^{b}	123	<0.001
Vitamin B_{12} (µg/d)	5.5	4.7	6.3	6.3	5.4	5.6	0.089
Vitamin C (mg/d)	88^{a}	77	81^{b}	99	64^{c}	70	<0.001
Vitamin D (µg/d)	4.3^{a}	3.31	3.61^{b}	3.25	2.74^{c}	2.87	<0.001
Iron (mg/d)	16.3^{a}	6.7	13^{b}	4.6	11.3^{c}	4	<0.001
Calcium (mg/d)	1077^{a}	284	959^{b}	280	805^{c}	297	<0.001
Females							
n	223		451		375		
Body Weight (kg)	62.4^{b}	10.6	65.6^{a}	13.7	63.2^{b}	13.2	<0.003
Height (m)	1.63^{a}	0.06	1.62^{ab}	0.06	1.61^{b}	0.07	<0.025
Energy (MJ/d)	7.71^{a}	1.57	7.22^{b}	1.82	6.39^{c}	1.82	<0.001
Thiamin (mg/d)	1.54^{a}	10.14	0.63^{b}	2.46	0.35^{b}	0.56	<0.001
Riboflavin (mg/d)	1.2^{a}	2.23	0.66^{b}	2.64	0.39^{b}	0.72	<0.001
Niacin (mg/d)	38.3^{a}	41.4	30.9^{b}	11	27^{c}	9.4	<0.001
Vitamin B_6 (mg/d)	1.64^{a}	6.82	1.71^{b}	9.45	1.53^{c}	9.57	<0.001
Folate (µg/d)	247^{a}	73	219^{b}	75	192^{c}	68	<0.001
Vitamin B_{12} (µg/d)	4.6^{a}	4.7	4.4^{a}	4.6	4.1^{b}	4.7	0.005
Vitamin C (mg/d)	83^{a}	69	78^{b}	104	62^{c}	96	<0.001
Vitamin D (µg/d)	3.37^{a}	2.93	3.13^{b}	3.2	2.26^{c}	2.46	<0.001
Iron (mg/d)	15.3^{a}	16	10.5^{b}	8	9.7^{c}	8.6	<0.001
Calcium (mg/d)	878^{a}	244	762^{b}	246	601^{c}	240	<0.001

Values with different superscripts within a row are significantly different ($P < 0.05$; Least Significant Difference test).

The results indicate that breakfast makes a significant contribution to the intakes of a number of micronutrients which are not compensated for in subsequent meals if breakfast is missed. Furthermore if breakfast includes a fortified cereal there are additional benefits for most micronutrients.

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A comparison of two techniques to find out whether 5 to 8-year-olds in deprived schools in Southampton skip breakfast. BY JACQUELINE LANDMAN[1] * and VAL BOX[2], [1]*Department of Human Nutrition* and [2]*Health Education Unit, University of Southampton, Southampton SO9 3TU.*

The cognitive function of young deprived hungry children may be at risk (Simeon & Grantham-McGregor, 1990). Therefore, parents' worries that such children skipped breakfast were taken seriously. As a basis for planning nutrition health promotion, we decided to find out how many children missed breakfast and what they ate in the morning hours. In this study, breakfast included any food or drink consumed in the morning before leaving home. In order to obtain a sample large enough to detect the prevalence of skipping breakfast, the sample had to include at least 750 children from several schools. Seven of the 11 eligible schools which served central inner city and other low income areas agreed to participate. They had a mean uptake of free school meals of 35.5 % (5.6 % SEM) indicating the presence of social deprivation.

We conducted the survey using 2 kinds of short interview with 5 to 8-year-olds (*n* 935), individually or, if 5 years old, in a small group of two or three, between January and April 1993. Interviews were conducted at the schools before noon, 61.9% before the mid-morning break. The structured interviews included a questionnaire with closed questions, pre-coded answers to which were based on pre-tests a school which did not participate in the survey. In the interviews, children were asked to tell interviewers, in 'secret' the answer to questions such as "What did you eat before you left home this morning?". Responses to questions about feeding during break enabled answers to be cross-checked by direct observations and also provided data useful for programme planning. Further questions about feeding on the way to school and in the playground contributed to a narrative which did not focus exclusively on breakfast, and which we reasoned would reduce the chance of rehearsed answers.

We compared structured interviews with an illuminative 'Draw and Write' technique developed by Wetton (1978) to investigate children's concepts of health. Each child drew cartoons about all activities that morning. Each child was then asked to tell the interviewer in 'secret' about his or her drawing. In these semi-structured interviews particular attention was paid to feeding before leaving home and at school on that morning. The responses were recorded in the same way as the structured interviews.

To obtain some information about the quality of both kinds of interviews, each interviewer rated each child's fluency and interview rapport on a three point scale. They also recorded whether responses were volunteered or prompted, and the response to a question about ice-cream used as a distracter. Similar results were obtained when a subset of interviews were repeated after 2 months The 'Draw and Write' method gave more volunteered responses than direct questioning.

Excluding interviews with bad rapport and poor fluency, there were 844 (90.3%) interviews of good quality. Of these, 4.1% children reported skipping breakfast on the day of interview (95% confidence interval 2.8 - 5.4%). Similar prevalences of skipping breakfast were found using direct questioning (3.2%) and "Draw and Write" (5.6%) with semi-structured interviews. The frequency of skipping breakfast is in keeping with previous reports in this age group (e.g. Bender *et al.* 1977). The most commonly reported breakfast was cereal (67%) usually with milk (61%), followed by bread (33%). Of the 69% who said they had drinks, tea (27%) and milk (22%) or juice (22%) were most common; 7.8% reported only one or two items such as tea and/or biscuits which we judged to comprise 'poor' breakfasts. About half the children (48%) reported snacks, usually fruit or crisps at school.

The results suggest that interviewing 5 to 8-year-olds may be a reliable method to investigate nutrition health education in this age group, which now requires validation.

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Few differences in mean daily intake associated with a high intake of snacks in school children.

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There is evidence that snack foods make a substantial contribution to energy intake in children (Magarey *et al.* 1987; Adamson *et al.* 1992) but few studies have addressed whether snacking has an adverse effect on energy intakes, macronutrient balance and micronutrient intakes in children.

Dietary data on 136 Scottish 7 to 8-year-olds were collected in 1990/91 using a 7 d weighed inventory. Details on the methodology and the social class distribution of the sample are reported elsewhere (Ruxton *et al.* 1993). Data were analysed using COMP-EAT 4 and SPSS for Windows.

Snacks, defined as a food or drink taken outwith recognized meal times, contributed the following percentages of mean daily intakes: 26% energy, 14% protein, 29% fat, 26% carbohydrate, 25% starch, 28% sugars, 28% non-starch polysaccharide (NSP), 10% retinol equivalent (eq), 10% thiamin, 11% riboflavin, 14% pyridoxine, 6% cyanocobalamin, 15% folate, 21% ascorbic acid, 13% cholecalciferol, 15% Ca and 21% Fe. The most popular snack, taken by 92% of the sample, was potato crisps. Children were arbitrarily classified as high snackers (n 76) if snacks contributed >25% of the daily energy intake, while children were classified as low snackers (n 60) if snacks contributed ≤25% of the daily energy intake. No significant differences in mean daily energy, absolute macronutrient intakes and percentage energies from fat, carbohydrate, starch and sugars were seen between the two groups using a two-tailed Student's t test. However, the high snacking group had lower intakes of retinol eq ($P<0.05$), thiamin ($P<0.05$) and Se ($P<0.05$). An additional finding was that there were no significant differences in height, weight or body mass index between the two groups.

To determine whether differences in dietary intakes from meals existed between the two snacking groups, mean daily energy and nutrient intake from snacks was subtracted from total mean daily intake. Using a two-tailed Student's t test, the high-snacking group was found to have lower mean daily intakes of energy ($P<0.001$), protein ($P<0.005$), starch ($P<0.001$), carbohydrate ($P<0.001$), fat ($P<0.001$), NSP ($P<0.001$), retinol eq ($P<0.01$), thiamin ($P<0.001$), riboflavin ($P<0.05$), pyridoxine ($P<0.005$), cholecalciferol ($P<0.01$), folate ($P<0.005$), nicotinic acid eq ($P<0.005$) and Ca ($P<0.005$) from meals compared with the low-snacking group.

The results suggest that, in this sample, children with a mean intake of energy from snacks in excess of 25% total energy had overall diets which were not greatly different from those of children whose mean intake of energy from snacks was ≤25% total energy. This is partly attributed to the finding that the high snackers had significantly lower energy and nutrient intakes at mealtimes. Even when the data were analysed to focus on more extreme snacking groups, i.e. ≥35% energy from snacks (n 18) vs. ≤15% energy from snacks (n 12), the same conclusions were reached. Further research is needed to investigate whether this finding applies to other groups of children.

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Determination of energy intake in 7-10 year old children: use of a food record and interview technique. By P.J.MOYNIHAN, T.J.BUTLER and A.J.RUGG-GUNN, The Dental School, University of Newcastle upon Tyne, NE2 4BW.

Data on the intake of energy and nutrients of pre-adolescent children is sparse and existing data are over 10 years old (Cook et al. 1973; Durnin, 1984). One reason for the lack of data is the potential difficulty in obtaining information from this age group, who are gaining independence in food choice yet may be unable to record their own food intake unassisted. The current study investigated the suitability of a food record and interview method, which has successfully been used with adolescents (Hackett et al. 1983), for assessing the intake of energy in the 7 to 10-year-old age group.

Twenty-five children of both genders completed a 3 d food diary on two occasions. Child and parent were instructed on how to complete the diary during a home visit. Using household measures to estimate amounts, a record was kept of all food and drink consumed over a 3 d period in a purpose-designed diary. On the fourth day the parent and child were interviewed to clarify the information recorded. Intake of energy was derived using food tables (Holland et al. 1991) and purpose-written programmes.

Forty children were invited to participate, twenty-seven volunteered and twenty-five completed all aspects of the study. Records were made by both the child and the parent in nine cases, just the parent in eleven cases and just the child in five cases. Thirty minutes was required for each home visit. Energy intakes and the proportion of energy derived from different nutrients are tabulated below.

		% Energy from nutrients		
	Energy intake (MJ)	Carbohydrate	Fat	Protein
	Mean SD	Mean SD	Mean SD	Mean SD
Boys (*n* 13)	9.61 0.79	49.5 3.6	38.6 2.8	12.7 1.9
Girls(*n* 12)	7.92 1.43	48.8 6.1	38.5 5.4	13.4 3.3

Boys had a higher energy intake compared with girls (P=0.0013, student's t test). The mean energy intake of girls was identical to the Estimated Average Requirement (EAR), that obtained for boys was slightly above the EAR of 8.24 MJ. No significant difference in body weight between boys and girls was observed, mean weights were 31.0Kg (SD 6.6) and 33.7Kg (SD 9.4) for girls and boys respectively. No differences in the proportion of energy from nutrients were observed between genders. Intakes of fat were above the dietary reference value of 35% energy.

The intake of energy and nutrients of the 7--10-year-old age group may be assessed using the 3 d food record and interview method, however, further validation studies are required.

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The influence of social privilege on food and nutrient intakes in adolescent females. By J. CADOGAN[1], C. HYLAND[1], N. JONES[2] and M. BARKER[1], [1]*Centre for Human Nutrition and* [2]*Medical Care Research Unit, University of Sheffield, Sheffield S5 7AU*

The aim of the present study was to investigate the impact of social privilege on the dietary intake of a group of female adolescents. The study sample comprised eighty-two 11-12-year-old Caucasian girls, recruited from four schools chosen to give a cross-section of British social classes. The schools ranged from a fee-paying private school to a comprehensive school in a deprived area of Sheffield. None of the girls were following any special dietary regimens. Diet was assessed by the 7 d weighed inventory method using digital scales (Soehnle). Seventy-six dietary records were analysed using computerized food tables (FOODBASE, Institute of Brain Chemistry and Human Nutrition, London). Social class was assessed using the Registrar General's Standard Occupational Classifications (Office of Population Censuses and Surveys, 1990) based on the occupation of the head of household. For subsequent analysis, social classes were categorized into the broader classifications of 'manual' (M; *n* 35) and 'non-manual' (NM; *n* 41) social groupings. Height and weight were measured with the subject wearing light clothing, without shoes.

There were no significant differences in height and weight between the social groups. Mean intakes of all nutrients were calculated, and differences between the two groups were assessed using *t* tests. The only nutrients to show a significant ($P<0.05$) difference between the social groups were vitamin C and sugar intakes (higher in the NM group). To investigate differences in food intakes, analysis was carried out on twenty-five separate food groups. For the three food groups displaying a bimodal intake distribution (ice cream, fruit juice, and pasta/rice), chi-squared tests were carried out using a 'none' or 'some' intake classification. Ice cream and pasta/rice intakes were significantly ($P<0.05$) higher in the NM group (X^2 4.88 and 5.08 respectively, df 1). For all remaining food groups, Mann-Whitney tests were performed to test for social differences. The NM group had greater intakes of cakes/biscuits/puddings ($P<0.05$), fresh fruit ($P<0.01$) and white meat ($P<0.01$). Conversely, the M group had a higher intake of chips ($P<0.001$). Finally, using those food intake variables that had shown significant differences, logistic regression analysis was performed to determine the best predictors of social group. The best single predictor of social group was chips, followed by fruit then white meat. The ability of the logistic model to predict social group correctly increased from 62% for chips alone, to 66% when intakes of chips, fruit and white meat were combined. Additionally, when cakes/biscuits/puddings, pasta/rice and ice cream were added to the model, the predictive value increased further to 74%, despite these latter variables not achieving statistical significance in the model.

In conclusion, social advantage is associated with differentials in food intake and it is possible to predict social grouping from food intake information. However, most nutrient intakes were homogenous with respect to social group. These findings reflect the social gradients in food intakes found in studies of British adults (Barker *et al.* 1990), suggesting that food intake patterns are established early in life. These findings have implications for nutrition education programmes designed to catalyse dietary change.

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The relationship between urinary iodine excretion in 24 h urine collection and casual urine samples in Thai pregnant women. By S. PRUENGLAMPOO[1], P. LEELAPAT[2], T. VONGCHAK[2], V. LIKIT-EKARAJ[2], S. WORANUJ[2] and T. KUMRIN[2], *[1]Institute of Human Nutrition, University of Southampton, Southampton SO9 3TU and [2]Research Institute for Health Sciences, Chiang Mai University, Thailand*

Thailand not only has a high infant mortality rate (World Population Prospect ,1993) but it also has a high prevalence of low birth weight in infants, and of goitre in schoolchildren. Dietary I intake may be a risk factor for these health problems. Twenty-four hour urinary I excretion is an index commonly used for assessing the levels of I intake. However, it is not practical for large field studies. Thus most studies have used urinary I excretion in casual urine samples (μg I/g creatinine) to estimate the 24 h urinary I excretion and then evaluate the level of I intake. The studies undertaken to date have all been conducted on young, healthy, non-pregnant women.

In the present study, the correlation between I excretions in 24 h urine collection (μg I/d) and casual urine samples (μg I/g creatinine) and the correlation between I levels in 24 h urine collection (μg I/d) and total dietary I intake (μg I/d) were investigated in ten well-educated Thai pregnant women at a range of gestational ages (8-33 weeks). Casual urine samples were collected from subjects separately for each voiding from 06.00 hours of the study day until 06.00 hours on the following day. These urine samples were analysed for I and creatinine by using the modification methods of standard techniques (Henry, 1964; Wayne *et al.* 1964). The CV for estimate of I excretion and creatinine excretion were 4.49 % and 3.57 % respectively. The 24 h urinary I and creatinine excretions were calculated by adding those values from all urine samples together. The quantity of food intake of each subject was also recorded on the day of urine collection. These foods were collected and analysed for I contents using the modification method of Moxon & Dixon (1980). The total dietary I intake (μg I/d) of each subject was then calculated.

It was found that there was a positive significant relationship between total dietary I intake (μg I/d) and 24 h urinary I excretion (r 0.8123, $P<0.05$). The positive significant correlation between the urinary I excretion in casual urine sample (μg/g creatinine) of first voiding (>06.00-12.00 hours) and the 24 h urinary I excretion was found (r 0.679, $P<0.05$). There were also positive significant correlations between the 24 h urinary I excretion (μg I/d) and the mean of urinary I excretion (μg I/g creatinine) in casual urine samples during the periods >06.00-12.00 hours, >12.00-18.00 hours, >18.00-24.00 hours, and >00.00-6.00 hours at r 0.795, 0.860, 0.969 and 0.757 ($P<0.05$) respectively.

These results suggest that during pregnancy urinary I excretion in casual urine samples can be used for estimating the level of 24 h urinary I excretion.

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Vitamin B_{12} deficiency in rural Mexicans. By L.H. ALLEN[1], J.E. CASTERLINE[1], J.L. ROSADO[2] and P. LOPEZ[2], *[1]Department of Nutrition, University of California, Davis, CA 95616, USA and [2]Instituto Nacional de la Nutricion, Mexico, DF*

While vitamin B_{12} deficiency has been assumed to be rare in developing countries, older assays substantially overestimated the plasma concentration of biologically active cobalamins. During the Nutrition Collaborative Research Support Program (CRSP) in rural highland Mexico, 170 km north west of Mexico City, we found deficient (< 103 pmol/l) or low (103-148 pmol/l) plasma vitamin B_{12} concentrations in 75% of preschoolchildren (aged 18-30 months, *n* 28), 47% of schoolchildren (aged 7-9 years, *n* 49) 38% of non-pregnant, non-lactating women (*n* 21), 62% of pregnant women (*n* 42), 30% of lactating women (*n* 55) and 41% of men (*n* 22; Allen *et al.* 1992). Because these high prevalences were likely to be caused by malabsorption of the vitamin, rather than low intake alone, in a subsequent study on preschoolchildren in the same region we included measurements of plasma holotranscobalamin II (holo TC II). Holo TC II is believed to be an indicator of recent absorption of vitamin B_{12} (Herbert *et al.* 1990).

In the present study, 219 children from five communities were enrolled between the ages of 18 and 36 months. Blood samples were collected at baseline, and after 6 and 12 months. Vitamin B_{12} in plasma was measured by radioassay (Magic B-12 ^{57}Co/Fol radioassay kit, Ciba-Corning, Medfield, MA, USA) with the holo TC II separated by binding holo TC I and holo TC III on microfine glass. The percentage prevalence of deficient and low values of plasma B_{12} was 8 and 33 at baseline, 3 and 22 at 6 months and 7 and 28 after 12 months. Low holo TC II concentrations occurred in 38%, 17% and 19% at 0, 6 and 12 months respectively. Among the three time periods, plasma B_{12} concentrations in the same children were consistent (r 0.58-0.73 for the five communities; $P<0.001$) but holo TC II concentrations were more variable (r 0.20-0.41; $P<0.05$). There was substantial variability among communities in both plasma B_{12} and holo TC II, which could not be attributed to dietary differences. For example, one community was consistently worse off than the others (50-60% with low or deficient B_{12} concentrations in the three periods) while in another 40% of children had low values at baseline but none were low in the subsequent two periods. Overall, between baseline and 6 months there was substantial improvement in both B12 and holo TC II concentrations, which accompanied a similar spontaneous improvement in anaemia and plasma ferritin. These data are consistent with our hypothesis that intestinal malabsorption is causing vitamin B_{12} deficiency in these communities.

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Calcium requirements of lactating women: effect of a calcium supplement on the breast-milk calcium concentration of rural Gambian women. By A. PRENTICE[1], L. M. A. JARJOU[1], B. DIBBA[1], Y. SAWO[1], T. J. COLE[1], D. M. STIRLING[1] and S. FAIRWEATHER-TAIT[2], *[1]MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ, and Keneba, The Gambia, and [2]BBSRC Institute of Food Research, Colney Lane, Norwich NR4 7UA*

Calcium intakes of lactating women in rural areas of The Gambia, West Africa, average 300-400 mg/d (Jarjou *et al.* 1993) and are considerably below recommended levels. The possibility that such low Ca intake during prolonged lactation may result in low breast-milk Ca concentrations has been investigated by a randomized, double-blind, placebo-controlled supplementation study.

Sixty Gambian mothers from the rural villages of Keneba and Manduar were randomized to receive a Ca supplement or placebo from 10 d postpartum for the first 12 months of lactation. The supplement consisted of two tablets of orange-flavoured, chewable $CaCO_3$ (each containing 500 mg Ca; Calcichew, Shire Pharmaceuticals); the placebo was two dextrose tablets of similar taste and texture (Dextrosol, CPC). Tablets were delivered to the subjects in the early evening, between meals, 5 d/week, and consumed in front of a member of the research team. Days missed by subjects were made up at weekends, and overall compliance was 100%. By the end of the study, each subject in the Ca group had received an extra 714 mg Ca/d averaged over the year, increasing their intake threefold (mean dietary Ca intake measured at 3 months was 293 (SD 119) mg/d with no significant differences between groups (Jarjou *et al.* 1993)).

Breast-milk samples (1-2 ml) were collected at 6, 13, 19, 26, 39, 52, 65 and 78 weeks postpartum. Calcium concentrations were measured in whole-milk samples, after ashing and acid digestion, using a spectrophotometric method validated against atomic absorption spectroscopy (Laskey *et al.* 1991). Running precision and accuracy controls were included with each batch of assays.

Mean breast-milk Ca concentrations at the various sampling times are given in the Table for supplemented and placebo groups separately. Analysis of variance demonstrated that there were highly significant effects of stage of lactation and of the individual (nested by group; $P<0.0001$). The Ca supplement had no effect on breast-milk Ca concentration after adjustment for stage of lactation or on the pattern of change over time ($P>0.5$).

		Breast-milk Ca concentration (mg/l)							
Weeks of lactation...		6	13	19	26	35	52	65	78
Supplemented group	Mean	224	215	199	188	178	158	159	149
	SE	5	5	4	3	4	5	5	4
Placebo group	Mean	225	204	201	186	177	158	156	150
	SE	6	5	4	3	4	4	4	4

The results of this study demonstrate that increasing the current Ca intake of lactating mothers habituated to low Ca diets has no effect on breast-milk Ca concentration.

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Dietary sources of calcium and the contribution of flour fortification to total calcium intake. By A.J ADAMSON, P.J. MOYNIHAN, A.J. RUGG-GUNN, D.R.APPLETON AND T.J. BUTLER, The Medical Faculty, University of Newcastle, Framlington Place, Newcastle upon Tyne,NE2 4BW

Calcium intake by adolescents is sub-optimum (Department of Health, 1989; Adamson *et al.*1992). In order to increase Ca intake by this age group, information on the current dietary sources of Ca is essential to enable change to build on existing habits. The aims of the present study were twofold: first, to identify the dietary sources of Ca in adolescents and, second, to determine the importance of fortification of flour with Ca to present-day Ca intakes.

In 1990, 379 Northumbrian adolescents aged 12 years completed two 3 d dietary records. They were interviewed the day after completion of each diary to verify the information given and, with the help of food models, obtain a quantitative record of food intake. Computerized food tables were used to calculate the contribution of different food groups to total Ca intake. The Ca content of each food was subdivided into naturally occurring Ca and Ca from fortification, and data were analysed to give the daily intake of each.

Mean daily intakes of Ca were 789mg (SE 19) and 763mg (SE 16) for boys and girls respectively. The four most important sources of Ca were milk (25.2%), beverages (11.6%), puddings (9.5%) and bread (9%). No major differences in rank order of importance of different food sources to total Ca intake existed between boys and girls. Fortification of flour accounted for 13% of total Ca intake. When the contribution of fortification was removed, the proportion of subjects with intakes of Ca below the LRNI (lower reference nutrient intake) increased threefold, to 15% of subjects on average, but to as high as 21% in girls from the lower social class group.

These analyses show that milk is contributing less to Ca intake than in the past (Hackett *et al.*1983) and increased consumption should be encouraged. Ca fortification of flour remains an important source of Ca. Therefore, caution is needed to ensure that consumption of unfortified products from outside the UK does not lead to a further reduction in Ca intake.

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Influence of diet on summertime bone mineral density in healthy pre-menopausal women. By S. A. NEW[1], C. BOLTON-SMITH[2], D. A. GRUBB[3] and D. M. REID[1], [1]*Osteoporosis Unit, City Hospital, Aberdeen AB9 8AU.* [2]*Cardiovascular Epidemiology Unit, Ninewells Hospital, Dundee DD1 9SY.* [3]*Computing Department, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The influence of present and past dietary intake on summertime bone mineral density (BMD) was investigated in pre-menopausal women aged 45-49 years who had been randomly selected for screening during the months of April to September 1991-1993 (Garton *et al.* 1992). From the 1575 women scanned, 640 were eligible for the present study (41%), the main exclusion criteria being uncertainty of menstrual status.

BMD was measured using dual energy x-ray absorptiometry (DXA; Norland XR-26; Software Version 2.4). Dietary intake was assessed using a postal food frequency questionnaire (FFQ) which had been developed for the study (Lanham *et al.* 1993). Six questions were included on the past intake of foods considered important to peak bone mass attainment and included milk, milk products and fruit. Two age groups were chosen, childhood (up to 12 years) and early adulthood (20-30 years). Answers to the questions were categorized into low, medium and high intakes. A total of 520 FFQ were returned (82% response rate with one reminder letter).

Significant differences were found in lumbar spine (LSBMD) and femoral neck (FNBMD) BMD in women who consumed a low intake of milk, milk products and fruit in their early adulthood compared with a medium or high intake as shown in the Table below. These differences remained significant after adjustment for age, weight and height. BMD increased significantly with increasing intakes of the three food groups. Differences were also seen at the greater trochanter (GTBMD) and Ward's area (WABMD) BMD, but were not significant ($P<0.06$). Differences were also found in LSBMD in women who had consumed a low intake of milk and milk products in their childhood compared with a medium or high intake.

		Intake of food in early adulthood								
		Milk (pints/d)			Milk products (d/week)			Fruit (d/week)		
		Low	Medium	High	Low	Medium	High	Low	Medium	High
		<0.5	0.5-1	>1	1-2	3-4	>5	1-2	3-4	>5
LSBMD(g/cm^2)	Mean	1.051a	1.075	1.089b*	1.057a	1.065	1.116b*	1.045a	1.070	1.084b*
	SD	0.148	0.178	0.174	0.151	0.165	0.213	0.160	0.162	0.178
FNBMD (g/cm^2)	Mean	0.870a	0.891	0.909b*	0.879	0.883	0.910*	0.858a	0.896b	0.889b
	SD	0.115	0.139	0.130	0.126	0.128	0.140	0.117	0.137	0.120

a,b Values with unlike superscripts are significantly different $P<0.05$: * F-test for linearity $P<0.05$.

Present nutrient intakes were grouped into quarters and the mean BMD at each site was calculated. Adjustments for total energy intake were made by expressing each nutrient as a density (amount per 4.12MJ). No differences in BMD were found for intakes of Ca, but differences were seen in LSBMD between the lowest and highest quarters for Fe intake ($P<0.05$). Differences in LSBMD were also found for cholecalciferol intake (without supplements), but were not significant ($P<0.08$). These findings support, in part, the results from the wintertime BMD study (New *et al.* 1994). The importance of dietary intake in peak bone mass development is further highlighted.

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Plasma antioxidant nutrients in Belfast and Toulouse. By D.I.THURNHAM[1], N.R. WILLIAMS[2], A.E. EVANS[3], J.P. CAMBOU[4] and A.N. HOWARD[2], [1]*Human Nutrition Research Group, University of Ulster, Coleraine, BT52 1SA,* [2]*COAG Laboratory, Papworth Hospital, Cambridge CB3 8RE,* [3]*Belfast Monica Project, The Queen's University, Grosvenor Road, Belfast BT12 6BJ,* [4]*Project Monica, INSERM Unite 326-Orsmip, Purpan, F 31059, France*

A high intake of fruit and vegetables is inversely associated with the risk of coronary heart disease (CHD; Acheson & Williams, 1983) and several workers have suggested that the protective factors in these foods are the antioxidant nutrients, vitamins A, C, E and ß-carotene (Riemersma *et al.* 1991; Gey, 1993). We have recently reported that the carotenoid lutein appears to have good antioxidant activity in low-density lipoprotein (Chopra & Thurnham, 1993), but there is little epidemiological information on other carotenoids.

The mortality from CHD is 3-fold greater in Belfast than Toulouse (Gey, 1993). We therefore measured fasting lipids and the nutrients shown in the Table on plasma from randomly selected subjects (45-64 years) in Belfast (men 90, women 81) and Toulouse (men 101, women 110).

Measurement	Belfast (*n* 167)		Toulouse (*n* 206)		
	Mean	SE	Mean	SE	$P<$*
Total cholesterol (mmol/l)	6.46	0.09	6.13	0.06	0.01
HDL cholesterol (mmol/l)	0.99	0.02	1.20	0.02	0.001
Ascorbate (μmol/l)	31.4	1.18	32.9	0.97	NS
Retinol (μmol/l)	2.05	0.05	1.92	0.04	NS
α-Tocopherol (μmol/l)	30.2	0.88	27.9	0.43	NS
γ-Tocopherol (μmol/l)	2.41	0.10	1.57	0.06	0.001
Lutein (μmol/l)	0.278	0.010	0.590	0.018	0.001
α-Cryptoxanthin (μmol/l)	0.072	0.004	0.114	0.005	0.001
ß-Cryptoxanthin (μmol/l)	0.147	0.012	0.280	0.012	0.001
Lycopene (μmol/l)	0.479	0.025	0.447	0.018	NS
α-Carotene (μmol/l)	0.105	0.007	0.156	0.010	0.001
ß-Carotene (μmol/l)	0.461	0.025	0.504	0.031	NS

HDL, high-density lipoprotein; NS, not significant.
* Data corrected for age, sex and body mass index.

Men had lower ascorbate, α-, ß-cryptoxanthin, α- and ß-carotene values than women in both centres ($P<0.02$). Total cholesterol was 5% lower (men NS, women $P<0.001$) and HDL cholesterol 27% higher (both sexes $P<0.001$) in Toulouse. Ascorbate, retinol, α-tocopherol, lycopene and ß-carotene were not different between the two centres. In contrast, the hydroxy-carotenoids lutein, and α- and ß-cryptoxanthin were considerably higher in Toulouse in both sexes. Lutein correlated with green vegetable intake and α- and ß-cryptoxanthin with orange and fruit juice intake.

Oxidation of lipoproteins may be important in the aetiology of CHD. High density lipoproteins (HDL) may have important antioxidant properties (Durrington, 1993) and we suggest the hydroxy-carotenoids, which are more concentrated in HDL, may contribute to these properties.

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Plasma selenium concentration of the Scottish population in decline. By A. MacPHERSON[1], R. SCOTT[2], M.N.I. BARCLAY[1] AND J. DIXON[1], [1]*Scottish Agricultural College, Ayr KA6 5HW and* [2]*Royal Infirmary, Glasgow G4 0SF*

Since the identification of a declining trend in the dietary provision of Se to the Scottish population (Barclay et al. 1986; Barclay & MacPherson, 1992) plasma Se status of various population groups has been monitored to determine whether it reflects this decline in intake (MacPherson et al. 1993). The present paper describes a continuation of this study including results for 1994.

Plasma Se was measured in 354 heart disease patients and matched controls in 1985; in 478 volunteers from the Scottish Heart Health Survey of Ayrshire in 1988; in sixty-five and sixty-nine coronary heart disease patients and matched controls in Glasgow and Ayrshire respectively in 1991; in thirty-six young men in Glasgow in 1992 and eighteen in 1994 and forty mixed age males in Ayrshire in 1994. Se was determined by atomic absorption spectrometry following hydride generation and the method verified by analysis of a Community Bureau Reference sample and a standard serum sample.

The mean concentrations (μg Se/l) are presented in the Table.

Ayrshire 1985		Ayrshire 1988		Glasgow 1991		Ayrshire 1991		Glasgow 1992		Glasgow 1994		Ayrshire 1994	
Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
119	4	90.8	2.5	92.1	5	92.1	5	84.3	4.3	76.6	3	73.8	3.3
n 354		478		65		69		36		18		40	

There was a marked drop in plasma Se concentration between 1985 and 1988 amounting to almost 23% of the initial value. Concentrations remained stable for the next 2 or 3 years but have shown a further decline from 1992 to the present time. Sample size has been considerably smaller in the latter years and may therefore not be fully representative of the current situation. However each group exhibited a normal distribution pattern and the difference between the directly comparable 1992 and 1994 Glasgow samples approached significance ($P = 0.078$) thus supporting the suggestion of a real downward trend. The 1994 Ayrshire group was distinguished by uniformly low values with 60% below 80 μg/l and only 20% above the threshold value of 90 μg/l. These samples came from a group of healthy employed professional males who would be expected to have a diverse food intake and therefore maximum opportunity of consuming at least some of the relatively few Se rich foods available. That this was manifestly not the case suggests that the received wisdom that the human population is unlikely to suffer any micronutrient deficiency is at least open to question.

These results suggest that the decline in dietary Se in recent years is being reflected in falling plasma Se concentrations. They also highlight the need to establish the extent of the deficiency in a much more comprehensive survey.

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Selenium status in term and preterm infants. By L.A. DANIELS, R.A. GIBSON and K. SIMMER, *Department of Paediatrics and Child Health, Flinders Medical Centre, Adelaide, South Australia, Australia 5042*

Selenium (Se) is an essential trace element required for the enzyme glutathione peroxidase (GSHPx;EC 1.11.1.9), a component of cellular antioxidant defence. This helps protect infants against oxygen-induced, free-radical damage to lung and other tissue. Evidence suggests that preterm infants, particularly those who receive parental nutrition (PN) are Se-depleted which may be associated with increased risk of oxidative lung injury and chronic lung disease (CLD; Lockitch *et al* 1989).

Se status was assessed in sixty-three preterm infants with mean birth weight of 1572 g and mean gestational age 30.7 weeks. Plasma Se and erythrocyte Se and GSHPx were measured at week 0 (2.8) and week 1 (8.6) and weekly thereafter. Twenty-seven percent of infants had CLD and received 13.6 (range 1-105) ventilation, 26.9 (range 1-228) oxygen and 13.4 (range 0-45) total PN. Plasma Se was measured in healthy term reference infants at birth (cord), day 5 (week 0) and week 6. Results for plasma Se are shown in the table.

	Plasma Se (μg/l)											
	Term infants									Pre-term infants		
	Breast-fed			Formula-fed			All infants*					
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
Cord							52	12^{2}	48			
Week 0	32	12^{1}	23	40	11^{1}	8	33	11^{1}	46	31	13^{1}	62
Week 6	49	$11^{a,2}$	23	32	$11^{b,2}$	8	45	$14^{a,2}$	32	21	$11^{C,2}$	34

* Repeated measures analysis.

Means with different superscript are significantly different ($P<0.01$). Alphabetic superscripts refer to feed or gestational age effects and numeric superscripts refer to postnatal age effects.

Mode of feeding of preterm infants was not consistent from week to week and many infants received a combination of feeds. At week 6 predominantly breast-fed infants (>75% average daily energy from breast-milk for the week) had a mean plasma Se of 27 (SD11) μg/l (n 7) compared with 15(SD 14) μg/l (n 9) for formula-fed infants ($P<0.05$) and 22 (SD 9) μg/l (n 16) for those receiving mixed feeding. There are no infants receiving more than 75% energy from PN at week 6. At week 4 the plasma Se levels of PN-fed, breast-fed and formula-fed preterm infants were 17 (SD7) (n 5), 27(SD 15) (n 14) and 27 (SD 14) (n 11) μg/l respectively.

At week 0 preterm GSHPx levels (0.97 (SD 0.37) IU/gHb) were lower ($P<0.001$) than terms (1.34 (SD 0.65) IU/gHb). Term GSHPx levels had not changed at 6 weeks but the preterm levels increased ($P=0.007$) to week 6 levels similar to term infants. Infants with CLD (n 15) showed a 30% decline in plasma Se over the first month of life ($P=0.013$) while those without CLD (n 40) showed a smaller decline of 16% ($P=0.043$).

In summary term and preterm infants had similar plasma Se levels in the first few days of life. However levels increased with postnatal age (breast-fed) or remained the same (formula-fed) in term infants whereas levels fell in preterm infants regardless of mode of feeding. Plasma Se levels in preterm infants were half the levels of the healthy term reference group in 6 weeks postnatal age. This decline is also more pronounced in infants with CLD which has been documented by others (Darlow *et al* 1994). Overall, Se levels of Adelaide infants are low (Lockitch *et al* 1989; Sluis *et al* 1992). GSHPx activity, considered a functional marker of Se status did not reflect changes in plasma Se. Se levels in umbilical venous plasma were 50% higher than day 5 heel-prick neonatal plasma Se levels and therefore may not be relevant as baseline data for Se status studies in infants.

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Influence of iron supplementation on markers of infection in Pakistani infants. By C.A. NORTHROP-CLEWES[1], U.J. McLOONE[1], P.I. PARACHA[2] and D.I. THURNHAM[1], [1]*Human Nutrition Research Group, Faculty of Science, University of Ulster, Coleraine BT52 1SA,* [2]*Department of Human Nutrition, NWFP Agricultural University, Peshawar, Pakistan*

Iron given to severely malnourished children has caused death (McFarlane *et al.* 1970) and when given either orally (Smith *et al.* 1989) or parenterally (Oppenheimer *et al.* 1986) to children living in malaria-endemic areas, increased the prevalence of malaria. Fe will catalyse the formation of free radicals and evidence suggests that the body scavenges Fe from the circulation in chronic disease and increases storage Fe (Thurnham, 1990). Hence low haemoglobin in Third World children may reduce the severity of infection, in which case Fe supplementation may have the opposite effect.

An Fe-supplementation study was carried out in Peshawar Province, Pakistan in which 300 children below 2 years were given either oral Fe (15 mg as $FeSO_4$) or placebo daily for 12 weeks starting in August 1993. We measured retinol, lutein, α and ß-carotenes and α-tocopherol, the acute-phase markers, α_1-antichymotrypsin (ACT) albumin, caeruloplasmin (ceru) and the immunoglobulins (Ig) A, G and M in plasma collected before and after Fe supplementation.

Variable g/l	Pre-supplementation¶			Changes Post-supplementation§					
	Combined groups			Fe-supplementation			Placebo		
	n	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
ACT	265	0.50	0.01	102	0.046	0.03	107	0.003	0.02
Albumin	266	38.65	0.36	102	1.36	0.89	108	2.27	0.97
Ceru	265	0.37	0.01	102	-0.003	0.01	108	-0.00	0.01
IgG	265	9.72	0.15	102	0.59*	0.24	107	0.78*	0.23
IgM	265	1.24	0.02	101	0.02	0.49	107	0.067	0.04
IgA	264	0.82	0.02	102	0.113*	0.03	106	0.107*	0.03
Retinol (μmol/l)	266	0.66	0.01	103	0.12*	0.03	104	0.12*	0.03
Lutein (μmol/l)	266	0.102	0.015	103	0.116*	0.01	104	0.111*	0.01
ß-Carotene (μmol/l)	266	0.011	0.006	103	0.005	0.003	104	0.008	0.004
α-Tocopherol (μmol/l)	266	7.85	0.21	103	3.06*	0.44	104	2.61*	0.36

* Significantly different from pre-supplementation mean values, $P<0.05$.

¶ No significant differences between means of the two groups at the start.

§ No significant differences between the changes of the two groups after supplementation.

Plasma retinol increased post-supplemention in both groups and thus is unlikely to be due to the Fe supplement. August to October is associated with an increased intake of fruits and vegetables and the increase in the carotenoid lutein confirms the likely role of diet in explaining the increase in retinol and α-tocopherol.

ACT is a marker of chronic infection and the levels reported are slightly higher than those in British toddlers (D.I.Thurnham, National Diet and Nutrition Survey, unpublished data). The small differences in the changes in ACT between the two supplement groups were investigated using multiple regression. In the Fe group, variance (r^2) was explained by the changes in retinol (-ve, 20%), ceru (+ve, 10%) and albumin (-ve, 3%) while in the placebo group IgA (+ve, 18%), ceru (+ve, 12%) and albumin (-ve, 7%) were the main determinants. Retinol only influenced ACT in those infants receiving Fe where the greatest increases in ACT were associated with reductions or small increases in serum retinol. We suggest therefore that the Fe supplement did increase the severity of disease but because the supplementation study coincided with a period of increased vitamin A bioavailability, the children were protected from the trauma of the additional Fe.

The iron-intervention study was funded by USAID/UNICEF.

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The exclusion of folic-acid-fortified foods significantly reduces folate status.

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On the basis of a major study confirming the protective effect of pteroylglutamic acid (folic acid) in preventing neural-tube defects (NTD; MRC Vitamin Study Group, 1991), the Department of Health (1992) has published new folic acid recommendations for the prevention of both recurrence and first occurrence of NTD. Regarding the latter, women planning a pregnancy are advised to increase their intake by 400 μg folic acid/d over and above current intakes by increasing consumption of folate-rich foods, taking supplemental folic acid in tablet form or the consumption of folic-acid-fortified foods. The aim of the present study was to examine the contribution to folate status of foods fortified with folic acid by investigating the changes in dietary, erythrocyte and serum folate levels in response to their removal from the diet over a 3-month period.

Fifty-three subjects (aged 18-40 years) were recruited who were neither pregnant nor planning a pregnancy, who were neither sufferers of NTD nor first degree relatives of a sufferer, and were not taking vitamin or mineral supplements or drugs known to interfere with folate metabolism. Subjects received individual dietary counselling (along with written advice) on the exclusion of folic-acid-fortified foods and their substitution with equivalent non-fortified foods, for an exclusion period of 12 weeks. Dietary responses were assessed by comparing habitual intakes (recorded on recruitment) with those recorded during the exclusion period (diet-history method supplemented with food frequency questionnaire). Erythrocyte and serum folate concentrations, measured using microbiological assay techniques (O'Broin & Kelleher, 1992) were compared in samples obtained before (initial) and following (final) the exclusion diet. Data were transformed as appropriate before analysis (paired *t* test).

	Dietary energy (MJ/d)		Dietary folate (μg/d)		Erythrocyte folate (μg/l)		Serum folate (μg/l)	
	Habitual	Exclusion	Habitual	Exclusion	Initial	Final	Initial	Final
Mean	8.2	8.0	226	192**	370	250***	8.4	5.9***
SD	1.4	1.5	75	49	130	56	4.0	2.8
n	50	50	50	50	50	50	50	50

Significantly different from habitual or initial value; $^{**}P < 0.01$; $^{***}P < 0.001$.

The results show that while dietary energy was not significantly altered as a result of the removal of foods fortified with folic acid, dietary, erythrocyte and serum folate were all significantly decreased. Haematological indices (both initial and final) were within the normal ranges for all subjects but dietary folate levels were well below the new Department of Health (1992) recommendations.

These data demonstrate that fortification contributes significantly to folate status. A wider availability of folic-acid-fortified foodstuffs may therefore offer an effective means of achieving the increased intakes currently being recommended for all women planning a pregnancy (Department of Health, 1992).

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Surviving in Sarajevo: winter nutrition monitoring 1993-1994. By F. WATSON[1], J. VESPA[1] and I. KULENOVIC[2]. [1]*World Health Organisation Nutrition Unit, Zagreb Office, Croatia and* [2] *Medical Faculty, University of Sarajevo, Bosnia*

The World Health Organisation (WHO) is currently monitoring nutritional status and household food security in besieged Sarajevo. The main aim is to collect objective, longitudinal data in order to detect change in the nutrition and food situation and provide early warning of deterioration before nutritional status is compromised.

A total of 414 household members in 143 households from different 'functional' groups (residents, refugees in collective centres, refugees in private accommodation and elderly living without younger family members) are being followed. Four locations within Sarajevo were purposively selected and households were randomly selected within each location. Two collective centres were randomly selected. Households have been visited each month since December 1993. At each visit, data are gathered through structured questionnaires on food and fuel stocks, food aid, recent sickness, access to water and heating, occupation and income. All household members are weighed and have their height measured. Clinical signs of micronutrient deficiencies are noted. Weight-for-Age Z scores are calculated for children aged from 6 months to 11.5 years and compared with a reference population. The nutritional status of adults (18-59 years) and the elderly (>59 years) is assessed by calculating body mass index (BMI). Demispan is used as a proxy for height in the elderly.

While the nutritional status of adults and children has consistently remained normal (5.0-5.5% BMI<18.5 for adults and 0.0% weight for age<2Z for children), high levels of undernutrition have been detected among the elderly, particularly those living without younger family members (10.5-18.8% BMI<18.5). Between December and February, adults lost a significant amount of weight averaging 260 g (paired *t* test, $P<0.05$). Some of this weight (220 g) was regained during March after the cease-fire. Household food stocks declined from 8.6 kg/person to 6.7 kg between December and March. Less than 1% comprised fruit and vegetables. The major source of food stocks came from food aid (80-86% of total) while the remainder was largely bought on the black market. Some black market commodities may have been "recycled" food aid. The quantity of food aid received per person met only 65-69% of estimated requirements. The cost of a basic food basket, meeting 100% of energy needs for one person, dropped from 88 DMS/week in January to 41 DMS/week in March. An average monthly salary is 2 DMS. Weekly expenditure on food showed little variation (between 6 and 10 DMS/person). About half the sampled population (47.5%) were cultivating gardens in March, but only 36% of refugees in collective centres had access to land.

Some households have been cushioned against the effects of unemployment, low salaries, high food prices and inadequate food aid through their access to resources (e.g. cash savings, family support from outside in the form of food parcels and cheques, cultivation of gardens). Others, however, have been forced to sell or exchange goods and personal possession in order to survive. These range from gold rings and fur coats to food and cigarettes. .

Whilst there was no nutritional disaster in Sarajevo over the winter 1993-1994, there were signs that capacity to cope was weakening. The age group identified as the most nutritionally vulnerable were the elderly. Undernutrition may have been precipitated in this group by sickness, cold, stress and problems related to food preparation. The most food insecure group were refugees in collective centres who were highly dependent on food aid, were less likely to have relatives outside Sarajevo who could support them, had fewer possessions to sell and were least likely to have gardens. Though food prices have dropped, the cost of a basic food basket remains exorbitant and with the transition away from dependence on humanitarian aid and towards reliance on self-production and the market economy, the vulnerable will be those who lack resources and so access to food, health care and a clean, warm environment. It is concluded that the impact of peace on the population of Sarajevo may have more devastating nutritional consequences than the impact of war.