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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at the University of Nottingham on Monday–Thursday, 12–15 July 1993, when the following papers were presented.

Calcium absorption from cow's milk and soya milks in young rats. By K. CASHMAN and A. FLYNN, <u>Department of Nutrition, University College, Cork, Republic of Ireland</u>

Soya-bean 'milks' (soya milks) are widely available as substitutes for cow's milk. Soya milks generally contain less Ca than cow's milk and also contain phytic acid which may inhibit the absorption of Ca. This study compares the absorption of Ca from cow's milk and six soya milks and examines the effect of phytate on Ca absorption in young rats.

Milks were extrinsically labelled with ^{47}Ca (1 µCi/ml) and 0.2 ml administered orally to 16-d-old Wistar rats (five rats per group), previously fasted for 16 h. Animals were killed 14 h later and ^{47}Ca in the stomach with contents (S), the contents of small intestine, obtained by perfusing with 0.6 ml 0.15 M-NaCl (SI-per) and caecum-colon with contents (C) was determined in a well gamma counter and expressed as % dose. Absorption of ^{47}Ca was calculated as:

Absorbed ^{47}Ca (% dose) = 100 - (S + SI-per + C).

Similar absorption studies were performed in rats aged 17 - 28 d old for two of the milks (cow's milk and soya milk C). Ca absorption as a function of phytate concentration was also determined in 16 d-old suckling rats from cow's milk-based infant formula (400 mg Ca/l) and from a solution of $CaCl_2$ (400 mg Ca/l), each containing 0 - 20 mM-sodium phytate.

| Milk	Milk Ca (mg/l)	Milk phytate (mmol/l)	Molar ratio Phytate:Ca.	Absorbed ^{47}Ca (% dose)* Mean SEM	Absorbable Ca[†] (mg/l)
Cow's (low fat)	1243	-	-	99.0 0.1	1228
Soya milk					
A	115	1.6	0.56:1	95.3[a] 0.2	110
B	135	2.2	0.65:1	99.0 0.1	134
C	766	0.8	0.04:1	97.3[a] 0.3	745
D	187	1.0	0.21:1	96.4[a] 0.7	180
E	1260	1.0	0.03:1	94.7[a] 0.2	1193
F	126	1.8	0.57:1	95.5[a] 0.4	120

*Five rats per group.
[†]Estimated from %^{47}Ca absorption and Ca concentration in milk.
[a]Significantly different from cow's milk (*Student's t-test*), p<0.05.

Absorption of ^{47}Ca was high from both cow's milk and soya milks in 16 d-old rats and remained at this level up to 28 d of age. Phytate inhibited Ca absorption in a dose-related manner (Figure).

The results show that under conditions used in these studies (high Ca need, low Ca load) essentially all Ca in both soya and cow's milks is available for absorption and absolute absorption is determined by Ca content of the milk. All soya milks, except that which was fortified with Ca to the level of cow's milk, are poorer sources of Ca than cow's milk.

This is due to their low Ca contents rather than to the presence of phytate, which seems to be a relatively weak inhibitor of Ca absorption.
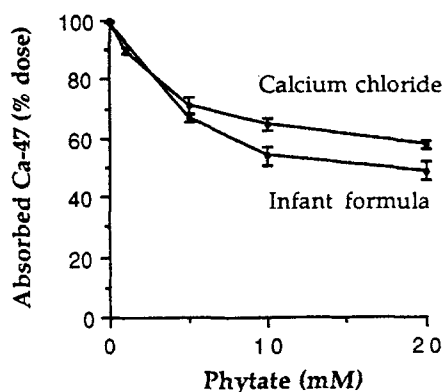
In utero exposure to maternal low-protein diets induces hypertension in rats.

By S.C. LANGLEY and A.A. JACKSON. Department of Human Nutrition, University of Southampton, Southampton S09 3TU.

Epidemiological analyses of the British population have demonstrated a close association between low birthweight and risk of adult cardiovascular disease (Barker *et al.* 1990). These findings suggested that adverse conditions *in utero* may lead to irreversible physiological changes which subsequently promote the development of the hypertensive state (Law *et al.* 1993). In the present study we demonstrate a rat model of hypertension induced by maternal dietary restriction that may be applicable to the determination of the mechanisms underlying the association of birthweight and heart disease.

Female Wistar rats were fed diets containing 18, 12, 9 or 6 % protein, as casein with methionine supplementation, for a period of 14 d before mating. Feeding of the diets continued throughout pregnancy. Within 12 h of giving birth the dams were placed on standard laboratory chow (20 % protein). This diet was fed throughout the lactation period, and the offspring were weaned onto the same chow when aged 4 weeks. Differences in the body weights of pups born to mothers fed 6 % casein were observed at 7 d old. These pups remained significantly smaller than those in all other groups throughout the 15 weeks of the study. Pups born to dams fed 12 or 9 % casein showed altered patterns of growth relative to the 18 % casein control group, but were grossly normal. At 9, 12 and 15 weeks old the systolic blood pressure (SBP) of the animals was measured non-invasively, using a tail-cuff method. Rats were placed in a pre-warmed, darkened restraint tube, and tail artery pulses recorded following inflation of a 15 mm cuff to 300 mm Hg, with deflation at a rate of 3 mm Hg/sec. The animals were assessed 3-4 times at each age, and the mean SBP recorded (Table).

Systolic Blood Pressure (mm Hg)

Age (wks)	*n*	Dietary Protein (%)							
		18		12		9		6	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
9	11-15	137[a]	4	152[b]	3	153[b]	3	159[b]	4
12	4-7	139[a]	8	152[a]	9	155[a]	8	158[a]	6
15	4-8	129[a]	4	155* 138*		149[b]	4	149[b]	4

* individual values for 2 rats.

Different superscripts indicate statistically different to 18 % (P<0.05 ANOVA).

SBP was significantly elevated in the low-protein groups, relative to the 18 % casein controls at both 9 and 15 weeks of age. At 12 weeks a similar trend was observed, but this failed to achieve statistical significance.

These data are consistent with the concept that fetal exposure to inadequate nutrition may induce physiological or metabolic changes that contribute to the development of adult cardiovascular disease. The precise mechanisms underlying these changes are yet to be evaluated.

Barker, D.J.P., Bull A.R., Osmond C., Simmonds, F.J. (1990). British Medical Journal 301 259-262.

Law, C. de Swiet, M. Osmond, C., Fayers, P.M., Barker, D.J.P., Cruddas, A.M., Fall, C.H.D. (1993). British Medical Journal 306 24-27.

The digestibility of macronutrients in the growing domestic cat. By H.S. MUNDAY and B.K. LOWE, Waltham Centre for Pet Nutrition, Waltham-on -the-Wolds, Melton Mowbray. Leics, LE14 4RT

A series of trials was carried out in young, growing domestic cats to investigate the changes in digestibility of the macronutrients in the diet with age. Comparable trials were also carried out in adult cats.

Three commercially prepared complete canned cat foods were selected for the trial with proximate analyses as follows (as fed):

	Diet 1	Diet 2	Diet 3
Moisture (g/kg)	739	821	805
Protein (g/kg)	126	75	72
Fat (g/kg)	103	62	80
NFE (g/kg)	4	23	24
ME (MJ/kg)	5.64	3.37	3.82

NFE, Nitrogen-free extract; ME, metabolizable energy.

Four kittens were assigned to each of the three diets. Equal numbers of both sexes were used on each trial. Kittens were weaned by the age of 8 weeks and during week 8 they were trained to defaecate in one part of a large kitten cabin. Daily faecal collections were carried out during the kitten's ninth week of age and then again during weeks 11, 13, 15 and 17. During the intervening weeks no collections were carried out and the kittens were able to socialize together in communal rooms. For comparative purposes, digestibility trials were also carried out on six adult cats fed on the same food.

Weekly bodyweights and daily food intakes were recorded for each kitten. Faecal samples for each kitten and test week were pooled, subsampled and analyses carried out (proximate and bomb calorimetry) to determine apparent digestibilities of energy and nutrients.

Statistical analyses were carried out to assess the effect of age and diet on digestibility of nutrients in cats. The following Table indicates the mean digestibility coefficients for cats of different ages (results of cats on all three diets, n=12).

Digestibility	Dry matter		Crude protein		Fat		Energy	
Age	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Week 9	0.73[a]	0.04	0.77[a]	0.03	0.81[a]	0.05	0.77[a]	0.04
Week 11	0.76[ab]	0.03	0.79[ab]	0.02	0.85[ab]	0.04	0.79[ab]	0.03
Week 13	0.77[ab]	0.04	0.80[abc]	0.03	0.87[abc]	0.04	0.82[bc]	0.04
Week 15	0.79[ab]	0.04	0.82[bc]	0.03	0.87[abc]	0.04	0.82[bc]	0.03
Week 17	0.81[b]	0.04	0.83[bc]	0.03	0.90[bc]	0.04	0.84[c]	0.03
Adult	0.80[b]	0.04	0.82[c]	0.02	0.91[c]	0.04	0.84[c]	0.03

[a,b,c] Values with unlike superscripts are significantly different (ANOVA): $P<0.05$

The statistical tests (ANOVA) show increasing digestibility for all nutrient groups and energy with age. The foods chosen contained different raw materials to achieve the different nutrient profiles, and dietery effects were consistent at the different ages.

The results show good digestibility of solid foods by newly weaned kittens, but this increases significantly over the 10 weeks post-weaning as physiological developments are occurring.

Effect of hypothyroidism on insulin-like growth factor (IGF-1) status in the postnatal lamb. By J.J. GATE, C.J. DARBY, M.A. LOMAX and M.E. SYMONDS, <u>Department of Biochemistry and Physiology, University of Reading, Whiteknights, Reading RG6 2AJ</u>

Thyroid hormones are known to play an important role in the control of thermoregulation during postnatal development (Symonds & Lomax, 1992), but their possible role in the endocrine control of growth after birth has not been examined. This study investigates the interaction between plasma concentrations of thyroxine and IGF-1 and their relationship with growth rate in developing lambs.

Fourteen lambs (mean (SEM) birth weight 4.8(0·2)kg) were removed from their ewes on the morning after birth. All lambs were individually housed at an ambient temperature of 10-15° and were fed a 2 l volume of milk containing 200 g of milk replacer (VOLAC LAMLAC, Royston, Herts). Hypothyroidism was induced in seven lambs (Hypothyroid;H) by oral administration of methimalzole (50 mg/kg body weight per day). The remaining seven lambs were reared as controls (Euthyroid;E). Blood was sampled from sleeping lambs at 7 and 30 d of life, plasma concentrations of thyroxine and IGF-1 were then measured by radio-immunoassay.

| Age (d)... | | IGF-1 (ng/ml) | | | | Thyroxine (nM) | | | |
| | | 7 | | 30 | | 7 | | 30 | |
Group	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
E	7	124·6	5·8	122·2	29·6	102·9	6·9	95·5	10·2
H	7	39·2***	6·3	95·5	8·2	84·5*	7·8	29·8*	15·2

Significantly different from Group E (Students t test): * $P<0·05$, *** $P<0·001$.

Plasma concentrations of IGF-1 were 69% and 22% lower in H compared with E lambs at 7 and 30 d of postnatal life, respectively. At this age plasma thyroxine concentrations were 18% and 69% lower in H lambs. In E lambs plasma concentrations of IGF-1 and thyroxine were unaffected by age but IGF-1 levels increased 3-fold ($P<0·001$) and thyroxine levels decreased by 65% ($P<0·01$) between 7 and 30 d of age in H lambs. These adaptations were not associated with any differences in growth rate which were similar in both groups (E; 128 (7) g/d, H; 129 (8) g/d).

It is concluded that sustained plasma thyroxine concentrations may be required for the maintenance of circulating IGF-1 levels during early postnatal life, but that this effect disappears by 30 d of age. The differences in plasma concentrations of IGF-1 and thyroxine between E and H groups did not appear to be reflected in their growth rates, which were similar.

Symonds, M.E. & Lomax, M.A. (1992). <u>Proceedings of the Nutrition Society</u> **51**, 165-172.

Endogenous purine and pyrimidine derivative excretion in pregnant sows. By S. M. MARTIN, J. BALCELLS, J. A. GUADA and C. CASTRILLO, <u>Departamento de Producción Animal y Ciencia de los Alimentos, Facultad Veterinaria, Miguel Servet 177, Zaragoza 50013, Spain</u>.

Excretion of urinary derivatives of endogenous purine is an important fraction of the total urinary purine excretion and it must be taken into account in order to establish the relationship between duodenal supply and urinary recovery of purines. However, there is a lack information about changes in endogenous excretion in relation to physiological status. The aim of the present study was to compare endogenous excretion of purines and pyrimidines by pregnant v. non-pregnant animals.

Urine samples were obtained from six sows, three pregnant and three non-pregnant, fed on a semi-synthetic purine-free diet containing maize starch, glucose, sucrose, vegetable oil, kibbled straw, casein plus synthetic amino acids and a vitamin-mineral mixture. The purine derivative excretion (PD), allantoin, uric acid, hypoxanthine and xanthine and pseudouridine (Ψ, a non-salvageable pyrimidine derivative), were analysed by HPLC (Balcells *et al.* 1992). PD and Ψ excretion (mol/100 mol creatinine) throughout the pregnancy are shown in the Figure.
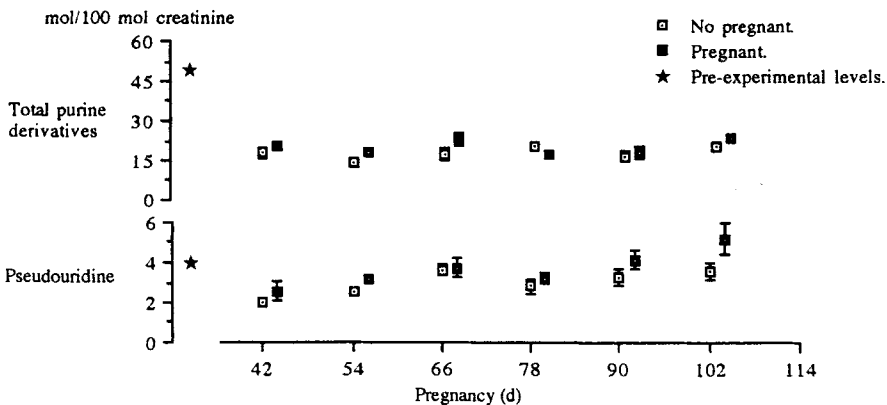
Following dietary purine suppression, allantoin and consequently total purine derivative excretion decreased from previous values of 401 and 482 μmol/kg live weight (LW)$^{0.75}$ respectively to constant basal levels of 128.31 (SE 21.69) and 199.19 (SE 33.66) μmol/kg LW$^{0.75}$, whereas excretion levels of allantoin precursors remained constant, averaging 38.66 (SE 6.53) and 32.22 (SE 5.44) μmol/kg LW$^{0.75}$ for uric acid and xanthine+hypoxanthine respectively. There were no differences in endogenous PD excretion between pregnant (197.46 (SE 13.77) μmol/kg LW$^{0.75}$) and non-pregnant (200.92 (SE 12.68) μmol/kg LW$^{0.75}$) animals, similar results were obtained when data were expressed in relation to creatinine excretion, averaging 20.15 (SE 1.48) and 17.98 (SE 1.00) mol/100 mol creatinine for both groups of animals. Pseudouridine excretion showed a slight increase throughout the experimental period, this increase tended to be higher ($p<0.10$) with pregnant (2.54 (SE 0.48)-5.21 (SE 0.76) mol/100 mol creatinine) than with non-pregnant sows (1.97 (SE 0.22)-3.59 (SE 0.43) mol/100 mol creatinine) between 42 and 102 d of pregnancy respectively.

It is concluded that pregnancy did not affect endogenous PD excretion in sows. Therefore equations, developed previously with non-pregnant animals (Balcells *et al.* 1991), which estimate the duodenal flow of purine derivatives from measurements of their urinary excretion, would not appear to be invalid when extended for use with pregnant animals.

Balcells, J., Guada, J. A., Castrillo, C. & Gasa, J. (1991). <u>Journal of Agricultural Science, Cambridge</u> **116**, 309-317.
Balcells, J., Guada, J. A., Peiró, J. M. & Parker, D. S. (1992). <u>Journal of Chromatography</u> **575**, 153-157.

Unilateral intramammary infusion of GH does not support a local galactopoietic action of growth hormone in goats. By K. SEJRSEN[2] and C. H. KNIGHT[1], [1]Hannah Research Institute, Ayr KA6 5HL, U.K. and [2]National Institute of Animal Science, Foulum, DK-8830 Tjele, Denmark

The effect of exogenous GH on milk yield in ruminants involves stimulation of mammary uptake and utilization of nutrients, but the mechanism whereby GH affects mammary function is not clear. Most evidence suggests the action is indirect; however, a recent report indicates that GH can affect mammary cell proliferation directly in vivo in late pregnancy (Collier et al, 1993). This raises the possibility that GH may also have local action on the lactating mammary gland.

To examine this hypothesis goats were given unilateral intramammary infusions of GH. Six goats with exteriorized mammary veins were used in a cross-over design with three treatments and three treatment periods. Treatment periods were 8 d and control periods were 4 d. The treatments were 10 mg bovine GH given as: (1) subcutaneous injection, (2) unilateral intramammary infusion or (3) unilateral intramammary infusion together with 300 mM-Na-EGTA; pH 6.8. In (2) and (3) the contra-lateral gland was treated with saline or EGTA alone. The dose of EGTA was adjusted for control period milk yields to be 3 ml/kg milk. The EGTA infusion was given to cause a short term disruption of the integrity of the tight junctions, allowing para-cellular flux of milk constituents across the mammary epithelium (Neville & Peaker, 1981) and thus enabling access of infused GH to the basal cell surface. The efficacy of the dose was tested before the experiment. Daily milk yields of individual glands were recorded. Milk samples of individual glands were collected daily. Blood samples were collected from the jugular vein every 2d and from the milk veins via indwelling catheters before and for up to 4 h after the first hormone administration in each treatment period. Milk and blood was assayed for GH and insulin-like growth factor-I (IGF-I).

Almost all the intramammary-infused GH could be recovered in the milk at the following milking, and milk vein GH levels were not increased by intramammary infusion either of GH alone or of GH with EGTA. In agreement, circulating levels of GH and IGF-I, as well as milk IGF-I concentration, remained at control period levels throughout the period of intramammary infusion. As expected, plasma levels of GH and IGF-I were increased during subcutaneous administration, but milk levels were not altered. Milk yields of individual glands are shown in the Table, which gives mean values for the first 4 and the last 4 d of the treatment periods, with all values adjusted for the pretreatment control period as covariate.

Route	Single gland milk yield (kg/d)					
	Subcutaneous	Unilateral intramammary		Unilateral intramammary		
Infusate	GH	Saline	GH + Saline	EGTA	GH + EGTA	SEM
Day 1-4	1.73	1.58	1.57	1.47	1.40	0.04
Day 5-8	1.82	1.53	1.47	1.40	1.25	0.05

Milk yield was increased approximately 25 % when GH was administered as subcutaneous injections compared with intramammary infusions ($P < 0.001$). Infusion of EGTA depressed milk yields by approximately 12 % compared with saline infusion ($P < 0.05$). Intramammary GH infusion had no effect on milk yield when compared in infused and non-infused glands ($P > 0.10$). The results, therefore, do not support a local galactopoietic action of GH on lactating mammary tissue of goats.

Collier, R. J., McGrath, M. F., Byatt, J. C. & Zurfluh, L. L. 1993. Livestock Production Science 35, 21-34.
Neville, M. C. & Peaker, M. 1981. Journal of Physiology 313, 561-570.

Energy cost of activity in normal weight and obese women. By J.M. HIBBERT[1], L.D. BROEMELING[1], J.N. ISENBERG[2] and R.R. WOLFE[1], [1]Shriners Burns Institute and [2]Department of Pediatric Gastroenterology, University of Texas Medical Branch, Galveston, Texas, USA

Few metabolic studies have examined energy cost of habitual physical activity (PA) in people of different body weights (Prentice *et al.* 1986; Bandini *et al.*1990; Welle *et al.* 1992). None has investigated what determines free-living total energy expenditure (TEE). This was previously investigated only in the confines of a respiratory chamber (Ravussin *et al.*1986), where fat-free mass and involuntary physical activity were shown to be the main determinants.

In the present study, TEE was measured in nine normal weight controls mean (SD) weight 60 (7) Kg and five obese women 101 (17) Kg by doubly-labelled water (DLW). Resting energy expenditure (REE) and thermic effect of food (TEF) were measured by indirect calorimetry and PA calculated by deduction, to quantify all components and identify determinants of free-living TEE.

PA best explained variability across the groups, contributing 76% to the variance of TEE after adjusting for covariances, whereas body weight was the single best determinant of TEE by variable selection regression analysis. TEE, PA and REE were elevated in obesity, whereas TEF was not significantly different between the groups, accounting for 7.6% of expenditure in obesity and 8% in controls (Table). TEE:REE was 1.95 for the obese, representing a considerable though not statistically significant increase (19%) over the control value (1.64).

Group	TEE (MJ/d) Mean	SD	REE (MJ/d) Mean	SD	TEF (MJ/d) Mean	SD	PA (MJ/d) Mean	SD	TEE:REE Mean	SD
Control	8.34	1.79	5.08	0.26	0.70	0.35	2.55	1.45	1.64	0.34
Obese	12.40	2.57	6.39	0.68	0.94	0.24	5.07	2.39	1.95	0.45
P value	<0.005		<0.0005		NS		<0.05		NS	

Significance was obtained by the student's T-test

These results demonstrate increased energy cost of habitual physical activity in obesity. TEE:REE for the obese was similar to that previously found for elite female runners (1.99) engaged in rigorous training (Schulz *et al.*1992). This comparison emphasizes the high PA in obesity. In contrast with previous work (Ravussin *et al.*1986) we have found that body weight and voluntary PA are the main determinants of free-living TEE. Consequently, energy requirements estimated from measurements restricted to a metabolic chamber may be inappropriate for application to the free-living situation.

Bandini, L.G., Schoeller, D.A. & Dietz, W.H. (1990). Pediatric Research 27, 198-203.

Prentice, A.M., Black, A.E., Coward, W.A., Davies, H.L., Goldberg, G.R., Murgatroyd, P.R., Ashford, J., Sawyer, M. & Whitehead, R.G. (1986). British Medical Journal 292, 983-987.

Ravussin, E., Lillioja, S., Anderson, T.E., Christin, L. & Bogardus, C. (1986). Journal of Clinical Investigation 78, 1568-1578.

Schulz, L.O., Alger, S., Harper, I., Wilmore, J.H & Ravussin, E. (1992). Journal of Applied Physiology 72, 23-28.

Welle, S., Forbes, G.B, Statt, M., Barnard, R.R. & Amatruda, J.M. (1992). American Journal of Clinical Nutrition 55, 14-21.

Low carbohydrate oxidation during rest: a possible mediator of weight loss in the profoundly inactive? By A.P.M. MCKENNA, P.R. MURGATROYD, G.R. GOLDBERG and A.M. PRENTICE, MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL

Body carbohydrate (CHO) stores are of limited capacity, and as fat synthesis from dietary carbohydrate is believed to be induced only by very high levels of carbohydrate intake, dietary carbohydrate intake and carbohydrate oxidation must be matched to maintain carbohydrate balance. In the normal range of activity this may be achieved by regulation of food intake or by modulation of the substrate mixture oxidized. At the extremes of activity the freedom to modulate the oxidation mixture may be limited. In endurance exercise, such as marathon running, for example, carbohydrate stores are insufficient to support oxidation in proportion to their normal dietary contribution. We have examined the contribution of carbohydrate to energy expenditure at the opposite extreme of activity, sleep. During sleep the demand by the brain for carbohydrate remains relatively constant while resting muscle depends primarily on fat. This may limit the extent to which the oxidation mixture may be adjusted to reflect the composition of the day's dietary intake.

We have examined patterns of carbohydrate oxidation within 24 hour periods in a number of groups studied by whole-body indirect calorimetry and have been struck by the consistency of carbohydrate oxidation overnight. In Study 1 subjects received a meal at 20.00 hours and retired to bed at 23.00 hours. The meal was of typical British dietary composition; 47 % CHO, 40 % fat, 13 % protein by energy. Carbohydrate oxidation rose as expected during the post-prandial period but with the onset of sleep fell dramatically. Just 31.3 (SD 8.2) g of CHO were used overnight. In Study 2 a similar meal was fed to five males and five females at 17.30 hours. The subjects retired to bed at midnight. The overnight CHO oxidation rate averaged 40.2 (SD 10.6) g. We conclude that carbohydrate oxidation during sleep or extreme inactivity may fall to a basal level. The level we have observed is consistent with the minimum level of carbohydrate oxidation needed to support the function of the brain and major organs.

Study	n	Overnight CHO oxidation rate (g/d)		% Energy from CHO	
		Mean	SD	Mean	SD
1	15 (male)	107.4	28.3	24.8	6.4
2	5 (male)	129.3	29.8	30.2	7.8
2	5 (female)	111.9	34.4	31.9	10.1

We have also examined the proportion of energy derived from carbohydrate in the sleeping state and have observed that this too falls dramatically when sleep begins, to a level where only about 30 % of energy is derived from carbohydrate, in contrast to the 47 % supplied in the diet. This leads us to suggest that if a state of extreme inactivity was maintained for an extended period of time and was accompanied by a level of dietary intake which maintained carbohydrate balance then a negative fat balance would be induced with consequent weight loss. This would mirror the situation seen in endurance athletes where the balance between substrate availability and oxidation is distorted by the demand for the oxidation of fat, resulting in a very lean physique.

We suggest that these observations be considered in the context of the inactive and immobile elderly who often exhibit profound weight loss which could derive partly from a low carbohydrate requirement.

The metabolic effects of acute hyperketonaemia on whole body and forearm glucose disposal. By J. WEBBER, E. SIMPSON, H. PARKIN and I. A. MACDONALD, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH

During starvation there is a progressive fall in blood glucose levels, whilst levels of ketones and free fatty acids rise. Using the hyperinsulinaemic euglycaemic glucose clamp it can be demonstrated that insulin-mediated glucose oxidation falls during starvation, but glucose storage is unaffected, although muscle glucose uptake is virtually abolished (Mansell & Macdonald, 1990). The present study was designed to examine the effects of acutely raising blood ketone levels to those seen after 72 h of starvation on whole body glucose disposal and substrate utilization.

Ten healthy subjects (mean age (SE) 28.0 (0.75) years, mean body mass index (BMI) 21.6 (0.8) kg/m^2; five men), were recruited. They each attended on two occasions after an overnight fast. Baseline measurements of resting metabolic rate (RMR), respiratory exchange ratio (RER), forearm blood flow (FBF), arterialized blood glucose and deep venous blood glucose were made. Then on one occasion a stepped infusion of sodium β-hydroxybutyrate was commenced (6 mg/kg per min for 20 min and then 10 mg/kg per min for a further 100 min), whilst on the other occasion an equivalent volume of saline (9 g NaCl/l) was infused. An insulin-glucose clamp was started after 20 min of the higher rate of the ketone or saline infusion and continued for the duration of the infusion, with blood glucose clamped at around 4.3 mmol/l. The Table shows the mean values with standard errors during the insulin-glucose clamp.

	Total Glucose Disposal (mg/kg per min)		Clamp AV Glucose difference (mmol/l)		Clamp FBF (ml/100ml per min)		Forearm Glucose Uptake (μmol/min per litre forearm)		Increment in RMR in Clamp (kJ/min)		Clamp RER	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Saline	9.58	0.56	1.68	0.23	4.40	0.54	70.9	12.4	0.441	0.073	0.94	0.02
Ketone	13.68[a]	1.48	0.51[b]	0.12	8.30[c]	0.78	40.2[a]	9.5	1.970[c]	0.118	0.83[b]	0.01

Significantly different from saline (paired t test): [a]$P <0.05$; [b]$P <0.01$; [c]$P <0.001$

During the ketone infusion there was a reduction in muscle glucose uptake. At the same time total body glucose disposal was increased, but there was no significant rise in RER, thus the glucose load must mainly have been stored with the energy cost of the storage being reflected in the higher metabolic rate response to the clamp during the ketone infusion.

Acute hyperketonaemia causes a similar inhibition of carbohydrate oxidation to that seen after more prolonged starvation.

Mansell, P. I. & Macdonald, I. A. (1990). *Metabolism* **39**, 502-510.

Dietary fat utilization by the exercising forearm. By K.N. FRAYN, A.J. GRIFFITHS, S.M. HUMPHREYS and M.L. CLARK. Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford, OX2 6HE

Both carbohydrate and fat are used as fuels during muscular work. The contribution of fat to energy production appears to be limited to around 50% of maximal sustained power output. Endurance performance is enhanced if the plasma non-esterified fatty acid (NEFA) concentration is elevated by heparin injection (Costill *et al* 1977). Few attempts have been made to modulate the supply of fat to the muscle by dietary means, mainly because consumption of carbohydrate, which is known to be beneficial, will lower the plasma NEFA concentration. However, we have recently shown that consumption of a meal high in both carbohydrate and fat results in maintenance of plasma NEFA concentrations despite prolonged elevation of plasma glucose and insulin concentrations; we believe that this represents direct liberation into the plasma of fatty acids released from chylomicron-triacylglycerol (TAG) by the action of lipoprotein lipase (LPL; *EC* 3.1.1.34) (Griffiths *et al* 1993). In addition, the presence of a high concentration of chylomicron-TAG might in itself be beneficial; chylomicron-TAG is a good substrate for muscle LPL, whereas VLDL-TAG is a very poor one (Potts *et al* 1991).

We have therefore investigated the effects of ingestion of carbohydrate (80 g) and fat (80 g) (with 18 g protein) on the metabolism of the working forearm, in ten normal subjects; each subject was also studied after overnight fast ('control'). Exercise was started 3 h after eating the meal. Arterio-venous differences were measured across the forearm before and at 15 min intervals during 60 min of isometric flexion of the fingers (alternate 5 sec contractions and 5 sec relaxation) to 50 % of maximal voluntary contraction. Forearm blood flow was measured at 15 min intervals by strain-gauge plethysmography during brief interruption of exercise.

| | Plasma TAG (μmol/l) | | | | Plasma NEFA (μmol/l) | | | | Plasma glucose (mmol/l) | | | |
| | Control | | Fat meal | | Control | | Fat meal | | Control | | Fat meal | |
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Arterial concentration	790	80	1690**	160	490	60	570	60	4.8	0.1	5.0	0.2
Potential contribution to O_2 (%)	0	9	47**	28	-6	29	18	4	39	4	58	18
Combined potential contribution to O_2 (TAG, NEFA, glucose, ketones) (%):									35	29	128**	48

Significantly different from control (Mann-Whitney test): **$P<0.02$.

During the period studied, plasma concentrations of glucose and NEFA were similar after the meal (compared with overnight fast) whilst plasma TAG concentrations were considerably higher. During forearm exercise, forearm blood flow and O_2 consumption each increased 5 - 6-fold; there were no differences between nutritional states. The net exchange of NEFA across the forearm was variable in the overnight-fasted state but there was consistent uptake in the fed state; nevertheless, differences in uptake between nutritional states were not significant. In contrast, forearm extraction of plasma TAG was consistently greater in the fed state ($P<0.02$). In order to overcome uncertainties associated with measurement of blood flow, net substrate uptake was expressed as percentage potential contribution to forearm O_2 consumption. The potential contribution of TAG was close to zero in the overnight-fasted state but significantly greater in the fed state (Table). The sum of the potential contributions of glucose, NEFA, TAG and ketone bodies to O_2 consumption was greater both before ($P<0.01$) and during (Table) exercise in the fed compared with the fasted state, implying sparing of endogenous forearm fuels.

The studies were sponsored by Mars, Incorporated.

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The effect of test meal fatty acid composition on postprandial triacylglycerol and lipoprotein lipase responses in normal subjects.

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Since postprandial lipaemia is suggested to be an important factor in atherosclerosis (Patsch, 1987), a human study was carried out in order to compare postprandial triacylglycerol (TAG), and post-heparin lipoprotein lipase (LPL; EC 3.1.1.34), responses to test meals of different fatty acid compositions.

On three separate occasions, eleven male volunteers consumed a standard breakfast and lunch and at 19.00 hours were given one of three test meals in random order, which provided 5.65 MJ (1350 kcal), 35 g of protein, 208 g of carbohydrate, and 40 g of the test oil under investigation (corn oil, fish oil [MaxEPA], or a mixed oil, the latter designed to represent the current fatty acid composition of a typical UK diet). Blood samples were taken at 0 min (pre-prandial sample) and at 30, 60, 120, 180, 240, 300, 360, 420, 480, 540, 600, and 660 min following the consumption of the test meals. After the end of the postprandial period (660 min), blood samples were taken 5 and 15 minutes, after injection of heparin, for measurement of post-heparin LPL activity.

Calculation of incremental areas under the TAG response curve showed that postprandial response was significantly decreased ($P<0.05$) following the fish oil meal (365(SD 145)), compared with that following the mixed oil meal (552(SD 141)), whereas the TAG responses to the corn oil meal were not statistically significantly different from the other meals. Two postprandial peaks were observed, the first one between 30 and 120 min and the second between 240 and 480 minutes postprandially; the most pronounced biphasic pattern was observed following the mixed oil meal. Duncan's range test showed that post-heparin LPL activity was significantly higher ($P<0.01$) in plasma obtained 15 min after the injection of heparin following the fish oil test meal (223 (SD 40) nmol oleate released/min/ml of plasma), compared with 15 minute LPL activity following the mixed oil meal (161 (SD 33) nmol oleate released/min/ml of plasma). Postprandial insulin and gastric inhibitory polypeptide (GIP) responses did not differ following the three test meals.

In conclusion, one of the mechanisms by which fish oils may decrease postprandial lipaemia is by increasing LPL activity and therefore increasing clearance of dietary TAG.

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Influence of the intensity of prior exercise on postprandial lipaemia in man. By R.A. BURNETT, H.E. ALDRED AND A.E. HARDMAN. <u>Department of Physical Education, Sports Science and Recreation Management, Loughborough University, Loughborough, Leicestershire LE11 3TU</u>

Exercise before eating may attenuate postprandial lipaemia (Aldred *et al.* 1993) but it is not known whether this effect is influenced by the intensity of the exercise. The purpose of the present study was to compare the effects of low and high intensity exercise on postprandial lipaemia.

Five normolipidaemic, endurance-trained men aged (mean (range)) 19.7 (19.2 - 20.2) years were studied. Serum concentrations (mmol/l) of lipids, determined after an overnight fast, were: triacylglycerol (TAG) 1.03 (0.80-1.48), total cholesterol 4.50 (3.74-5.04), high density lipoprotein cholesterol (HDL) 1.12 (0.92-1.33), HDL_2 cholesterol 0.38 (0.16-0.51). Subjects ingested a high-fat test meal on three separate occasions, in a balanced, repeated measures design. On two trials, before consumption of the test meal, subjects ran on a motorised treadmill for 90 min at either 48 (40-54) % or 66 (62-79) % of previously determined maximal oxygen uptake (VO_2max). During the other trial no exercise was undertaken (control). Food intake was weighed and recorded for 2 d prior to the first trial and replicated for the 2 d before the second and third trials. Exercise habits were standardized for the 3 d leading up to each trial. Subjects arrived at the laboratory after an overnight fast and then either exercised or rested quietly for 90 min. At 15 min after completion of the exercise, and after a capillary blood sample had been obtained, subjects ingested a meal of cereal, fruit, nuts, chocolate and whipping cream (Schlierf *et al.* 1987). This was given according to body mass and, for a typical 70kg man, had a total energy value of 4968 kJ and contained 85.2 g of fat. Capillary blood sampling was repeated 1 h after consumption of the meal and thereafter at hourly intervals for 6 h. Samples were separated and plasma analysed for TAG. Inter-trial comparisons were made using the Wilcoxon matched pairs signed rank test.

Mean respiratory exchange ratio, heart rate and blood lactate concentration were all higher ($P<0.05$) during high intensity exercise than during low intensity exercise. The total lipaemic response, determined as the area under the TAG v. time curve normalized to the zero hour level, was lower ($P<0.05$) in both exercise trials than in the control trial (66% VO_2max, 2.5 (0.9-4.6) mmol/l.h; 48% VO_2max, 2.0 (0.7-3.0) mmol/l.h; control, 5.2 mmol/l.h (4.2-9.2)) but did not differ between exercise trials. Peak serum TAG concentrations were not significantly different between trials (66%VO_2max, 1.9 (1.4-2.6) mmol/l; 48% VO_2max, 1.8 (1.5-2.0) mmol/l ; control, 2.7 (1.4-4.0) mmol/l).

These observations suggest that the exercise-stimulated attenuation of the total lipaemic response to a high-fat meal may be independent of intensity.

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Microscopical location of guar galactomannan in mid-jejunal biopsies from the pig: potential long-term immunological implications. By F.G. ROBERTS[1,2], A. TURVEY[2], A.G. LOW[2] and P.R. ELLIS[1]. [1]Macromolecular Structure and Function Research Group, Division of Life Sciences, Kings College London, London, W8 7AH.and [2]AFRC Institute of Grassland and Environmental Research, Shinfield, Reading, RG2 9AQ

Dietary supplements of guar gum, containing the polysaccharide galactomannan, are of therapeutic use in the treatment of diabetes. Recent studies with pigs fed on either fully-hydrated solutions of guar gum or guar bread meals, showed substantial increases in viscosity of digesta sampled from the mid-jejunum (Roberts *et al.* 1990 *a, b*). This, together with microscopical evidence suggesting that galactomannan inhibits the digestion of starch by acting as a physical barrier to hydrolysis, supports the idea that galactomannan reduces glucose absorption rates and ultimately postprandial hyperglycaemia. Glucose absorption may also be affected by reductions in the rate of release of starch from the bread matrix, and the inhibition of maltose uptake at the mucosal surface. In this study we have examined mucosal biopsies from guar-fed pigs to determine the topological relationship between the galactomannan fraction of the diet and the epithelial surface of the jejunal mucosa, which is likely to improve our understanding of the physiological effects of guar gum.

Three Large White X Landrace boar pigs were fitted with re-entrant cannulas 2.0m distal to the pylorus. Pigs received meals of wheat bread (C) or wheat bread containing low, medium or high molecular weight (M_w) guar gum flour added at a level of 100g/kg dry ingredients. The bread was chopped into pieces (about 1cm^3) and added to water 15 min. before feeding (2.5kg water/kg diet). Diets were allocated on a random basis and each pig received each diet for 5 d. On the 5th day of each treatment period mucosal biopsies were removed via the distal portion of the cannula from approximately 20cm along the small intestine using a Watson intestinal biopsy capsule (Kik *et al.* 1988). Samples were fixed in a buffered 40g/l formalin solution, stained with each of the chosen fluorescein isothiocyanate conjugated lectins, and veiwed using fluorescence microscopy (Baldo *et al.* 1982).

Stained sections of the mucosal biopsies from pigs fed control or high M_w guar bread meals revealed no visible fluorescence. However, in pigs given low M_w guar bread meals galactomannan appeared to be present in the brush border, goblet cells and lymphatic vessels of pig mucosa. Samples taken from pigs fed medium M_w guar bread also indicated the presence of galactomannan in some sites of the mucosa but at a much reduced intensity of staining. These results indicate that some low M_w species of galactomannan (approximately 500,000 daltons) may be perabsorbed across the mucosal epithelium. Normally, perabsorption is the process by which an individual can be rendered immunologically unresponsive to food components, although in susceptible individuals it may produce intolerance. The extent to which it is valid to extrapolate the current results in growing pigs to the clinical use of guar gum in man is difficult to assess at present. However, since low M_w guar gum remains one of the preferred options of dietary supplements in the treatment of diabetes in terms of palatability and patient compliance, further work is required to evaluate the long-term nutritional and immunological implications of food products containing guar gum and similar non-starch polysaccharides.

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The use of microscopy in the evaluation of the relationship between guar galactomannan and starch in guar wheat bread: subsequent physiological implications in the pig. By C.S. BRENNAN[1], F.G. ROBERTS[1,2], A.G. LOW[2] and P.R. ELLIS[1], [1]Macromolecular Structure and Function Research Group, Division of Life Sciences, Kings College London, London W8 7AH and [2]AFRC Institute for Grassland and Environmental Research, Shinfield, Reading RG2 9AQ

It is now well documented that guar gum has the capacity to reduce postprandial blood glucose and plasma insulin levels in both man and animal models (Jenkins *et al.* 1978; Roberts *et al,* 1992). The mechanism(s) by which these physiological effects are exerted are believed to be related to physico-chemical properties attributed to the galactomannan polysaccharide, most notably viscosity (Jenkins *et al.* 1978). It has long been recognised that the same sources of guar gum may exhibit markedly different physiological effects depending on their mode of administration in the diet (Peterson, 1985) and current evidence would suggest that the incorporation of guar gum into wheat bread will prove a preferred option in terms of both its therapeutic effectiveness and long-term patient compliance. However, to date, little attention has been focused on the exact relationship between guar galactomannan and other nutritional components (e.g. starch and protein) when guar flour is incorporated into whole food products such as bread. This is due, at least in part, to difficulties in identifying galactomannan polysaccharide in the native guar seed, bread and digesta.

Using the modified techniques of Baldo *et al.* (1982), it was possible to identify both the galactomannan deposits of guar seed endosperm, and the starch and galactomannan components of bread both prior to ingestion and also in digesta sampled postprandially at 2m from the pylorus of pigs fitted with jejunal re-entrant cannulas. Samples were removed at 10, 20, 30, 40, 50, 60, 90, 120, 150, 180, 210 and 240 min postprandially and immediately dried onto microscope slides and fixed. Four fluorescein isothiocyanate conjugated lectins were used as appropriate labels for locational studies using fluorescence microscopy.

Examination of sectioned guar seeds revealed discrete, irregular inclusions occupying the greater proportion of the endosperm cell which stained positive and were found to absorb water during controlled experiments. It was concluded that these bodies were the galactomannan deposits. Examination of guar bread sections revealed starch granules enveloped within the bread matrix covered by a layer of galactomannan mucilage. This is in contrast to control bread where starch granules appeared distinct from the loaf matrix. The assumption is that the galactomannan disperses during the baking process and was evident as a fluorescent 'halo' when examined using fluoresence microscopy. In pig digesta, intact starch grains still enveloped by this galactomannan mucilage were observed up to 180 min postprandially following the guar bread meals. In contrast, digesta removed from animals receiving the control wheat bread showed no evidence of intact starch grains after 60 min. Earlier data reported in the same animals (Roberts *et al.* 1990) indicated that pigs fed on guar bread showed an increase in postprandial jejunal digesta viscosity relative to the control diet. Thus, the mechanism by which guar gum reduces the absorption of glucose from bread meals is potentially two-fold. Initially, enzyme-substrate interaction may be inhibited by the presence of the galactomannan mucilage coating the starch granules and secondly, when starch digestion does occur, the observed increase in digesta viscosity may impede the absorption of maltose from the aqueous phase of digesta.

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The effect of dietary lipid manipulation on the natural killer cell activity of rat lymphocytes. By P. YAQOOB, E. J. SHERRINGTON, E. A. NEWSHOLME and P. C. CALDER, <u>Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU</u>

Natural killer (NK) cells are cytotoxic lymphocytes which play a role in lysis of microbial, tumour and virus-infected cells and in transplant rejection. Fish oils and their component n-3 polyunsaturated fatty acids (PUFA) have been shown to suppress many immune cell functions including proliferation, cytokine production and antigen presentation (Calder & Newsholme, 1993). In addition, the PUFA contained in fish oils (eicosapentaenoic and docosahexaenoic acids) have been shown to inhibit the NK cell activity of human peripheral blood lymphocytes *in vitro* (Yamashita *et al.* 1991). There is little information regarding the effect of dietary lipid manipulation upon NK cell activity. In the present study the effects of different dietary lipids upon the NK cell activity of rat spleen lymphocytes were investigated.

Weanling 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 of either hydrogenated coconut oil (HCO), olive oil (OO), safflower oil (SO), evening primrose oil (EPO) or menhaden oil (MO). All diets contained a further 10 g corn oil/kg to prevent essential fatty acid deficiency, 1.2 g vitamin E/kg as antioxidant and adequate amounts of vitamins, minerals and fibre. Spleen lymphocytes were prepared and the NK cell activity determined using a standard chromium release assay: 2×10^4 ^{51}Cr-loaded target cells (murine YAC-1 lymphoma cells) were incubated with spleen lymphocytes at lymphocyte:target (L:T) cell ratios of 100:1, 50:1, 25:1 and 12.5:1. After 4 h the release of ^{51}Cr into the medium was measured; spontaneous release was measured in the absence of added lymphocytes and total release was determined by adding Triton instead of lymphocytes. NK cell activity is expressed as % cytolysis:

$$((^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release})/(\text{total } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release})) \times 100$$

Diet L:T ratio	Cytolysis (%)							
	100:1		50:1		25:1		12.5:1	
	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
LF	46.5¶	2.0	29.7¶	2.8	17.7¶	2.1	7.4¶	1.2
HCO	40.4¶	2.4	22.9¶	1.5	11.5*¶	0.6	4.5	0.8
OO	36.4*	2.8	20.8*¶	1.7	9.3*¶	0.9	3.4*	0.4
SO	42.1¶	1.5	24.2¶	1.3	11.2*¶	1.1	3.9	0.7
EPO	40.7*¶	0.7	19.1*	2.6	8.9*¶	0.2	3.2*	0.4
MO	29.4*	2.1	13.6*	0.7	5.0*	0.3	3.1*	0.8

Data are mean and SEM for four animals fed each diet.
Statistical significance ($P < 0.05$; Student's t-test) compared with *LF or ¶MO.

Spleen lymphocytes from animals fed on the MO, OO or EPO diets had lower NK cell activities than those from animals fed on the LF diet. The NK cell activities of spleen lymphocytes from animals fed on the MO diet were lower than those from animals fed on the other high fat diets. These results confirm the *in vitro* observations of Yamashita *et al.* (1991) but extend these findings to include dietary manipulation with a range of lipids. The potent inhibition of NK cell activity could contribute to the immunosuppressive effect of dietary fish oils which may make them beneficial in the treatment of inflammatory and autoimmune disorders and in prolonging graft survival.

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Effect of the addition of guar gum to an acute test meal on post-prandial lipaemia in healthy young adults. By J.A.LOVEGROVE, L.M.MORGAN, S.G.ISHERWOOD & C.M.WILLIAMS, Nutritional Metabolism Research Group, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH.

The inclusion of a soluble non-starch polysaccharide (NSP) into the diet has been shown to lower post-prandial hyperglycaemia (Morgan *et al.* 1979), to be an effective treatment for diabetics (Jenkins *et al.* 1980) and to lower fasting plasma cholesterol levels (Jenkins *et al.* 1979). However the effect of the addition of soluble NSP to an acute test meal on post-prandial lipaemia has not been extensively studied.

The aim of the present study was to determine the effect of incorporating guar gum, a galactomanan derived from the seed of an Indian cluster bean (*Cyamopsis tetragonoloba*) , into an acute test meal on post-prandial concentrations of triacylglycerol in healthy young adults.

On two occasions, six healthy young male subjects (18-24 years) attended the investigation unit at 09.00 hours after an overnight fast and having standardized their food intake the previous day. They were randomly allocated to take a standard meal consisting of 49 g fat, 49 g protein and 116 g carbohydrate and containing 4564 KJ with (GUAR) and without (CONTROL) the addition of 10 g guar gum (Guarem; Rybar Laboratories). Hourly blood samples were taken for an 8 h period post-prandially and triacylglycerol was determined using an automated spectrophotometric assay.

The time of appearance of the peak post-prandial triacylglycerol concentration was significantly later with the GUAR meal compared with the CONTROL meal ($p<0.05$) (CONTROL 140 (SEM 6) vs GUAR 240 (SEM 20) mins). There was a trend to a lower mean post-prandial peak triacylglycerol concentration with the GUAR meal compared with the CONTROL meal (CONTROL 2.39 (SEM 0.41) vs GUAR 1.88 (SEM 0.28) mmol/l) although this did not reach statistical significance. Fasting triacylglycerol levels were reached with both meals 8 h post-prandially (CONTROL 1.17 (SEM 0.01) vs GUAR 1.11 (SEM 0.13) mmol/l). A small second peak was observed at 5 h in the triacylglycerol profile after the guar gum-containing meal, although the significance of this second peak is not known.

The present study has demonstrated that guar gum supplementation into a test meal containing solid foods significantly lengthened the time of appearance of the peak post-prandial triacylglycerol concentration, with a trend to a reduced mean post-prandial peak triacylglycerol concentration in healthy young subjects. Recent evidence for atherogenic effects of postprandial lipoproteins suggest that further postprandial studies in individuals with elevated triacylglycerol concentrations and long-term guar gum supplementation are warranted.

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Measurement of apolipoprotein B-48 with a novel specific antibody reveals two postprandial peaks of intestinal origin. By A.S.PEEL, A.ZAMPELAS, B.J.GOULD, E.AH-SING, J.C.CHAKRABORTY, R.J.HOWLAND and C.M.WILLIAMS <u>Nutritional Metabolism Research Group, School of Biological Sciences, University of Surrey, Guildford, GU2 5XH</u>

Measurement of apolipoprotein B-48 (apo B-48) could provide a specific marker for intestinally derived lipoproteins. To date, these have largely been studied using the analysis of ingested retinyl palmitate, although this method has been criticised (Krasinski *et al.* 1990). Until recently, measurement of apo B-48 was hampered by the lack of a specific antibody (Peel *et al.* 1992).

On two occasions, eleven normal subjects were given an identical breakfast and lunch. At 19.00 hours, they were allocated on a random basis to take an acute test-meal containing either 40 g mixed oil or 40 g fish oil. These were incorporated as part of a normal meal and labelled with retinyl palmitate (700 IU / kg body weight). Hourly blood samples were taken and used to prepare triacylglycerol-rich lipoproteins (TRL) by ultracentrifugation. In this fraction: apo B-48 was separated using denaturing polyacrylamide gel electrophoresis and immunodetected following transfer to a nitrocellulose membrane, triacylglycerol was measured on an automated analyser by a colorimetric assay and retinyl palmitate was detected using high performance liquid chromatography.

For both meals, triacylglycerol had a primary peak at 1 to 2 h after the meal and a secondary peak at 5 to 7 h. Apo B-48 also had two peaks, at 1 to 2 h and 5 to 8 h. There was only one peak in retinyl palmitate which occurred at 5 to 7 h. For the first peak, the incremental area under the curve for apo B-48 was the same following both meals, but for the second peak it was significantly higher ($P<0.05$, Student's t-test) after mixed oil compared to fish oil. Retinyl palmitate levels were identical in the declining phase after both meals. However, apo B-48 returned to baseline 7 to 8 h following the fish oil meal, but was still elevated at this time after mixed oil.

Members of our group have not previously observed an early triacylglycerol peak in subjects studied following an overnight fast (Zampelas *et al.* 1993). To the best of our knowledge the present study is the first demonstration of two postprandial peaks clearly shown to be of intestinal origin. This is probably because previous studies have either been conducted in subjects after an overnight fast, have only measured samples taken every 3 h, or have used retinyl palmitate for chylomicron analysis. The evidence suggests that the early peak in triacylglycerol and apo B-48 is from an earlier meal, but the source of the second peak is from the meal that was ingested at 19.00 hours. Furthermore, the results suggest that apo B-48 may be a more reliable measure for detecting clearance of intestinally derived lipoproteins and that this is accelerated following a fish-oil meal compared to a mixed-oil meal.

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Central and peripheral cardiovascular responses to meals of different composition measured over a 3 h period.

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Deleterious effects of food ingestion have been observed in angina patients (Cowley *et al.* 1991) and also in healthy elderly individuals (Sidery *et al.* 1993). As yet, measurement of the cardiovascular responses to a meal have been limited to a short (60-90 min) postprandial period. The aim of the present study was to record the central and peripheral cardiovascular responses to meals over a 3 h period after food.

Seven healthy subjects (mean age 26.3 years, mean body mass index $21.4kg/m^2$) were studied on three occasions. Measurements of heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure by oscillometry, cardiac output (CO) using a pulsed wave Doppler ultrasound technique (Digidop; Scimed, Bristol), calf blood flow (CBF) by venous occlusion plethysmography, and superior mesenteric artery blood flow (SMABF) by Duplex Doppler ultrasound, were made before and for 3 h after either a high-carbohydrate (HC), or a high-fat (HF) meal, both 2.5MJ or water. The meals consisted of solid food.

There was a significant rise in HR after the HC meal (10.3b/min. 95% CI 7.2 to 13.4b/min) and after the HF meal (7.7b/min. 95% CI 2.5 to 12.9b/min; $P < 0.001$) and also in CO after the HC meal (1.58l/min. 95% CI 0.66 to 2.5l/min; $P < 0.0001$) and after the HF meal (1.36l/min. 95% CI 0.51 to 2.21l/min; $P < 0.0001$) occurring at 30 min after both meals. HR and CO were still significantly ($P < 0.05$ and $P < 0.02$ respectively) elevated at 180 min after the HF meal but had returned to baseline 120 min after the HC meal. SMABF was maximal 15 min after HC (95% CI of the change 260 to 647ml.min^{-1}) and 30min after HF (95% CI of the change 373 to 614ml.min^{-1}). SMABF was significantly greater after the HF meal compared with the HC meal 150 min into the postprandial period ($P < 0.05$).

CBF fell significantly in the first 30 min only after the HF meal (95% CI -0.04 to -1.2ml/100ml per.min). CBF then recovered towards baseline. In contrast CBF rose after the HC meal, peaking 150 min postprandially (95% CI 0.2 to 2.9ml/100ml per min). These observations are reflected in the changes in calculated vascular resistance which fell significantly after the HC meal at 150 min (95% CI -1.7 to -14.9). No significant change occurred after the HF meal.

SBP initially rose 30 min after the HC meal (95% CI 1 to 10.3 mmHg) and then fell to a nadir 150 min postprandially. DBP also fell significantly 150 min after the HC meal (95% CI -1 to -13 mmHg). There was no significant change in blood pressure after HF.

The present study is the first to examine the central and peripheral cardiovascular responses to food ingestion for 3 h after solid meals in young subjects. The study confirms the differential effect of HC and HF meals on the mesenteric bed, but also demonstrates that the cardiovascular responses to food are still evident 3 h postprandially, particularly after the HF meal. The falls in both SBP and DBP 150 min after food in the young after the HC meal is a novel observation. Increases in skeletal muscle blood flow have been observed after liquid meals and the present study suggests that this rise in blood flow is delayed on ingestion of solid food.

Cowley, A.J., Fullwood, L.F., Stainer, K., Harrison, E, & Hampton, J.R. (1991). British Heart Journal **66**, 147-150.
Sidery, M.B., Cowley, A.J. & Macdonald, I.A. (1993). Clinical Science **84**, 263-270.

Modest *in utero* protein restriction modulates responses to endotoxin in seven week old rats fed standard chow. By S.C. LANGLEY, M. SEAKINS, R.F. GRIMBLE and A.A. JACKSON. Department of Human Nutrition, University of Southampton, SO9 3TU.

Epidemiological studies (Barker *et al.* 1990) have shown a close association of low birth weight with hypertension, diabetes mellitus, ischaemic heart disease and chronic lung disease in adulthood. A rat model to investigate the mechanisms underlying these associations has recently been developed (Langley & Jackson, 1993). Female Wistar rats were fed on a diet of normal protein content (18 % casein, plus 10 g methionine/kg), or low protein diets containing 12, 9 or 6 % casein, plus 10 g methionine/kg. The diets were fed for two weeks before mating, and throughout pregnancy. All dams received standard rat chow (CRMX, 20 % protein) during lactation, and the offspring were subsequently weaned onto this chow diet at the age of 4 weeks. At the age of weaning the offspring of the dams that had received 18, 12, 9 and 6 % casein had gained (Mean (SE) 46 (2), 53 (1), 51 (2) and 46 (1) g respectively (P < 0.05 for 12 and 9 % casein vs 18 and 6 % casein). Responses of male animals aged 7 weeks, from each dietary group, to an intraperitoneal injection of endotoxin (E; 200 μg/kg body weight) were examined. Controls from each group received saline injections (S) and were pair-fed. Animals were killed at 24 h post injection and liver, lung and spleen removed. Reduced glutathione (GSH) in liver and lung, and Zn in liver, lung and spleen were determined.

| | | Dietary protein (%) | | | | | | | |
| | | 18 | | 2 | | 9 | | 6 | |
	Injection	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Liver GSH (μmol/g)	S	4.4[a]	0.6	3.6[a]	0.4	2.4[b]	0.4	3.4[b]	0.2
	E	5.1[a]	0.5	5.0[a]	0.1	4.7[a]	0.3	5.2[a]	1.2
Lung GSH (μmol/g)	S	0.9[a]	0.1	0.8[a]	0.1	0.8[a]	0.1	0.9[a]	0.1
	E	1.2[a]	0.1	0.6[b]	0.1	0.8[c]	0.1	0.8[c]	0.1
Liver Zn (μg/g)	S	44[a]	4	41[a]	3	35[a]	2	37[a]	2
	E	55[a]	3	57[a]	4	40[b]	1	40[b]	2
Spleen Zn (μg/g)	S	20[a]	2	19[a]	1	20[a]	1	16[a]	2
	E	24[a]	1	23[a]	1	26[a]	1	17[b]	3

Values with different superscripts a,b,c are significantly different (P < 0.05, ANOVA).
n 4-5 animals.

Prenatal exposure to various degrees of protein restriction resulted in modified inflammatory responses in adult animals. These were characterized by an inability to increase lung GSH in offspring from the low-protein groups, a diminished gain in hepatic Zn in the 9 and 6 % casein groups, and a failure to increase total spleen Zn in the 6 % casein group. Prenatal exposure to modest as well as, severe protein restriction *in utero* has long lasting effects on the response of lung and liver, and the components of the reticulo-endothelial system to an inflammatory agent.

Barker, D.J.P., Bull, A.R., Osmond, C., Simmonds, F.J. (1990). British Medical Journal **301** 259-262.
Langley, S.C. & Jackson A.A. (1993). Clinical Science (In the Press).

The respective roles of gastric distension and intestinal nutrients in the induction of post-prandial sensations. By M. I. KHAN, C. FEINLE and N. W. READ, Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Herries Road, Sheffield S5 7AU

The aim of the present study was to investigate the subjective sensations induced by gastric distension alone, and the modulation of these sensations by the infusion of lipid into the duodenum. In ten healthy male volunteers (20-34 years old) distensions of the stomach with air at a rate of 100 ml/min were carried out, using a latex balloon. To measure intragastric pressure changes, the balloon was fitted with a manometric catheter. The study consisted of three consecutive infusions: 9 g NaCl/l, 200 g Intralipid/l and 0.9 g NaCl/l. These were administered into the duodenum, at a rate of 1.1 ml/min, using a nasoduodenal tube. By 30 min into each infusion (with the infusion continued) the stomach was distended. During distension, subjects were asked to report and describe when balloon perception and fullness occurred and when distension became uncomfortable, and whether the discomfort was associated with nausea. Preliminary studies showed these sensations to occur reproducibly, during distensions. In addition, during infusions subjects were periodically asked to describe any changes in hunger and drowsiness.

During the lipid infusion subjects reported balloon perception, fullness and discomfort at higher volumes of air than during the control infusions (Table 1, data is expressed in ml).

Table 1

Infusion	n	Balloon perception mean SE	Fullness mean SE	Discomfort mean SE
Control 1	10	93 13	302 43	456 44
Test	10	166 32[*]	406 45	598 62[*]
Control 2	10	125 15	335 63	497 87

[*] = significantly different from control 1, $P < 0.05$, analyzed by Kruskall-Wallis analysis of variance followed by Wilcoxon rank-sum test

The lipid infusion also caused an increase in gastric compliance (δ volume/ δ pressure, $P < 0.05$), and decreased the amplitude and frequency of gastric phasic activity ($P < 0.01$). Moreover, subjects said that the fullness sensation during the lipid infusion was more 'meal-like', as opposed to localized pressure felt during the saline infusions. Two subjects described it as being pleasurable. The effect of lipid upon other subjective sensations are summarized in Table 2 (the data shows the number of subjects experiencing each sensation):

Table 2

Infusion	n	Decreased hunger	Drowsiness	Nausea
Control 1	10	0	0	2
Test	10	7[*]	6[*]	9[*]

[*] = significantly different from control 1, $P < 0.01$, analyzed by chi-square test

In conclusion, the presence of lipid (and other nutrients?) in the small intestine acts to bring about gastric accomodation and produces 'post-prandial satisfaction'.

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The potential value of *Bacillus stearothermophilus* spores as a gut transit time marker. By J.C. MATHERS and S. CARTER, Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU

Whilst several markers are in widespread use for measurement of gut transit time (TT), none is entirely satisfactory. The primary aim of the present study was to investigate the use of a novel marker, a pulse dose of the spores of the thermophilic bacterium *Bacillus stearothermophilus* (BSS), to estimate TT in the rat. Marteau *et al.* (1990) used BSS in acute studies of small intestinal absorption in man. In the present study, comparisons were made with a water soluble marker (CrEDTA) also given as a pulse dose. The secondary aim was to determine the response in TT to alterations in starch supply to the large bowel by feeding raw potato starch (RPS). Details of the rats and diets are provided by Mathers & Smith (1993).

After 15 d consumption of the experimental diets, the rats were given 2 ml Cr EDTA solution and 0.9 ml BSS suspension (containing 10^8 spores/ml) mixed with the diet at 22.00 hours. All faeces were collected 4 times daily for the first 2 d and then twice daily for a further 5 d and freeze-dried. Aqueous faecal slurries were prepared, diluted appropriately, spread on nutrient agar (Oxoid CN3; Unipath, Basingstoke, Harts) plates and incubated at 65° for 12–24 h.

BSS germinated at 65° to produce small, white colonies with a raised appearance. No other thermophiles could be consistently cultured from faeces under these conditions. From both faecal CrEDTA and BSS concentrations whole gut TT was calculated as:

$$Whole\ gut\ TT = \frac{\Sigma m_i t_i}{\Sigma m_i}$$

where m_i is the number of spores (or quantity of Cr EDTA) excreted at time t_i since dosing.

Dietary RPS (g/kg)...	0	80	160	240	SEM	Statistical Effects
Whole gut mean TT (h)						
Spores	33	34	29	35	2.2	NS
Cr EDTA	19	21	18	21	1.5	NS

NS, not significant

Estimates of whole gut TT were on average 13 h longer with BSS than with Cr EDTA but no between-diet differences were found for either marker. Although there was a clear difference in estimates of TT for the two markers, for individual rats, the values obtained were strongly correlated:

Spore TT = 9.3 (SD 3.54) + 1.2 (SD 0.18) Cr EDTA TT   ($r^2 = 0.72$)

This initial study has shown that BSS may be used to estimate TT but further studies are needed to determine whether they are to be preferred over chemical markers.

Marteau, P., Fluorie, B., Pochart, P., Chastang, C., Desjeux, J.F. & Rambaud, J.C. (1990). *British Journal of Nutrition* **64**, 71–79.
Mathers, J.C. & Smith, H. (1993). *Proceedings of the Nutrition Society* [In the Press]

Digestibility in Labrador Retrievers during growth. By M.S. GILHAM, D. BOOLES, J.V. JOHNSON and V. LEGRAND-DEFRETIN, <u>Waltham Centre for Pet Nutrition, Waltham-on-the-Wolds, Melton Mowbray, Leics. LE14 4RT</u>

The effect of age on digestibility of macronutrients was investigated in a group of Labrador Retrievers. Four male and twelve female puppies (8 weeks of age) were allocated to the trial. Littermates of the same sex and similar weight were randomly assigned to two different complete commercial dry diets to give an equal male, female, weight split on each diet. The puppies were housed in pairs and trained to eat individually before the start of the trial. Daily food intakes and weekly bodyweights were recorded for each puppy. Assessment of digestibility was performed on each pair of puppies at age 11-12, 15-16 and 19-20 weeks by a 4d faeces collection. Food and faeces samples were analysed for proximate nutrients and energy (bomb calorimetry) to determine apparent digestibilities. Statistical analysis (ANOVA) was carried out to assess the effect of age and diet on digestibilities of nutrients in puppies. Proximate analysis of the two diets were as follows:

Nutrients (g/kg air-dry diet)	Diet 1	Diet 2
Moisture	73.0	75.0
Protein	328.0	353.0
Fat	209.0	98.0
NFE	326.0	420.0
Predicted Metabolizable Energy (MJ/kg air-dry diet)	17.0	14.8

The following Table indicates the mean digestibility coefficients with their standard errors for puppies of different ages fed on the two dry diets:

	Week	12		16		20	
	Nutrient	Mean	SE	Mean	SE	Mean	SE
Diet 1:	Dry matter	0.82	0.009	0.83	0.007	0.84	0.008
	Crude protein	0.80[a]	0.014	0.82[a]	0.013	0.82[a]	0.007
	Fat	0.97	0.001	0.97	0.001	0.97	0.003
	NFE	0.79	0.014	0.81	0.012	0.82	0.014
	Energy	0.86	0.007	0.87	0.004	0.87	0.005
Diet 2:	Dry matter	0.85	0.007	0.83	0.002	0.86	0.014
	Crude protein	0.78[a]	0.008	0.73[b]	0.007	0.81[a]	0.014
	Fat	0.95	0.005	0.96	0.004	0.95	0.012
	NFE	0.90	0.015	0.90	0.005	0.90	0.012
	Energy	0.85	0.005	0.86	0.029	0.87	0.012

[a,b]   Values with unlike superscripts are significantly different (ANOVA): $P < 0.05$

The results showed good digestibilities of all the nutrients in Labrador Retriever puppies. There was no effect of diet or age on dry matter, fat, carbohydrate and energy digestibilities. The only statistical difference observed was for crude protein digestibility in puppies fed diet 2 at 16 weeks of age. This is probably not related to an age effect. In conclusion, digestibility in Labrador Retriever puppies between 12 and 20 weeks of age does not seem to be affected by the physiological development of the intestine occurring during growth.

The effect of increased ammonia supply on post-prandial hepatic metabolism in growing steers fed either forage or cereal-based diets. By S.A. MALTBY[2], L.A. CROMPTON[1], M.A. LOMAX[1], D.E. BEEVER[2] and C.J. PIPPARD[1] [1]Department of Biochemistry and Physiology, University of Reading, Reading, RG6 2AJ and [2]AFRC Institute for Grassland and Environmental Research, Hurley, Maidenhead, Berkshire, SL6 5LR

Ruminant diets containing a high proportion of forages are associated with poor efficiency of utilization of metabolizable energy (ME) and dietary N for growth (Beever et al. 1988; Reynolds, 1992). A possible cause of this effect is an increase in hepatic amino acid deamination as a result of the higher rates of urea synthesis necessary to detoxify the increased portal ammonia supply on forage-based diets (Fitch et al. 1989).

In the present study we examined the effect of increased ammonia supply to the liver on net hepatic metabolism in four Friesian steers (live weight 169 kg) using venous-arterial difference and blood flow rate procedures. Animals were fed twice daily in a cross-over design either grass silage (S, 700 g/kg total diet) with dried grass pellets (300 g/kg) or rolled barley (B; 700 g/kg total diet) with dried grass pellets (300 g/kg). Average ME and N intakes were 38.5 and 40.0 MJ/d, and 93.8 and 78.8 gN/d for S and B respectively. After adaptation to diets blood samples were taken 0.5 h before and for 6.5 h after the morning feed. The sampling procedure was then repeated after adding urea (U) to the diets for 7 d (SU 49.1, BU 23.5 gN/d).

Net hepatic flux (mmol/min)	S	SU	B	BU	SEM
Ammonia	-2.50	-3.55	-1.28	-2.68	0.273
Urea	1.13	2.21	0.99	1.88	0.243
Total amino acids	-0.42	-0.48	-0.48	-0.66	0.330

Hepatic ammonia uptake was higher for diet S than for diet B ($P<0.001$) and was increased with the addition of U to both basal diets ($P<0.001$). Hepatic urea production was increased by U addition ($P<0.01$) but there was no difference between S and B. On an incremental basis, the increase in hepatic ammonia N uptake due to addition of U (SU 1.05, BU 1.40 mmol/min) accounted for a lower proportion of the increment in hepatic urea N production (SU 2.14, BU 1.78 mmol/min) for diet S than for diet B . Total hepatic amino acid removal was not altered by diet or U and could not account for the hepatic urea N not apparently derived from ammonia when U was added to the diets.

The results suggest that hepatic amino acid deamination is increased more in cattle fed a roughage diet compared with a cereal-based diet during an increased supply of ammonia to the liver over the postprandial period but this occurs without alteration in net hepatic amino acid removal.

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Reynolds, C.K. (1992). Journal of Nutrition **122**, 850-854.

Fitch, N.A., Gill, M., Lomax, M.A. & Beever,D.E. (1989) Proceedings of the nutrition society **48**, 76A.

Adaptation to a diet by cats and dogs: implications for protocol design. By H.M.R. NOTT, S.R. RIGBY, J.V.JOHNSON and S.J.BAILEY, Waltham Centre for Pet Nutrition, Waltham-on-the-Wolds, Melton Mowbray, Leics. LE14 4RT

The domestic cat is an obligate carnivore, whereas the dog has adapted to a more varied diet. Previous studies have shown differences in the digestibilities of diets in cats and dogs (Kendall *et al.* 1982), although adaptation to the diet over time was not examined.

A diet designed to meet the nutrient requirements of both cats and dogs (moisture 71.8%, protein 11.4%, fat 8.5%, carbohydrate 6.1%, ash 2.2%) was fed to four adult cats and six adult male beagles for 21 d. Faeces were collected over three time periods; days 4-7, days 8-14 and days 15-21. Food and faeces were analysed for dry matter, gross energy (bomb calorimetry), protein, fat and ash to determine apparent digestibilities. The results are shown in the Table.

Period (days)	4-7		8-14		15-21	
	mean	SEM	mean	SEM	mean	SEM
Dry Matter Digestibility						
Dog (n6)	0.85	0.073	0.85	0.002	0.85	0.003
Cat (n4)	0.85	0.335	0.89	0.016	0.85	0.013
Energy Digestibility						
Dog (n6)	0.90	0.005	0.91	0.001	0.91	0.002
Cat (n4)	NA		0.94	0.011	0.91	0.009
Protein Digestibility						
Dog (n6)	0.87	0.006	0.87	0.003	0.87	0.004
Cat (n4)	NA		0.91[a]	0.013	0.89[b]	0.013
Fat Digestibility						
Dog (n6)	0.97	0.002	0.97	0.001	0.97	0.001
Cat (n4)	NA		0.97	0.003	0.97	0.004
Nitrogen Free Extract Digestibility						
Dog (n6)	0.81	0.011	0.81	0.004	0.80	0.003
Cat (n4)	NA		0.90	0.009	0.84	0.010
Faeces Dry Matter %						
Dog (n6)	38.0	0.85	38.0	0.51	37.5	0.55
Cat (n4)	56.4[c]	8.71	50.8	8.54	38.2[d]	0.62
Wet faeces ouput (g per 100g DM eaten)						
Dog (n6)	40.2	1.62	39.6	0.7	40.1	0.84
Cat (n4)	33.8	7.18	24.3	8.49	39.9	6.67

NA, data not available due to small sample weights. a,b and c,d are significantly different, $P<0.05$.

There were no significant differences between the different collection periods in any of the variables measured in dogs. In the cat, dry matter and energy digestibilities were consistent between the collection periods but protein digestibilities were significantly lower ($P<0.05$) over days 15 - 21 than the collection over days 8 -14. During the collection over days 4 - 7 the cats produced quite dry faeces, reflected in the faeces dry matter percentages. This had stabilized to more typical levels during the last collection.

Individual variability in the dogs was relatively low resulting in small standard errors, whereas the cats were much more variable. In contrast to Kendall *et al.* (1982) we found no significant differences between the cats and dogs for any of the variables measured.

These results suggest that a 4 d collection period following a 3 d adaptation is sufficient to measure apparent digestibilities in dogs. However in the cat this short period of collection was insufficient to enable full faecal analysis. In addition there is evidence to suggest that cats may require a longer adaptation period if accurate digestibilities are to be measured.

Kendall, P.T., Holme, D.W. & Smith, P.M. (1982). *Journal of Small Animal Practice* **23**, 577-587.

**Effects of overfeeding on protein-energy metabolism and body composition of high genetic
potential boars.** By R. URQUHART[1], J. McEVOY[2] and K.J. McCRACKEN[1,3], [1]Department of
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The studies of Campbell & Taverner (1988) and Rao & McCracken (1992) showed linear increases in
protein deposition and weight gain up to the appetite ceiling in pigs of high genetic potential for lean
growth and it has been suggested that appetite has become a limiting factor for protein depositions in
pigs selected for lean carcasses. The present study investigated the effects of increasing food intake
above the appetite limit by use of intragastric feeding.

Four replicates of four littermate, pedigree, Landrace boars were studied between 40-90 kg. One
pig from each litter acted as a control and was fed *ad lib*. The remaining pigs were cannulated in the
fundic region of the stomach and fed at 100, 120 or 140 % of the intake of the control pig via the
stomach cannula. The diet contained 240 g crude protein (CP; N x 6.25) and 15.1 MJ metabolizable
energy (ME)/kg dry matter. The control pig was given pelleted feed but the diet was ground and mixed
with water for the cannulated pigs which were fed five times daily. Pigs were killed at 90 kg and body
composition determined. Energy retention from 40-90 kg was calculated from protein and fat gain
using values of 23.8 and 39.3 MJ/ kg respectively. Results were analysed using one-way ANOVA.

Feeding Level...	Control	100 %	120 %	140 %	SEM	*P*
ME intake (MJ/d)	34.4	36.3	42.2	47.5	0.72	<0.001
EBG (kg/d)	1.14	1.17	1.27	1.27	0.048	0.180
FCR (ME:EBG)	30.6	31.1	33.3	38.3	1.29	0.008
CP gain (g/d)	181	181	170	170	10.8	0.796
Fat gain (g/d)	172	198	242	295	13.4	<0.001
ER (MJ/d)	11.1	12.1	13.6	15.6	0.66	0.006

EBG, empty body gain; FCR, feed conversion ratio; ER, energy retention.

Growth rate tended to increase with the high levels of energy intake but the effect was not
significant. FCR was significantly poorer ($P<0.01$). There was no significant effect of treatment on
protein gain but fat gain increased ($P<0.001$) with increasing level of feed. Consequently ER also
increased ($P<0.01$).

The results demonstrate that the pigs fed *ad lib*. in the present study were already achieving
maximum rates of protein deposition. They, therefore, cast doubt on the hypothesis that appetite is
limiting protein depostion in pigs of high genetic potential, at least under conditions of individual
feeding.

Campbell, R.G. & Taverner, M.R. (1988). Journal of Animal Science, 66, 676-686.

Rao, D.S. & McCracken, K.J. (1992). Animal Production, 54, 83-93.

Energy restriction affects muscle fibre area and distribution selectively during growth.
By A.P. HARRISON, K.A. BURTON and M.J. DAUNCEY, Department of Cellular Physiology, AFRC Babraham Institute, Cambridge CB2 4AT

Energy deficiency over a 2 week period results in a reduction in size of fast-twitch but not slow-twitch fibres in *triceps brachii* and *quadriceps femoris* muscles of exercising human adults (Henriksson, 1990). This selective preservation of slow fibre area during reduced energy availability could be energetically advantageous, since the energy expenditure per unit tension developed is lower in slow-than in fast-twitch fibres. The present study aimed to determine first, whether such a mechanism operates in young growing animals and second, whether changes in the relative distribution of fibre types occur during this period of particularly dynamic myofibre differentiation.

Six pairs of 3 week old littermate pigs, mean weight (SEM) 5.0 (0.5) kg, were housed individually at a temperature of 26°, which is close to thermal neutrality. Within each pair, one animal was given a high energy intake over the next 4 weeks, while the other was fed a restricted intake amounting to half that of its littermate. Intakes at 7 weeks were 9.8 and 4.9 MJ/d for the high and low diets respectively and mean (SEM) body weights for the two groups were 12.5 (0.3) and 8.7 (0.6) kg. Animals were killed humanely for sampling of *longissimus dorsi* (LD) and *rhomboideus* (RH) muscles. Histochemical staining of frozen sections for myosin ATPase (EC 3.6.1.3) and succinate dehydrogenase (EC 1.3.99.1) activities was used to evaluate contractile (slow/fast) and metabolic (oxidative/glycolytic) properties. Most fibres could be classified as type I (slow oxidative; SO), IIA (fast oxidative glycolytic; FOG) or IIB (fast glycolytic; FG), with less than 5% being IIC (fast oxidative glycolytic, intermediate). Measurements of fibre size and the proportion of each fibre type were made using an image analysis system (Seescan, Cambridge).

Muscle ...	*Longissimus dorsi*						*Rhomboideus*					
Fibre type ...	I (SO)		IIA (FOG)		IIB (FG)		I (SO)		IIA (FOG)		IIB (FG)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Fibre area (μm^2)												
High energy	1027	56	908	51	1116	85	1160	143	1367	98	1516	162
Low energy	802*	50	611**	25	863**	46	928	70	1071	89	1314	115
Distribution (%)												
High energy	8.0	1.0	37.1	1.8	53.3	2.6	35.0	2.1	41.7	2.3	19.0	3.2
Low energy	9.0	1.0	37.4	2.5	52.4	2.0	49.9*	3.6	36.7	2.0	10.8**	2.1

Significantly different from high energy diet (Student's paired t test); *$P<0.05$, **$P<0.01$.

Results were markedly dependent on muscle type. Areas of all fibres in LD muscle were smaller on the low than on the high intake; the reduction was similar to the 30% lower body weight in animals on the restricted diet. Fibre area tended to be affected similarly in RH but there was wide individual variation and none of the comparisons was significant. Most striking was the finding that in RH muscle the reduced energy intake resulted in a greater proportion of slow oxidative fibres and fewer fast glycolytic fibres. No such effects were observed in LD muscle. It is concluded that energy restriction in the early postnatal period does not result in selective preservation of fibre area but can significantly influence myofibre type differentiation. The changes in proportion of fibre types in RH muscle would improve the energetic efficiency of contraction on the reduced food intake.

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The energy intakes of growing canaries. By E. J. TAYLOR, E. LAMBERT and N. MOODIE. Waltham Centre for Pet Nutrition, Waltham-on-the-Wolds, Melton Mowbray, Leics, LE14 4RT

A study using twelve pairs of breeding canaries was carried out to determine growth rates and energy intakes of canary chicks. Each individually-caged pair of breeding birds was offered a standard seed diet and egg-based breeding diet until the end of the egg incubation period. Once chicks hatched the birds were offered boiled, mashed egg (chicken) twice daily in addition to the basal diet. The total daily food consumption per cage was recorded. This was done until each pair of birds had successfully reared two clutches.

To obtain a record of the growth rate of canary chicks the present study was carried out on a subsample of five newly-hatched chicks from the same clutch. On the third day following hatching each chick was marked and weighed. The chicks were weighed every second day until the age of 21 d, then on days 25 and 30. Although a larger subsample would have been preferable, the potential disruption to the breeding colony precluded handling of a large number of chicks from different clutches. Regular handling of young passerines has previously been shown to result in premature fledging (Coles, 1985). The growth rate of the canary was shown to be rapid with the maximal growth period occurring during the first 10 d following hatching. Fitting the data to a saturation kinetics model (Mercer et al. 1991) predicted an asymptote of 20.1 g of which 90% was attained at or around day 11. After this point the rate of growth slowed considerably and began to plateau around day 17 as shown in the Table. Fledging occurred immediately after this.

Following hatching the mean daily energy intake by individual canary chicks was found to be approximately 3 kJ for the first 48 h. By day 5 this increased to 15 kJ per chick and continued to increase so that by day 10 the energy intake was equivalent to that of an adult bird (50 - 60 kJ/d). Peak energy demand occurred around day 9 when the intake was equivalent to 3.68 kJ/g bodyweight (see Table). This is approximately 16% higher than the demand of an adult canary.

Day	3	5	7	9	11	13	15	17	19
Bodyweight (g)	3.6	6.7	10.2	13.2	16.3	17.5	17.9	18.8	19.0
SE	0.28	0.48	0.52	0.43	0.40	0.34	0.18	0.28	0.09
Energy intake (kJ/g bodywt)	2.73	2.31	3.03	3.68	3.31	2.84	3.27	2.79	3.17
SE	0.73	0.36	0.25	0.31	0.19	0.11	0.16	0.14	0.22

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Maternal manipulation of iodothyronine 5'deiodinase activity in the ovine foetus.
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Chronic maternal cold exposure induced by winter shearing during the final month of gestation stimulates the thermogenic activity of brown adipose tissue in the newborn lamb (Symonds *et al.* 1992). The present study extends these findings by investigating the extent of which altering the maternal metabolic environment may influence the ability of newborn lambs to thermoregulate as a result of changes in liver development.

Twenty-one Bluefaced Leicester x Swaledale ewes were individually housed at ambient temperature (-6 to 19°) and fed daily on a diet comprising 200 g barley concentrate and 1.0 kg chopped hay. At 4 weeks before predicted lambing date eleven ewes were shorn. Foetal and neonatal livers were sampled from shorn (S) and unshorn (US) groups at 140 and 145 d of gestation and within 2 h after birth for measurement of iodothyronine 5'deiodinase (I5'D) activity (Wu *et al.* 1990) and glycogen content. In newborn lambs both twins from a S and US were sampled, plus one twin from four US and three S ewes.

Stage of gestation...	140 d				145 d				Birth			
	US		S		US		S		US		S	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Liver weight (g)	115	18	112	11	105	17	108	12	88*	9	118	7
I5'D (pmol/mg protein per h)	1063	248	1071	394	1495	394	1838	662	2112*	264	2602	368
Total glycogen (mmol)	30	10	24	6	26	12	29	4	12	5	21	9

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

Over the final week of gestation there was no difference in liver weight, I5'D activity or glycogen content between S and US groups. Between day 145 of gestation and birth a decrease in liver weight and glycogen content occurred only in US group, resulting in lambs born from S ewes possessing 25% larger livers which contained 43% more glycogen than the US group. The mean (SEM) plasma concentration of triiodothyronine (T_3) was also 27% greater (S 6.45 (0.60); US 4.68 (0.44) nM $P < 0.05$) in lambs born from S ewes and their hepatic I5'D activity was 19% higher.

It is concluded that chronic maternal cold exposure increases hepatic I5'D activity, glycogen content and plasma levels of T_3 at birth which are all likely to contribute to the increased ability of newborn lambs from S ewes to thermoregulate.

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The interaction between plane of nutrition and response to growth hormone on fat and protein metabolism in cattle. By J M DAWSON[1,5], H GREATHEAD[1], D L HACHEY[2,5], P J REEDS[2,5], P J BUTTERY[1], J M PELL[3] and D E BEEVER[4], [1]University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, [2]ARS/USDA Children's Nutrition Research Centre, Houston, Texas 77030, USA, [3]AFRC Babraham Institute, Babraham, Cambridge CB2 4AT and [4]AFRC Institute of Grassland and Environmental Research, Hurley, Maidenhead SL6 5LR.

The effect of plane of nutrition and endocrinological stimulation on whole-body leucine and palmitate flux rates was investigated in young cattle. Seven Hereford × Friesian steers (approximately 168 kg) were fed a pelleted diet of dried grass and barley (70:30;w:w) at 0.8, 1.2, 1.6, 2.0, 2.4, 2.8 or 3.2 × maintenance. After 2-3 weeks, each animal received continuous intravenous infusions of 1-[^{13}C] palmitate and 1-[^{13}C] leucine for 5 h to determine basal plasma flux rates after which they were subcutaneously injected with bovine growth hormone (GH; 0.55 mg/kg body weight) and the infusion continued for a further 3 h to assess the acute response to GH. The infusion was then terminated and repeated the following day, 19-24 h after the GH was administered. This was timed to coincide with the increase in plasma insulin-like growth factor-1 (IGF-1) concentration that has been shown to occur in response to GH injection. Basal plasma IGF-1 concentrations increased linearly with intake from 50 ng/ml at 0.8 × maintenance to 188 ng/ml at 2.4 × maintenance and plateaued at higher intake levels. Plasma IGF-1 concentrations were elevated by 70-250% at the time of the second infusion in response to the GH with the maximum stimulation being observed in the animal fed at 1.6 × maintenance and lower responses at the higher intake levels.

Plasma palmitate flux rate was considerably higher in the poorly-fed animals (128 μmol/kg$^{0.75}$ per h) than in those fed at the higher intake levels (48-60 μmol/kg$^{0.75}$ per h) suggesting increased lipolysis in these animals; this was corroborated by higher plasma non-esterified fatty acid (NEFA) concentrations. GH increased palmitate flux rate and plasma NEFA concentration by 50% within 3 h of administration in the most poorly fed animal (0.8 × maintenance) suggesting a direct effect of GH on lipolysis in this animal. No acute effect on flux rate or plasma NEFA concentration was observed in animals fed at levels above maintenance. Lipolytic rates were, however, elevated at all intake levels up to 2.8 × maintenance 19-24 h after GH administration with again, maximal stimulation (260%) being observed in the animal fed at 1.6 × maintenance. This delayed response was unexpected but plasma GH concentrations were still elevated at this time and could, therefore, still be mediating this effect.

Plasma leucine flux rates increased with level of feeding from 390 μmol/kg$^{0.75}$ per h at 0.8 × maintenance to approximately 900 μmol/kg$^{0.75}$ per h at intakes above 2.4 × maintenance. No response to GH was observed either acutely or 19-24 h after administration, when plasma IGF-1 concentrations were elevated. In all animals plasma leucine concentration was lower during the second infusion i.e. 19-24 h after GH administration. There appeared to be a diet/GH interaction in as much as plasma concentration was reduced by 15% (to 173 μmol/l) in animals fed close to maintenance but by 34% (to 219 μmol/l) in animals fed at 2.8 - 3.2 × maintenance. This change in plasma leucine concentration without any change in plasma flux rate suggests that metabolic clearance rate was increased following GH administration.

These data demonstrate that plane of nutrition has a marked influence on the response of whole-body protein and fat metabolism to exogenously administered growth hormone.

Urea salvage and protein synthesis during pregnancy in normal English and Jamaican women.
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[1]Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU and [2]Tropical Metabolism Research Unit, University of the West Indies, Kingston, Jamaica.

The protein requirements for normal pregnancy are not clear. The simplest definition of overall protein status might be given by an assessment of N balance, but more dynamic measurements such as protein turnover and urea kinetics are needed to give a full picture (Jackson, 1992). We and others have measured protein turnover and the results appear to be divergent, which might be explained either on a methodological basis or as genuine biological differences Between Jamaica and England (de Benoist *et al*, 1985, Thompson & Halliday, 1992). In this study, we have used the same method to measure urea kinetics longitudinally during pregnancy in Jamaica ($n10$) and England ($n9$). The metabolic demand for protein might be expressed as the intake of N in the diet, plus the urea-N salvaged through the lower bowel (intake + salvage).

Trimester....	Protein synthesis				Intake + salvage		
	I	II	III		I	II	III
England	108	111	90 (Thompson & Halliday, 1992)		107	109	111
Jamaica	204	151	115 (de Benoist *et al*, 1985)		145	158	129

Values expressed as a percentage of the non-pregnant results

These data show that the pattern of change in protein metabolism, measured either as protein synthesis or the metabolic demand for N measured as the sum of N intake and urea-N salvage is similar within England and within Jamaica, but that the pattern in Jamaica is different from that seen in England. In England the intensity of protein metabolism is increased relative to the non-pregnant state by about 10% during pregnancy. Overall there is little difference between trimesters, but the greatest change is towards the end of pregnancy. This contrasts with Jamaica where there is a significant increase in the rate of protein metabolism during the first trimester with the rate falling towards non-pregnant values as pregnancy advances. That similar patterns of change are seen for measures of protein synthesis and for urea kinetics suggest that the differences are unlikely to be explained simply by methodological variation and are more likely to represent true biological differences. The basis of the difference is not clear at this time, but one important factor might be the nutritional plane at which mothers enter pregnancy, with women in Jamaica having a worse nutritional status than that of the women in England.

In rat studies we have shown that differences in dietary protein during pregnancy can influence the development of hypertension in the offspring (Langley & Jackson, 1993). The hypertension associated mortality in Jamaica is high. These data emphasize the need for assessing the dynamics of N and protein interactions using standard methodologies in cross-cultural and geographically varied settings.

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New analytical techniques and considerations for measuring ^{13}C-labelled amino acids at sub-micro molar levels. By E. MILNE[1], G.J. ATKINS[2] and S.T. BROOKES[2] [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB and* [2]*Europa Scientific Ltd., Crewe CW1 1ZA*

L-[1-^{13}C]-amino acids are commonly used for measuring tissue protein synthesis by either constant infusion or 'flooding dose' procedures. Measurement of incorporated label into protein is made on $^{13}CO_2$ liberated by ninhydrin from the carboxyl group of isolated amino acid with ninhydrin (Read *et al.* 1984). Measured ^{13}C increases can be as low as 10‰ (delta ^{13}C; relative to Pee Dee Belemnite) and therefore require precise isotope ratio mass spectrometry (IRMS). Conventional IRMS instruments, fitted with cryogenic CO_2 purification units and dual capillary inlets require a relatively large amount of sample (3 μmol C), equivalent to tissue biopsies of approximately 50 mg wet weight.

In the present study continuous flow-isotope ratio mass spectrometry (CF-IRMS) was investigated as a technique for measuring small amounts of [1-^{13}C] leucine, valine or phenylalanine. Each amino acid was analysed at natural abundance and from ^{13}C-enriched standards. Samples (each *n* 5) were dried into septum-capped containers to give either 3, 1 or 0.5 μmol. Each was reacted with 0.5 ml of saturated ninhydrin solution and the $^{13}CO_2$ measured by a CF-IRMS which incorporated a gas chromatograph purification stage (Prosser *et al.* 1991). A separate batch of samples (0.3 μmol) was similarly prepared and reacted with 0.1 ml of saturated ninhydrin solution and analysed by CF-IRMS using a thermal desorption purification stage (Brooks *et al.* 1993). The results of the measurements are shown in the Table and expressed as difference in ‰ between natural abundance and enriched amino acids.

	3 μmol C		1 μmol C		0.5 μmol C		0.3 μmol C	
	mean	SD	mean	SD	mean	SD	mean	SD
Leucine	33.86	0.34	34.53	0.51	34.58	0.81	33.98	1.73
Valine	30.41[a]	0.32	29.99[a]	0.26	30.36[a]	0.87	34.79[b]	1.50
Phenylalanine	53.40[b]	0.15	51.60[a]	0.22	52.36[a]	0.50	54.61[c]	1.13

a,b,c within row $\pm$ mean values with unlike superscripts are significantly different (one-way ANOVA) $P < 0.05$.

CF-IRMS when coupled to a gas chromatograph purification stage gave accurate values down to 0.5 μmol of amino acid, an improvement in sample size requirement over conventional cryogenic approaches. The precision of the system is shown by the low variability and allowed identification of numerically but significant differences for phenylalanine. Below 0.5 μmol care must be exercised to reduce or eliminate sources of contamination (e.g. air leaks, reagent quality) as exemplified by the greater variability of samples analysed by the more sensitive thermal desorption CF-IRMS. In consequence, the values given in the table are corrected for the contribution of reagent blanks.

In conclusion, these data suggest that CF-IRMS allows measurement of ^{13}C enrichment in lower amounts of isolated amino acid than hitherto. In consequence, smaller tissue biopsy samples (as low as 5 mg) from experimental subjects are required, with all the attendant advantages.

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The metabolic and cardiovascular responses to infused adrenaline in lean and obese subjects. By J. WEBBER[1], M. DONALDSON[2], D. FORSTER[1], S. P. ALLISON[3] and I. A. MACDONALD[1]. [1]Department of Physiology and Pharmacology, [2]Department of Dietetics and [3]Department of Medicine Medical School, Queen's Medical Centre, Nottingham, NG7 2UH

Abnormalities in the sympathoadrenal system (SAS) have been implicated in the aetiology and maintenance of the obese state (Astrup *et al.* 1990). Although resting metabolic rate (RMR) is not low in obesity, the response to thermogenic stimuli such as food, cold and catecholamines may be subnormal, thus resulting in a predisposition to positive energy balance. However, there is increasing evidence for discrete activation of the SAS and it is, therefore, of interest as to whether cardiovascular responses as well as metabolic responses may be different in obesity.

We studied twenty healthy subjects, ten lean (five female, mean (SE) body mass index (BMI) 21.9 (0.7) kg/m^2, mean fat-free mass (FFM) 51.4 (3.1) kg, mean age 22.5 (1.1)), ten obese (eight female, mean BMI 35.9 (1.4), mean FFM 55.7 (4.1) kg, mean age 32.8 (2.6)). After an overnight fast basal measurements of RMR (indirect calorimetry), blood glucose, plasma adrenaline, blood pressure, heart rate (HR) and forearm blood flow (FBF; venous occlusion plethysmography) were made whilst the subject was resting supine for 1 hour. An adrenaline infusion was then commenced at 25 ng/kg per min in the lean subjects, and at 25 ng/kg ideal body weight per min in the obese subjects. Measurements were continued for a further 90 min.

Mean adrenaline levels achieved in the two groups during the final 60 min of the infusion were 2.41 (SE 0.10) nmol/l in the lean and 2.09 (SE 0.12) nmol/l in the obese (P 0.11). The Table shows mean basal values in each group and the increment in response to adrenaline with their standard errors.

Increment from basal during adrenaline infusion...		Lean				Obese			
Time (min)		Basal	0-30	30-60	60-90	Basal	0-30	30-60	60-90
RMR	Mean	95.3	11.7	13.0	14.4	96.3	11.0	13.7	12.7
(J/kg FFM per min)	SE	3.0	1.3	1.3	1.6	4.0	1.0	1.8	1.5
Glucose	Mean	4.33	0.78	0.88	0.94	4.42	0.57	0.74	0.69
(mmol/l)	SE	0.09	0.11	0.14	0.16	0.14	0.07	0.10	0.11
Heart Rate	Mean	61.4	14.0	16.3	17.0	69.7	13.7	15.6	16.8
(beats/min)	SE	2.7	2.4	2.6	2.7	2.7	2.1	2.3	2.4
FBF	Mean	3.66	3.47	3.53	3.89	2.85	1.43[b]	1.93[a]	2.05[a]
(ml/100ml per min)	SE	0.39	0.52	0.54	0.45	0.27	0.20	0.25	0.42

Significantly different from lean group value (unpaired t test): [a]P <0.05, [b]P <0.01

Blood pressure responses were similar in the two groups with a slight increase in systolic blood pressure and a fall in diastolic blood pressure. The only significant difference in response to the adrenaline infusion was in the increment in FBF which was reduced in the obese compared with the lean, even when expressed as percentage change from the basal value. This study, therefore, provides no evidence for a thermogenic deficit in established obesity, but does suggest that there may be discrete haemodynamic changes in adrenergic sensitivity in the obese state.

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Does dietary protein influence resting energy expenditure? By P.J. PACY, R.M. QUEVEDO, M. COX, N.R. GIBSON, G. PRICE and D.J. MILLWARD. Clinical Science Department, Nutrition Research Unit, St Pancras Hospital, 4 St Pancras Way, London NW1 0PE

Protein synthesis is a major component of resting energy expenditure (REE). We have reported that the REE of elite athletes is higher than that expected from predictive formulas derived from non-athletic populations (Quevedo, 1992). We have investigated the possibility that this finding reflects the greater protein intake of athletes by measuring the REE in healthy weight-maintaining non-athletic adults who consumed very different levels of dietary protein. In Study 1 four males, one female; age 43 (SD 11) years; weight 64 (SD 9) kg; body mass index 23 (SD3) kg/m^2) consumed, in consecutive order, increasing dietary protein from low (0.35 g/kg per d; LP) to medium (0.75 g/kg per d; MP) to generous protein (1.50 g/kg per d; GP) for 2 weeks with ad lib. feeding for 2 weeks between each intake. In Study 2 seven male adults; age 32 (SD 9) years; weight 77 (SD 10) kg; body mass index 23 (SD3) kg/m^2) who were habituated to high protein (1.9 g/kg per d; HP) switched to a medium intake (0.75 g/kg per d; MP) for 14 days. Resting energy expenditure was measured after an overnight fast by indirect calorimetry for at least 60 minutes at the end of each 2 week dietary period (Study 1). In Study 2 the REE was measured under identical conditions on the GP level and at intervals throughout the MP intake. Energy expenditure was determined by Weir's formula (Weir, 1949).

In Study 1 REE remained unchanged on each level of protein intake (LP, 6209 (461) kJ/d; MP, 6318 (310) kJ/d; GP, 6502 (306) kJ/d; paired t-test) as did respiratory quotient (RQ; LP, 0.83 (0.02); MP, 0.83 (0.05); GP, 0.82 (0.02). Their weights remained constant (64 (9) kg, 63 (8 kg and 64 (9) kg respectively) on the LP, MP and GP diets. In Study 2 REE remained unchanged during the switch from HP to MP (HP, 7356 (381) kJ/d; MP 7189 (427) kJ/d on day 3, 7264 (402) kJ/d on day 7 and 7306 (515) kJ/d on day 14) as did RQ (HP, 0.80 (0.02); MP, 0.79 (0.04) on day 3, 0.79 (0.04) on day 7 and 0.81 (0.01) on day 14). The REE expressed in terms of kJ/kg per d was constant at all levels of habituated protein intake (96 (8); 100 (4); 100 (4); 96 (4) on the LP, MP, GP and HP diets respectively). All figures are mean (SD).

In conclusion, these data provided little evidence that the REE is significantly influenced by level of habitual dietary protein intake.

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24h energy expenditure patterns in multiple organ failure. By CERI J. GREEN, P. McCLELLAND and I.T. CAMPBELL. Intensive Therapy Unit, University Dept Anaesthesia and Dept of Renal Medicine, Royal Liverpool Hospital, Liverpool L69 3BX.

After uncomplicated accidental or surgical trauma resting energy expenditure (EE) follows a predictable pattern with an elevation directly related to the severity of the pathological insult (Frayn, 1986). Unlike simple trauma, multiple organ failure (MOF) has no predictable time course and is a condition associated with a high mortality. Isolated measurements of EE in multiple organ failure have shown both elevations and depressions relative to basal EE (predicted from age, height, weight and sex), but there are no reports of serial measurements of 24h EE in multiple organ failure so it is not known how much EE varies over the course of this type of illness nor whether outcome is associated with any particular EE pattern.

29 patients (20M, 9F; 18-76(median 60)yrs) suffering multiple organ failure were studied. All were ventilated and fed intravenously. 18 were in acute renal failure undergoing haemodiafiltration. Admission APACHE II (Knaus et al., 1985) scores were 15-35(median 24). Seventeen died and 12 returned to the ward. EE was measured continuously, using the Engstrom Metabolic Computer (Gambro, Bromma, Sweden), from within 24h of the start of artificial ventilation (Engstrom Erica, Gambro, Sweden) until the patient was either weaned from the ventilator or died.

Variation in 24h EE of all 29 patients as denoted by the coefficient of variation (CV) over the whole of the period of illness ranged from 1.8 to 14.4 (median 6.2)%. Patterns of variation in 24h EE were analysed using the Spearman rank correlation coefficient of EE against time. In 22 patients there was no change in EE with time but in 7 EE did change significantly. In 4 patients EE increased and in 3 it decreased but changes bore no relationship to any change in APACHE II score. Variability in 24h EE in this group as denoted by the CV was greater than that seen in the group in which EE did not change (6-13.5(median 11.3)% v 3.5-14.9(median 6)% - $p<0.05$). Fifteen of the patients in whom energy expenditure did not change died compared with only 1 death in the group in which there was a significant change in EE ($p<0.05$).

Variability and change in 24h EE during the course of MOF appears to be associated with a lower mortality than a pattern of EE in which changes in 24h EE are only random. The explanation for this finding is not immediately obvious but is perhaps worthy of further investigation.

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Energy intake and energy expenditure in acute admissions to a geriatric unit. By
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Previous studies on long-stay and psycho-geriatric elderly patients have shown that negative energy
balance is common (Prentice *et al.*, 1989; Sutherland & Wootton, 1993). Relatively little attention has
focused on acutely ill elderly subjects but negative energy balance might be expected to be common
due to reduced food intake and/or raised metabolic rate related to acute illness, and the prevalence of
feeding or eating difficulties in this patient group.

The aim of the present study was to quantify energy balance in a group of twenty patients
(eight men, twelve women, age range 73-96 years; main diagnoses: myocardial infarct, stroke, falls)
with hospital stay ranging from 10 to more than 60 days. The study was one aspect of a survey of
nutritional status (n=72) of acute admissions to a geriatric unit from the community.

Basal Metabolic Rate (BMR) was measured by ventilated hood indirect calorimetry and energy
intake (EI) by three day weighed dietary record. Anthropometric data were collected. Body
composition was estimated from the sum of four skinfolds (Durnin & Womersley, 1974). All
measurements were carried out every two weeks on each patient until the seventh week of
hospitalization. Energy requirement (TEE; total energy expenditure) was estimated as 1.3 x BMR
(Reilly *et al.*, 1992) since patients were extremely inactive.

Anthropometric data on admission were as follows: mean body mass index (kg/m^2) 20.6 (*SD*
4.8) in men and 21.9 (*SD* 4.9) in women; mean body fatness (% of body weight) 18.4 (*SD* 7.6) % in
men and 29.9 (*SD* 6.9) % in women; mean body weight 59.2 (*SD* 16.9) kg in men and 50.3 (*SD* 9.9) kg
in women. Anthropometric variables showed neglible changes during hospitalization. Mean EI for
men (5.19, *SD* 1.53 MJ/d) and women (4.53, *SD* 1.10 MJ/d) was lower than estimated TEE for men
(6.72, *SD* 1.18 MJ/d) and women (5.83 *SD* 1.15 MJ/d) in sixteen of twenty cases. The difference
between EI and estimated TEE averaged 1.53 (*SD* 2.25) MJ/d for men and 1.30 (*SD* 1.28) MJ/d for
women. The median EI:BMR value was 0.99 (range 0.65-1.76).

We conclude that negative energy balance is common in this patient group and that these
patients are at risk of undernutrition during their hospital stay. The findings are consistent with those
reported for other elderly patient groups.

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A comparison of nutrient intakes and activity levels in female aerobics instructors and beginners. By L. MCKAY, C.A. BROWN and C. BOLTON-SMITH, Cardiovascular Epidemiology Unit,University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY

Many studies report on nutrition and exercise, however, the possible effects of a healthier (cardio-protective) diet in fit individuals has not been widely explored. In a pilot study, habitual eating patterns in relation to activity levels in two different exercising groups, aerobics instructors n 48; mean age 31.9 (range 18 - 61) years and their beginner class attendees n 107; mean age 31.2 (range 16 - 58) years were assessed using a food frequency questionnaire (Bolton-Smith et al. 1991). An activity questionnaire asked about work and leisure hours spent in active, moderate or inactive pursuits. From self-reported weight and height the mean Body Mass Index (kg/m^2) of instructors was 21.4 (1.7) and beginners was 25.9 (3.4).

Energy and nutrient intakes were calculated from standard portion sizes and the UK food composition tables (Paul & Southgate, 1978). Estimated energy expenditure was calculated from the minutes spent per day at each level of activity using published physical activity ratios (James & Schofield, 1990). Daily nutrient intakes which vary significantly between groups, and the activity data are reported in the Table.

	Instructors		Beginners		
	Mean	SD	Mean	SD	P Values[a]
Energy (MJ)	6.61	1.68	6.71	1.95	NS
Total fat (% energy)	29.8	6.2	32.8	6.6	0.01
Saturated fat (%energy)	12.3	3.4	13.7	3.9	0.03
Fibre (g)	25.2	6.5	22.7	7.9	0.02
Fibre (gND)	16.3	3.9	14.5	4.5	0.007
Active (min)	204	135	123	107	0.0001
Moderate (min)	328	207	307	166	NS
Inactive (min)	307	198	453	227	0.0002

[a]Analysis of variance performed on the appropriately transformed data.
ND, nutrient density (amount/4.18 MJ)

Tendencies for higher sugar and vitamin C intakes in instructors compared with beginners were non-significant, and although there were no significant differences in energy intake between the two exercise groups, there were highly significant differences in estimated energy expenditure (instructors 11.2 (1.38); beginners 10.3 (1.48) MJ/d P < 0.0007).

The wide difference between intake and energy expenditure will be partly due to the 53% of instructors and 76% of beginners who reported actively trying to lose weight (23% of instructors and 16% of beginners reported actual weight loss over the previous year.) A further proportion of the difference may be due to the methodological difficulties in assessing both diet and activity. Evidence has also been reported to suggest that appetite may be suppressed post exercise, leading to dietary restriction (Kissileff et al. 1990), and that energy metabolism may be altered in athletes such that energy requirements are less than expected (Mulligan & Butterfield, 1990).

The high degree of nutritional awareness in these two exercising groups was generally reflected in the nutrient quality of their diets and, as such, diet may be contributing to the 'healthy' effects of exercise.

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Low intensity exercise after consumption of a high-fat meal decreases postprandial lipaemia during the recovery period. By H.E. ALDRED and A.E. HARDMAN. Department of Physical Education, Sports Science and Recreation Management, Loughborough University of Technology, Leicestershire LE11 3TU

Lipoproteins of dietary origin may be atherogenic (Zilversmit 1979). The purpose of the present study was to examine the effect of 90 min of low intensity exercise on the lipaemic response to a high-fat meal.

Twelve (6 male, 6 female) adults aged (mean (SEM)) 24.5 (1.2) years participated in the study. Serum concentrations (mmol/l) of lipids, determined after a 12 h fast were: triacylglycerol (TAG) 0.82 (0.09), total cholesterol 3.82 (0.25), high density lipoprotein cholesterol (HDL) 1.12 (0.05) and HDL_2 cholesterol 0.41 (0.05). Each subject undertook 2 separate trials, an exercise trial and a control trial, approximately 7 d apart, in a balanced cross over design. In both trials subjects reported to the laboratory after an overnight fast and a cannula was introduced to a forearm vein. A blood sample was taken and the test meal was then ingested. This consisted of cereal, fruit, nuts, chocolate and cream (Schlierf et al. 1987) and was given according to body mass providing 1.2 g fat and 71 kJ/kg body mass. Further blood samples were obtained 1, 1.5, 2, 3, 4, 5 and 6 h after the meal. In the exercise trial subjects walked for 90 min at 40.1 (1.1)% maximal oxygen uptake, starting 1.5 h after consuming the test meal, and then rested quietly for the remaining 3 h. In the control trial subjects rested for the whole 6 h period. Subjects performed no physical exercise for 2 d before each trial and food intake was weighed and recorded for the 2 d before the first trial and replicated for the 2 d before the second trial. Serum was analysed for TAG, correcting for free glycerol. Three indices of postprandial lipaemia were adopted: (i) peak TAG concentration, (ii) total lipaemic response and (iii) the lipaemic response for specified time intervals. Indices (ii) and (iii) were calculated by the area under the TAG v. time curve, normalised to the zero hour level. Comparisons between trials were made using the Wilcoxon matched pairs signed rank test.

Serum TAG concentrations increased after ingestion of the high fat meal in both trials ($P<0.01$). There was no difference between trials in peak TAG concentration (Control 2.03 (0.23) mmol/l, Exercise 2.23 (0.28) mmol/l) but the total lipaemic response was 23.5 (11.3)% lower in the exercise trial then in the control trial. This difference was attributable to a lower lipaemic response during the recovery period, ie 3 to 6 h after ingestion. These results are shown in the table below.

	Lipaemic Response (mmol/l.h)							
	0-6 h		0-1.5 h		1.5-3 h		3-6 h	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	4.48	0.62	0.62	0.06	1.45	0.18	2.42	0.44
Exercise	3.22*	0.48	0.48	0.06	1.30	0.21	1.35**	0.32

significantly different from control, *$P<0.05$, **$P<0.01$

These results show that postprandial lipaemia is attenuated during the recovery from low intensity exercise in young normolipidaemic adults. One possible mechanism may be increased lipoprotein lipase activity in exercised skeletal muscle.

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Evidence that fat oxidation is modulated by fat intake in lean but not in obese women. By P.A. VOLSCHENK, P.R. MURGATROYD, G.R. GOLDBERG, A.E. BLACK and A.M. PRENTICE, MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge, CB2 1QL

The existence of a dose-response interaction between fat intake and oxidation remains a controversial issue (Flatt *et al.* 1985; Griffiths *et al.* 1992; Schutz *et al.* 1985). However, some of these studies have manipulated the fat content of the diet without keeping carbohydrate (CHO) constant. The failure or ability of dietary fat to elicit an oxidative response may be attributed to preferential CHO oxidation.

The hypothesis that there is a positive dose response between fat intake and fat oxidation on iso-carbohydrate diets of varying fat and energy content, was tested on four lean (mean (SD) weight) 65.66 (6.62) kg and five obese (80.73 (10.91) kg), untrained women each studied on three occasions for 20.5 h on a sedentary protocol, by whole-body indirect calorimetry. Mean (SD) fat intakes on the low- medium- and high-fat runs (LF:MF:HF) were 1.9 (0.2):3.3 (0.3):5.6 (0.4) MJ. The absolute amount of CHO (3.7 (0.3) MJ) and protein (0.7 (0.05) MJ) for each subject remained the same on all runs. The intakes varied between subjects according to their body size. Within each run dinner (20.30 hours), breakfast (09.00 hours) and lunch (13.00 hours) were of equal composition and energy. Substrate oxidation rates were calculated from urinary N excretion and respiratory gas exchange, using the coefficients of Elia & Livesey (1988). Oxidation rates are summarized in the Table.

	Energy expenditure (kJ/20.5 h)			Fat oxidation (kJ/20.5 h)			CHO oxidation (kJ/20.5 h)			Protein oxidation (kJ/20.5 h)		
	LF	MF	HF	LF	MF	HF	LF	MF	HF	LF	MF	HF
Lean												
Mean	6567	6583	6672	2696	2972	3771	3000	2745	2099	870	865	801
SD	811	435	447	412	678	438	426	693	457	230	105	146
Obese												
Mean	6405	6559	6329	2768	2968	2978	2822	2644	2393	814	946	954
SD	310	1035	669	1040	1424	697	894	662	497	226	109	152

Analysis of variance confirmed a significant autoregulatory responsiveness in fat oxidation in the lean ($F_{2,12}$ = 4.702; $P < 0.05$) but not in the obese women ($F_{2,13}$ = 0.055; NS). In previous studies this effect may have been masked by differences in CHO oxidation on diets of varying CHO content. The failure to increase fat oxidation in response to a raised intake in the obese may arise from higher levels of circulating free fatty acids which may mask the intake of dietary fat. This may be a factor contributing to the cause and maintenance of obesity in untrained individuals.

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The use of nutritional supplements in female high exercisers. By L. MCKAY, C.A. BROWN and C. BOLTON-SMITH, <u>Cardiovascular Epidemiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY</u>

Forty-eight aerobics instructors and 107 beginner class attendees (mean age 31.9 years and 31.2 years) were recruited through leisure centres and fitness clubs. They completed a food frequency questionnaire (Bolton-Smith *et al.* 1991) which included supplement use, and answered questions on time spent at work and at leisure on active, moderately active and inactive pursuits. Energy and nutrient intakes were calculated from standard portion sizes using the UK food composition tables (Paul & Southgate, 1978). The percentage of instructors and of beginners reported taking at least one type of supplement on a regular basis (daily or weekly) was 44 and 17% respectively. Three supplement-taking groups were identified: single supplement takers (e.g. Fe, vitamin C, vitamin E, Ca), multivitamin or mineral takers (with or without Fe) and an 'Other' supplement taker group which included fish and evening primrose oil, garlic capsules, royal jelly etc. The number and percentage of individuals taking each type of supplement are shown in Table 1.

	No. supplements/d		Single-supplement		Multi-supplement		Other-supplement	
Exercise Group	Mean	SD	*n*	%	*n*	%	*n*	%
Instructors	1.48	0.68	11	22.9	8	16.7	12	25.0
Beginners	1.94	1.00	9	8.4	11	10.3	15	14.0

No significant differences in energy intake, and only few differences in nutrients were observed between the exercise groups (McKay *et al.* 1993). Differences in diet irrespective of supplement use were assessed for supplement (+) *v,* non-supplement (-) takers. No significant differences in intake of energy or the macro nutrients were observed, however fibre and retinol intakes were higher in the supplement taking group when expressed as nutrient densities (ND; amounts/4.2 MJ) as shown in Table 2. Neither amounts per day nor nutrient density values differed significantly between the groups (at the 5% level) for the other vitamins reported.

	MJ/d		Fibre gND		Vit C mg ND		Vit E mg ND		Retinol µg ND	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
+Supplements	6.39	1.78	16.4[**]	4.6	59[*]	29.7	4.8	3.29	293[**]	164
-Supplements	6.80	1.87	14.6	4.2	50	27.2	4.7	2.90	236	131

Significantly different from non-supplement takers (ANOVA on appropriately transformed data) * $P < 0.10$; ** $P < 0.05$.

Supplement takers reported spending more time engaged in active leisure pursuits than non-takers, 311 (172) min/d and 220 (125) min/d respectively ($P = 0.0005$), but there was no difference in the estimated total energy expenditure.

These data agree with a previous report of higher intakes of fibre (and β-carotene) in supplement takers compared with non-takers (Kearney & Gibney, 1990) but do not suggest that the diets of supplement-takers are initially lacking in vitamins. Supplement takers appear to be more active in leisure time, but not sufficiently so to raise their total energy expenditure compared with non-takers. Heightened health perception and awareness is likely to account for the greater prevalence of supplement taking in the higher leisure activity group rather than an actual nutritional need.

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Between- and within-subject variability in energy expenditure during physical activity.
By S.A.WOOTTON, S.A.BOND and S.MAKI, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

Information on the energy cost of different occupational and recreational tasks expressed as a multiple of basal metabolic rate (Physical Activity Ratio; PAR) is required in order to improve our understanding of the energy needs of children and adults. The determination of PAR under field conditions has been limited by the availability of a portable calorimeter that can be used to measure energy expenditure at rest and during physical activity. The Deltatrac II (Datex Instrumentarium Corp, Helsinki Finland) which utilizes a ventilated canopy to measure energy expenditure at rest may be modified to determine automatically the volume and composition of expired air collected in ultralightweight bags during physical activity. The present study (1) examined the validity of measurements of oxygen uptake (VO_2) and carbon dioxide excretion (VCO_2) made using the modified Deltatrac II bag system and (2) examined the between- and within-subject variability in PAR during (a) a defined task of cycle ergometery (50W) and (b) a less structured task of self-paced load carrying (4kg load).

Mass flow transducers were used to fill bags (n 250) with known amounts of N_2, CO_2 and air to achieve differing final volumes (50-125 litres) and gas composition (RQ 0.7-1.0). Comparison of the measured VO_2 and VCO_2 against the known VO_2 and VCO_2 revealed that the system was accurate (percentage difference between measured and known (Mean $\pm$ SD): VO_2 -0.36 $\pm$ 1.78; VCO_2 1.27 $\pm$ 2.28; RQ 1.36 $\pm$ 1.66), linear (measured VO_2 ml/min = 1.01 x known VO_2 -1.6; SEE 5.8; R^2 = 99.9%) and highly repeatable (between bag variability <2%). Eight healthy female subjects (age 18-31 years) completed each task on two occasions in a random order. Basal metabolic rate was assessed before each task and the energy cost of the task was determined from the average of two successive 3 min gas collections taken over the final 7 min of a 12 min task. The energy cost of the task (EEt) and PAR value (EEt/BMR) are shown in the Table

	Cycle Ergometer				Load Carrying			
	Trial I		Trial II		Trial I		Trial II	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
EEt (kJ/min)	18.03	0.73	18.01	1.67	15.72	2.57	18.72	2.64[a]
PAR Value	4.86	0.32	4.85	0.49	4.22	0.64	4.92	0.81[a]

[a]significantly different from Trial I $P<0.05$

The range of PAR values for both tasks was large (ergometer 4.01-5.56; load 3.50-5.44); the between-subject variability in PAR for the defined ergometer task appeared less than that of the less structured load carrying in both trials (coefficient of variation: 6.6% and 10.0% v 15.0% and 16.0%). The within-subject variability in PAR was also greater for the less structured task (individual differences between trials expressed as a percentage of Trial I: ergometer 0.4-11.8%; load 0.6-57.6%) and was significantly increased on the second trial. These results suggest that the modified Deltatrac II can reliably be used to assess the energy cost of activity in addition to the energy expenditure at rest. The variability in energy expenditure, particularly in less structured self-paced tasks such as load carrying, should be taken into account when PAR values are used to estimate total energy expenditure.

Is the antioxidant activity of premature baby plasma modulated by plasma ascorbic acid concentrations? By K.M. SILVERS, H.J. POWERS, and A T GIBSON University Department of Paediatrics, Sheffield Childrens Hospital, S10 2TH

Babies born prematurely show greatly increased risk of developing diseases thought by many workers in the field to be associated with oxidation damage. Many factors appear to be important in the aetiology of the so called diseases of prematurity, including circulating levels of antioxidants. Free metal ions can initiate oxidative damage via the generation of free radicals. The ability to minimise the levels of trace Fe in the plasma may be particularly important to premature babies, among whom blood transfusions are frequent and for whom there is evidence for reduced antioxidant activity. Caeruloplasmin has an important role in this respect by virtue of its ferroxidase activity; ascorbic acid has been implicated as an inhibitor of this activity (Gutteridge, 1991).

We have measured, for at least 28 d from birth, the ability of the plasma of thirty premature babies to prevent lipid peroxidation of brain homogenate (Sullivan & Newton, 1988). In the same plasma samples we have measured ascorbic acid (Vuilleumier & Keck, 1989) and in fifteen babies we have also measured plasma caeruloplasmin.

The antioxidant activity of the plasma (Dmax) is expressed as the volume of plasma required to achieve maximal inhibition of lipid peroxidation. Dmax is inversely related to antioxidant activity and is largely a measure of the ability of the plasma to remove ferrous iron thereby making it unavailable for Fenton chemistry . Dmax values at birth were widely distributed and clearly skewed. Dmax at birth was significantly higher in five babies who died compared with twenty five survivers, (164 (SD 71) compared with 88 (SD 43) respectively) ($P < 0.05$, Mann Whitney U). Caeruloplasmin concentrations ranged from 13.8 to 140.9 mg/l at birth in contrast to the normal adult mean value of 313 (SD 44) mg/l. Values rose in the few days after birth, reaching levels above 400 mg/l by the second week of life in some cases. Thereafter there was, in all babies, a further fall to reach a stable value in the adult range in the third week of life. Plasma ascorbic acid concentrations at birth were in the range 32.4 - 179.2 μmolar, with a mean of 83.3 (SD 36.76) which is well above the lower limit of normality conventionally used for adults. Values fell transiently after birth. There was a wide range in the response to the feeding regimen; but after the transient fall values increased significantly and in some babies reached values above 200 μmolar. Dmax at birth showed a negative correlation with plasma caeruloplasmin ($P < 0.05$) and was strongly correlated with the plasma ascorbic acid : caeruloplasmin ratio ($P < 0.001$).

The antioxidant activity of the plasma at birth is partly determined by ferroxidase activity. However this may be modulated by high concentrations of ascorbic acid which inhibit the antioxidant activity of the plasma. The antioxidant activity of the plasma may be a useful predictor of short term outcome in sick premature babies.

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Plasma vitamin C concentrations in critically ill patients. By C.DOWNING, A.PIRIPITSI, A.BODENHAM and C.J.SCHORAH, Department of Clinical Medicine, University of Leeds, Leeds LS2 9JT

Plasma vitamin C concentrations are reduced in acute and chronic disease. Such changes could affect antioxidant status and impair recovery. It is unclear, however, whether this decrease is due to redistribution and cell uptake of the vitamin, as an appropriate part of an acute phase response, or oxidation of ascorbic acid (AA) via dehydroascorbic acid (DHAA) to diketogulonic acid, when antioxidant potential would be lost (Basu & Schorah, 1982). Using reverse-phase HPLC (Sobala et al. 1991) we measured plasma levels of AA, total vitamin C (C tot = AA+DHAA) and DHAA by difference in patients on an intensive care unit (ICU) and investigated the effects of sample storage on these levels.

Blood samples were taken from twenty-three healthy, active controls and thirteen ICU patients. After centrifugation, duplicate plasma samples were removed into two volumes of 2% (w/v) metaphosphoric acid (MPA) with or without dithiothreitol (for the measurement of C tot and AA respectively). Samples were stored at -40° within 20-60 min of venepuncture. Samples for the investigation of stability were stored at room temperature and agitated hourly for 4 h before plasma removal into MPA.

Group	n	AA (μmol/l) Mean SE	DHAA (μmol/l) Mean SE	Ctot (μmol/l) Mean SE	DHAA/Ctot(%) Mean SE
Controls	23	65.3 3.0	2.1 1.2	67.4 3.1	2.4 1.8
ICU patients	13	10.4 3.0	3.6 0.6	14.0 3.0	32.4 6.1

Plasma levels of both Ctot and AA were significantly lower in ICU patients than controls ($P<0.001$), while the DHAA/Ctot ratio was significantly higher in ICU patients ($P<0.001$). When whole blood was stored for 4h the loss of Ctot and AA was significantly greater ($P<0.01$) in the plasma of ICU patients (47%, 59%) than in the plasma of controls (22%, 21%). To further investigate storage loss, AA was added to the blood of ICU patients so that its concentration approached that of the controls and the samples were then stored at room temperature as both plasma and whole blood. After 4 h plasma was removed into MPA from both samples. The losses of Ctot and AA were lower when storage was in plasma (41%, 47%) compared with whole blood (68%, 77%), but loss in the latter was still considerably greater than in control samples stored as whole blood (see above), although the initial concentrations were now equivalent.

These results suggest that the loss of vitamin C from plasma is increased in the blood of ICU patients, and that this change is due to both cellular and humoral components. The concentration of vitamin C in the plasma of such patients is critically dependent on the conditions of storage of the sample prior to extraction.

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Lack of concordance between two biochemical indices of vitamin B_6 nutritional status.
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A number of studies have suggested, on the basis of measurement of either the plasma concentration of pyridoxal phosphate (PLP) or the activation of erythrocyte aminotransferases by PLP added *in vitro,* that between 10-30% of the population of developed countries are inadequately supplied with vitamin B_6 (Bender, 1989). None of these studies has reported results of both methods of assessing vitamin B_6 nutritional status in the same subjects.

Vitamin B_6 nutritional status has been assessed in two groups of people: 129 young women about to embark on a study of different types of oral contraceptive steroid preparation and ninety four elderly men undergoing transurethral resection of the prostate. The plasma concentration of PLP was determined fluorimetrically, and the activation of aspartate aminotransferase (*EC* 2.6.1.1) in erythrocyte lysate by PLP added *in vitro* was determined radiometrically, as described previously (Bender *et al* 1982). Plasma PLP < 41 nmol/l (men) or < 36 nmol/l (women) was considered marginal, and < 34 nmol/l (men) or < 23 nmol/l (women) indicative of deficiency; aspartate aminotransferase activation coefficient (ASTac) >1.25 was considered to indicate marginal status, and >1.5 to indicate deficiency (Leklem, 1988). The numbers of subjects in each group with apparently inadequate vitamin B_6 nutritional status are shown in the Table.

	Men (*n* 94)		Women (*n* 129)	
	n	%	n	%
Plasma PLP marginal	15	15.9	28	21.7
Plasma PLP deficient	4	4.3	1	0.8
ASTac marginal	31	32.9	41	31.8
ASTac deficient	8	8.5	17	13.2
PLP and ASTac marginal	9	9.6	9	6.9
PLP and ASTac deficient	1	1.1	0	0

These results are in agreement with previous studies showing up to 30% of the population with inadequate vitamin B_6 nutritional status by one criterion or the other, but they show poor concordance between the two indices. It is suggested that only people apparently marginal by both criteria should be considered to be inadequately supplied with vitamin B_6.

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Antioxidant status during liver transplantation. By HELEN F. GOODE, NIGEL R. WEBSTER, PETER D. HOWDLE & BARRY E. WALKER, Clinical Oxidant Research Group, St James's Hospital, Leeds, LS9 7TF, UK.

The fat soluble vitamins A and E, and the carotenoids provide an important line of antioxidant defence against free radical damage. Patients with chronic liver disease are likely to have both increased production of reactive oxidant species as a result of chronic inflammation (Goode & Webster) and lowered absorption and storage of fat soluble vitamins (Losowsky, Walker & Kelleher). During liver transplantation an additional insult of superimposed reperfusion injury when the transplanted liver is reperfused is also likely. The present study was undertaken to determine the antioxidant vitamin status of patients during liver transplantation, and the effects of reperfusion.

Twelve patients (five females, seven males) aged 23-68 years, who were undergoing elective liver transplantation were studied. Blood samples were obtained at the beginning and end of each of the three stages of the transplant, for simultaneous measurement of plasma vitamins A and E, β carotene and lycopene, using high performance liquid chromatography with diode-array spectrophotometric detection. Thiobarbituric acid reactive substances (TBARS) was quantitated fluorimetrically as an index of malondialdehyde formation and hence lipid peroxidation.

Concentrations of the antioxidant vitamins were markedly reduced in all patients at the start of the transplant, compared to our reference ranges for healthy subjects of similar ages: Vitamin E 4.3 (SD 2.7) mg/l, reference range 9.0-14.0 mg/l, $P < 0.001$; Vitamin A 141 (SD 87)μg/l , reference range 370-110 μg/l, $P < 0.001$; β carotene 0.29 (SD 0.18) mg/l, reference range 1.0-11.4 mg/l , $P < 0.001$; Lycopene 0.39 (SD 0.22) mg/l, reference range 1.5-8.9 mg/l, $P < 0.001$. TBARS was elevated at 2.6 (SD 1.6) μmol/l compared to the reference range of 0.1-2.1 μmol/l, $P < 0.05$.

On reperfusion of the transplanted liver, both vitamins A and E decreased slightly in all patients. These changes were significant using the Wilcoxon test for paired data: vitamin A $P < 0.01$, and vitamin E $P < 0.02$. No change was detectable in the already very low carotenoid concentrations, and levels of TBARS also remained constant.

These data indicate that patients undergoing liver transplantation have extremely low levels of antioxidant vitamins and that reperfusion of the transplanted liver with the concommitant release of oxygen radicals puts an additional strain on these already depleted antioxidant defences. The index of lipid peroxidation chosen was either insufficiently sensitive or possibly our results imply an alternative reaction pathway other than malondialdehyde formation.

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Lipid peroxidation and ultraviolet-B erythemal responses in patients consuming fish oil supplements. By S.O'FARRELL[1], L.E.RHODES[2], P.S.FRIEDMANN[2] and M.J.JACKSON[1], [1]Department of Medicine, [2]Department of Dermatology, University of Liverpool, P.O.Box 147, Liverpool, L69 3BX

Marine oils rich in the n-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been recommended as a therapy for disorders such as psoriasis (Bittner *et al.* 1988) and in patients with ischaemic heart disease (Kinsella *et al.* 1987). However, it has been proposed that the consumption of fish oils renders tissues more susceptible to free radical-mediated lipid peroxidation (Hu *et al.* 1989) and, hence, may be deleterious in tissues subjected to oxidative stress. One such example might be damage to the human epidermis induced by ultraviolet irradiation where peroxidation of skin tissue lipids can occur (Punnonen, 1991). We have therefore studied the effects of dietary fish-oil supplementation on the accumulation of EPA and DHA by skin tissue in comparison with the effects on tissue lipid peroxidation *in vitro* and the erythemal response induced by a graded dose of ultraviolet (UVB) light.

Six patients were given a 3 month course of a fish oil supplement in the form of ten MAXEPA capsules daily. Each 1g capsule contained 18% EPA and 12% DHA. The response of the epidermis to UVB-induced damage was assessed by exposing the skin of the lower back to a geometric series of ten doses of UVB. At 24 h the minimal erythemal dose (MED) was noted, which is the minimum dose of UVB required to bring about a visual response. A split-skin shave biopsy was performed at day 0 and after 83 d supplementation. Lipid peroxidation was assessed by measuring the amount of thiobarbituric acid reactive substances (TBARS) in untreated control skin and skin treated with a dose of 120mJ UVB/cm², while the susceptibility to lipid peroxidation was assessed by undertaking the same analysis following incubation of skin homogenates with $FeSO_4$ (50µM) and ascorbic acid (50µM).

Fish-oil supplementation had a dramatic effect on the tissue fatty acid composition with the mean amount of DHA varying from 0µg/g skin at day 0 to 77(SEM 30) µg/g at day 83. These changes resulted in a significant reduction ($P<0.01$) on the susceptibility of the skin tissue to UVB-induced damage, with a rise in the MED from 18.3mJ/cm² at day 0 to 35.5mJ/cm² at day 83. In addition, the TBARS produced on incubation were significantly higher in UVB-treated skin than control skin (at day 83 mean(SEM) values were control 9.9(1.6) A_{532}/g skin; UVB-treated 18.5(2.6) A_{532}/g skin).

These data indicate that MAXEPA supplementation brought about a substantial modification of erythemal fatty acid composition leading to increased susceptibility of skin tissue to lipid peroxidation induced *in vitro*. However, a comparable change was not apparent *in vivo*, where MAXEPA supplementation appears to exert a protective effect. PUFA n-3 supplementation may therefore be of benefit in the treatment of light sensitivity disorders in man.

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Dietary lipids modulate the physicochemical characteristics of rat hepatocyte plasma membranes.
By A. G. CLAMP[1], R. F. GRIMBLE[1], D. C. CLARK[2], S. LADHA[2] and E. K. LUND[2], [1]Institute of Human Nutrition, University of Southampton, Southampton, SO9 3TU, and [2]AFRC Institute of Food Research, Norwich Science Park, Norwich, NR4 7UA

Dietary lipids can modulate the inflammatory response to cytokines (Grimble, 1992). The mechanisms underlying this modulation are largely unknown. As a first step in the elucidation of these mechanisms, the influence of a variety of dietary lipids on the composition and lateral mobility of rat hepatocyte plasma membrane lipids was investigated.

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 10 % of the fat was provided as corn oil to prevent essential fatty acid deficiency. At the end of the 5 week period livers were removed and hepatocytes isolated.

The lateral diffusion rates and mobility of hepatocyte plasma membrane lipids were measured, as an indication of membrane fluidity, using fluorescence recovery after photobleaching (FRAP). The fatty acid composition of the membrane lipids was determined by gas-liquid chromatography. Membrane free cholesterol contents were assayed enzymically using cholesterol oxidase.

Diet...	Chow		Butter		Coconut		Maize		Fish	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
D	19.0	2.2	15.8	2.5	15.1	2.0	14.1	2.7	22.6	5.3
%R	63.7[a]	3.6	57.6[a]	3.0	61.5[a]	4.0	47.3[b]	2.4	50.0[ab]	5.5

D, Diffusion coefficient $(\times 10^{-9} cm^2/s)$; % R, percentage recovery (the % of membrane lipids which are mobile in the timescale of the experiment). a,b values in the same row with unlike superscripts are significantly different (student's t-test) $\underline{P} < 0.05$.

No significant differences in the lateral diffusion rates of plasma membrane lipids were found between animals on the different fat diets, despite diet-induced changes in their fatty acid composition. However, the proportion of lipid which was mobile did change with diet, being significantly greater in animals fed the chow, butter fat or coconut oil diets than in those fed maize oil. There was a significant correlation ($\underline{r}$ 0.43, $\underline{P} < 0.01$) between the proportion of membrane lipids which were mobile and rates of lateral diffusion.

Diet	Fatty acid (% by weight of total membrane lipid fatty acids)								C:PL
	C16:0	C18:0	C18:1($\underline{n}$-9)	C18:2($\underline{n}$-6)	C18:3($\underline{n}$-3)	C20:4($\underline{n}$-6)	C20:5($\underline{n}$-3)	C22:6($\underline{n}$-3)	
Chow	35.5	17.9	4.0	17.8	0.2	12.1	0.7	2.9	0.36
Butter fat	30.8	26.3	6.8	12.5	0.2	15.1	0.1	1.6	0.41
Coconut oil	23.2	26.1	5.4	10.6	0.0	23.4	0.0	4.1	0.27
Maize oil	28.9	23.3	5.2	15.8	0.0	19.2	0.0	1.6	0.50
Fish oil	41.6	11.3	14.2	9.2	0.6	3.5	4.5	5.1	0.17

C:PL = cholesterol : phospholipid ratio

Diffusion coefficients were correlated with the $\underline{n}$-3 : $\underline{n}$-6 fatty acid ratio in the membrane ($\underline{r}$ 0.89, $\underline{P} < 0.05$). The quantities of arachidonic acid (AA)(C20:4($\underline{n}$-6)) and eicosapentaenoic acid (EPA) (C20:5($\underline{n}$-3)) in the membrane were negatively correlated ($\underline{r}$ -0.89, $\underline{P} < 0.05$). Since these fatty acids are sources of eicosanoids, with those derived from AA being more potent mediators of inflammation than those from EPA, this is one possible mechanism for the modulation of the inflammatory response by dietary lipids. However, other aspects of membrane composition may also play a role, together with the observed changes in the physical properties of the hepatocyte plasma membranes.

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Estimates of the vitamin E requirement in rats fed long-chain n-3 fatty acids. By LUCIE POLLARD and T.A.B. SANDERS <u>Department of Nutrition and Dietetics, King's College, University of London, Campden Hill Road, London W8 7AH</u>

Estimates for the requirement for vitamin E are usually based upon an intake of 0.4 mg vitamin E/g polyunsaturated fatty acid (PUFA). While this may hold for linoleic acid, it may not be true for other PUFA particularly those with 4,5 and 6 double bonds which are found in fish oil. Danse & Verschuren(1978) showed that 0.4 mg vitamin E/g PUFA in cod-liver oil did not protect against steatitis. In the present study we have attempted to ascertain the requirement for vitamin E in rats receiving refined fish oil (Seven Seas Ltd, Hull) containing mainly 20:5n-3 and 22:6n-3. Male adult rats were dosed orally with 1ml oil stripped of vitamin E 5 d/week for 6 weeks: controls (n 12) received olive oil and experimental animals (six groups of five animals) received 1ml fish oil. The animals received 20 g/d of semisynthetic diets that varied only in their vitamin E content: the control diet provided 0.4 mg vitamin E/g PUFA and the experimental diets 0,0.4,0.8,1.6,3.2 and 12.8 mg vitamin E/g PUFA. At the end of the experiment, erythrocyte malondialdehyde (MDA) production in response to H_2O_2 was measured spectrophotometrically, plasma and erythrocyte α-tocopherol concentrations were determined by HPLC and sections of epidydimal fat were stained for lipofuscin and examined by light microscopy. Results are mean values with 95% confidence intervals.

	Control	n-3 Fatty acids					
Vitamin E (mg/g).... PUFA	0.4	0	0.4	0.8	1.6	3.2	12.8
Plasma α-tocopherol (μmol/l)	7.0	4.3	8.0	11.3	11.0	14.7	18.0
	5.7-8.4	3.4-5.4	6.5-9.9	9.9-13.0	8.1-15.1	10.5-20.7	11.8-27.9
Erythrocyte α-tocopherol (μmol/l)	4.7	2.4	1.9	4.3	7.6	7.6	8.6
	3.6-6.2	0.9-6.7	0.9-4.0	3.2-5.9	4.1-13.9	6.1-9.5	5.3-14.0
Erythocyte MDA production (%)	6	12	21	25	19	7	5
	5-8	9-17	17-27	19-34	14-25	4-13	4-6
Steatitis score (range 0-3)	0.25	2.4	2.2	2.2	2.0	1.6	0.2
	0-1	2-3	2-3	2-3	2-2	1-2	0-1

Plasma and erythrocyte α-tocopherol concentrations were lower in the groups receiving less than 0.8 mg vitamin E/g n-3 PUFA than in the controls but then increased dose dependently. Erythrocyte MDA production,which is a marker of susceptibility to lipid peroxidation, was elevated with intakes less than 3.2 mg vitamin E/g n-3 PUFA. Compared with controls, steatitis was more prevalent in all experimental groups except those receiving 12.8 mg vitamin E/g n-3 PUFA; moderate to severe steatitis was observed in animals receiving less than 1.6 mg vitamin E/g n-3 PUFA. The results of this study suggest that intakes in excess of 3.2 mg vitamin E/g n-3 PUFA are required to protect membrane lipids against lipid peroxidation and intakes as high as 12.8 mg vitamin E/g n-3 PUFA may be necessary to prevent steatitis.

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Effects of feeding a riboflavin - depleted diet on the morphometry of the small intestine of the weanling rat. By E.A. WILLIAMS, R.D.E.RUMSEY, and H.J. POWERS, University Department of Paediatrics, Sheffield Children's Hospital and Department of Biomedical Sciences, University of Sheffield, S10 2TH

It has been proposed that the effects of riboflavin depletion on Fe metabolism are exerted at the level of the small intestine (Powers et al. 1993).

An experiment was conducted to determine whether effects of riboflavin depletion observed in female weanling Norwegian Hooded rats could be reproduced in Wistar rats and to investigate further the effects of riboflavin depletion (RD) on the morphometry of the small intestine. Control animals were fed *ad lib* on a complete diet or were weight matched (WM) to the depleted animals . Microdissection of the upper small intestine confirmed an increased crypt depth ($P = 0.001$) but also revealed an increased villus base width ($P = 0.02$) and an increased villus tip width ($P = 0.02$) in the RD group when compared with the WM group. *Ad lib*. feeding of the complete diet was associated with an increased villus height ($P = 0.001$) and an increased villus base width ($P = 0.007$) compared with the WM group. WM animals consumed the same amount of diet as the RD animals but 18% less than those fed *ad lib*.

Variable	Riboflavin deficient (n 9)		Weight matched (n 8)	
	Mean	SEM	Mean	SEM
Villus height (μm)	801	17.7	698	49.5
Villus base width (μm)	671	22.0	574*	25.8
Villus tip width (μm)	393	12.8	346*	15.4
Crypt depth (μm)	268	18.7	184**	11.0
Crypt width (μm)	62	2.3	58	1.4

significantly different from RD (Mann Whitney U)   * $P < 0.05$;   ** $P < 0.001$

Riboflavin depletion at weaning leads to hypertrophy of both the crypts and the villi of the upper part of the small intestine. These effects cannot be explained by a difference in food intake which does appear to exert an independent influence.

Scanning electron microscopy revealed that RD rats had much less clearly defined individual villi than the WM rats. In addition whereas there was abundant mucus in the small intestine of WM rats it was scarce in the RD rats. The effects of riboflavin depletion on the structure and function of the small intestine may be mediated by an effect on normal mucus production.

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Plasma vitamin E levels of dogs with retinal pigment epithelial dystrophy. By P.WATSON[1], G. ANANTHARAMAN[2], J. VUICHOUD[2] AND P.G.C. BEDFORD[1], [1]Royal Veterinary College, University of London, Hatfield, AL9 7TA and [2]Nestec Ltd, (FRISKIES Research), 1000 Lausanne, 26 Switzerland

Retinal pigment epithelial dystrophy (RPED) is a degenerative disease of the canine retina characterized primarily by the accumulation of abnormal lipofuscin-like pigments in cells of the retinal pigment epithelium (Aguirre & Laties, 1976). The retinal abnormalities are similar to those seen in canine vitamin E deficiency retinopathy but RPED affected dogs do not have the disease changes in other body systems which occur in vitamin E-deprived dogs (Riis *et al.* 1981).

Dogs of a number of breeds show a genetic predisposition to RPED, but there is no clear pattern of inheritance. Ophthalmoscopic abnormalities are first seen in dogs of most RPED-affected breeds at 2-3 years of age but in one breed, the Briard, the age of onset varies greatly between 18 months and 9 years of age (Bedford, 1984). It has been suggested that nutritional factors and in particular vitamin E may be important in the pathogenesis of RPED but this has not previously been investigated.

Fasting blood samples were taken from control dogs of varying breeds and from RPED-affected and ophthalmoscopically normal Briards, cocker spaniels and Polish lowland sheepdogs (Nizzini). All groups were age-, sex- and diet-matched. Plasma vitamin E concentrations were measured by high pressure liquid chromatography (HPLC; Lehmann & Martin, 1982). Statistical analysis was by analysis of variance.

Plasma vitamin E concentrations (μmol/l) are shown in the Table.

	n	Mean	SE
Normal control dogs	18	34.0[a]	3.3
Ophthalmoscopically normal Briards	19	40.0[a]	8.2
RPED-affected Briards	14	19.3[b]	1.2
Ophthalmoscopically normal Nizzini / cocker spaniels	6	34.4[a]	1.1
RPED-affected Nizzini / cocker spaniels	6	2.8[c]	1.0

a, b or c values with unlike superscript letters are significantly different (ANOVA): ($p < 0.05$).

Plasma vitamin E levels were lower in RPED-affected Briards, Nizzini and cocker spaniels than in ophthalmoscopically normal dogs of these breeds. There was no significant difference between vitamin E levels of normal dogs of these breeds and those of control dogs.

These results suggest that RPED-affected dogs have abnormal uptake and/or transport of vitamin E. The higher levels in RPED-affected Briards than in RPED-affected dogs of other breeds may relate to the variation in age of onset of RPED in this breed. The presence of a measurable level of vitamin E in the plasma of all RPED-affected dogs may provide some antioxidant protection in most tissues such that, in contrast to vitamin E-deficient dogs, abnormalities only occur in the unique and highly oxidative milieu of the retina.

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Nutrient intakes and breakfast cereal consumption habits of British schoolchildren. By S. A. GIBSON[1] and K. R. O'SULLIVAN[2] [1]Leatherhead Food Research Association, Randalls Rd, Leatherhead, Surrey, KT22 7RY and [2]Kellogg Company of Great Britain Ltd, Talbot Rd, Manchester, M16 OPU

Breakfast cereals are generally low-fat nutrient-dense foods that could theoretically make a substantial contribution to nutrient intakes as well as reducing the proportion of energy derived from fat in the diet.

To examine the relationship between breakfast cereal consumption level and total nutrient intakes, the 7 d weighed dietary records of schoolchildren surveyed by the Department of Health in 1983 (Department of Health, 1989) were reanalysed. The 2705 children were grouped according to level of consumption of breakfast cereals (none; < 20 g/d; 21-40 g/d; > 40 g/d) and relationships between consumption and nutrient intake examined for each age group (age 10-11, and 14-15 years), for each sex. Intakes of energy and thiamin, riboflavin, Ca and Fe rose with increasing level of cereal consumption. However, cereal consumers also tended to have diets of higher nutrient density. The proportion of energy from fat fell with increasing cereal consumption, averaging 39-40% among non-consumers and 36-37% among children consuming over 40 g cereals/d ($P < 0.001$).

Daily intake of energy and other nutrients according to level of cereal consumption

Cereal intake (g/d)	Boys 10-11 years				Boys 14-15 years				Girls 10-11 years				Girls 14-15 years			
	0	<20	<40	>40	0	<20	<40	>40	0	<20	<40	>40	0	<20	<40	>40
n	100	211	315	272	93	80	141	191	166	301	242	120	144	103	160	66
Energy (MJ)	8.20ᵃ	8.35ᵃ	8.50ᵃ	9.00ᵇ	9.65ᵃ	9.92ᵃ	10.02ᵃ	11.1ᵇ	7.38ᵃ	7.44ᵃ	7.76ᵃ	8.34ᵇ	7.37ᵃ	7.86ᵃᵇ	7.97ᵇ	8.56ᵇ
Fat (% energy)	39.2ᵃ	38.5ᵃ	37.3ᵇ	35.9ᶜ	40.1ᵃ	37.8ᵇ	37.6ᵇ	36.3ᶜ	39.6ᵃ	38.8ᵃ	37.4ᵇ	35.9ᶜ	39.4ᵃ	39.4ᵃ	37.4ᵇ	36.8ᵇ
Fe (mg)	9.1ᵃ	9.5ᵃ	9.7ᵃ	10.6ᵇ	11.0ᵃ	11.5ᵃ	11.8ᵃ	13.2ᵇ	8.1ᵃ	8.1ᵃ	8.9ᵇ	9.6ᶜ	8.4ᵃ	9.3ᵇ	9.6ᵇᶜ	10.3ᶜ
Ca (mg)	710ᵃ	780ᵇ	860ᶜ	920ᵈ	750ᵃ	810ᵃᵇ	910ᵇ	1030ᶜ	630ᵃ	675ᵃ	755ᵇ	825ᶜ	595ᵃ	680ᵇ	735ᵇ	830ᶜ
Vitamin B₁ (mg)	0.8ᵃ	1.0ᵇ	1.2ᶜ	1.4ᵈ	1.0ᵃ	1.2ᵃ	1.4ᵇ	1.8ᶜ	0.7ᵃ	0.9ᵇ	1.1ᶜ	1.3ᵈ	0.8ᵃ	1.0ᵇ	1.2ᶜ	1.3ᵈ
Vitamin B₂ (mg)	1.1ᵃ	1.4ᵇ	1.7ᶜ	2.1ᵈ	1.2ᵃ	1.5ᵇ	1.8ᶜ	2.4ᵈ	1.0ᵃ	1.2ᵇ	1.6ᶜ	1.8ᵈ	1.0ᵃ	1.2ᵇ	1.6ᶜ	1.8ᵈ

ᵃ,ᵇ,ᶜ,ᵈ Within each age group, within sex, mean values with different superscript letters are significantly different (Multiple range test): $P < 0.05$.

Fe, Ca and riboflavin intakes of older girls particularly were identified as below the RDA in the original report (Department of Health, 1989). However, those who consumed more than 40 g cereals/d had mean intakes of Ca and Fe above the current Reference Nutrient Intakes (Department of Health, 1991).

These results suggest that nutrition education designed to encourage breakfast cereal consumption among adolescents may improve dietary quality and assist the achievement of dietary fat goals.

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CURRENT CALCIUM INTAKE AND BONE MINERAL CONTENT IN YOUNG MEN AND WOMEN. By T. J. PARSONS[1], A. PRENTICE[1], E. S. SMITH[3], T. J. COLE[1], M. A. LASKEY[2] and J. E. COMPSTON[3], [1]*MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*, [2]*Department of Nuclear Medicine, Addenbrookes Hospital, Cambridge CB2 2QQ, and* [3]*Department of Medicine, University of Cambridge, Cambridge CB2 2QQ.*

Seventy-seven normal, healthy young adults, thirty-seven male, forty female, age 18 - 21 years (in Cambridge) were recruited to investigate Ca intake and the development of peak bone mass after cessation of linear growth. Subject exclusion criteria included a history of renal disease, long term use of steroids, amenorrhea and anorexia.

Bone mineral content (BMC;g/cm) of the radius was measured at the midshaft and 5mm distal site using single-photon absorptiometry (SP2;Lunar Radiation Corporation, Madison, WI, USA), and at the spine, hip and whole body (BMC;g) using dual energy X-ray absorptiometry (DPX;Lunar). Ca intake was assessed using a food frequency questionnaire (Calquest; M. Nelson, King's College, London) and in fifty-seven subjects also by a 7d food record. Data on lifestyle factors were collected by questionnaire.

	Men		Women	
	Mean	SD	Mean	SD
Weight (kg.	68.5	7.3	60.9	6.8
Height (cm)	179.1	6.1	165.8	6.8
BMC: shaft (g/cm)	1.05	0.14	0.80	0.10
wrist (g/cm)	1.05	0.22	0.85	0.12
spine L1-4 (g)	70.8	11.7	64.4	9.4
femoral neck (g)	6.02	1.10	4.62	1.04
Ward's triangle (g)	3.61	0.94	2.71	0.47
trochanter (g)	13.8	3.4	9.45	1.89
Ca intake (mg/d) Calquest	1191	601	913	295

The range of Ca intakes was 176 - 3240 mg/d as assessed by the frequency questionnaire, corrected for the fact that Calquest only accounts for about 82% of Ca in the diet. The mean Ca intakes calculated from the food diaries were 1248 (SD 335) mg/d and 955 (SD 249) mg/d for twenty-six males and thirty-one females respectively. There was no significant difference between mean Ca intakes derived using the two different methods. At all sites BMC was significantly greater ($P<0.02$) in males than females but there was no association between BMC and calcium intake. After corrections for height, weight, age and scan area, multiple regression analysis showed that male BMC was significantly greater than female (7.3%;$P<0.01$) at the midshaft, but lower (-10.9%;$P<0.001$) at the spine (L1-4) with no difference at the wrist or hip. Corrected BMC at any site measured was not related to current Ca intake or, in females, age of menarche. Future longitudinal studies will provide more information about the impact of Ca intake on the development of peak bone mass in young adult life.

Factors contributing to goitre in pregnant women in Bangladesh. By S.M. FILTEAU[1], U. ANWAR[1], Z. REZWANA[1], A. JOHNSON[2] and A.M. TOMKINS[1], [1]Centre for International Child Health and [2]Division of Biochemistry and Genetics, Institute of Child Health, 30 Guilford Street, London WC1N 1EH

As part of a study on the effect of iodized poppyseed oil given to pregnant women on development of their infants, we analyzed presupplementation iodine status and the causes of goitre in these women. Goitrous women at various stages of pregnancy were recruited to the study. Clinical goitre grade was determined, anthropometric measurements performed and blood and urine samples were collected. Standard analyses were performed for urinary iodine and thiocyanate, serum thyroxine (T_4) and thyroid-stimulating hormone (TSH).

In spite of the high goitre prevalence in this area and the universal presence of goitre in the study women, urinary iodine results indicated only modest iodine deficiency with 14% of samples less than 0.16 μmol I/l and 51% less than 0.39 μmol I/l. Serum T_4 was weakly correlated with urinary iodine, expressed per g creatinine ($r=0.29$, $P=0.0001$). Most women (197/348) had T_4 and TSH within the normal ranges for pregnant women, thirty-three had hormone levels characteristic of hypothyroidism, and twenty-six women appeared to be hyperthyroid. In addition, thirty-seven women had elevated TSH levels in spite of normal T_4 levels and fifty-five had low concentrations of both hormones.

In an attempt to provide an explanation for the high goitre prevalence, we investigated goitrogens and other nutritional factors which might affect thyroid hormone metabolism. Thiocyanate-producing goitrogens were not an important factor as indicated by uniformly low urinary thiocyanate levels 14.5 μmol/l, SD 14.1, n=215). Analyses of a subset of the blood samples indicated that neither Se nor vitamin A deficiency was likely to be a major problem in these women. Mean serum Se was 0.13 mg/l (SD 0.02, n=21) and serum retinol was 1.36 μmol/l (SD 0.50, n=30). We conclude that goitre prevalence can be high even without severe iodine deficiency or high intake of thiocyanate-producing goitrogens. We suggest that the goitres may be due to a past iodine deficiency. Current iodine intakes, although adequate to support T_4 synthesis, are not, however, adequate to reverse the goitre.

Nutritional implications of reduced-fat (RF) food use in free-living consumers. By S.J. GATENBY, J.I. AARON, G.M. MORTON and D.J. MELA   Consumer Sciences Department, AFRC Institute of Food Research, Earley Gate, Whiteknights Road, Reading, RG6 2EF

Current dietary guidelines recommend a reduction in dietary fat and specifically in the percentage of energy derived from fat (Department of Health, 1992). In response to these and other recommendations the food industry has marketed a wide range of low- or RF products. Such products would appear to be a simple way to induce shifts in the nutritional quality of the diet without the need to alter substantially consumer behaviour or food selection/purchase patterns. Several studies have investigated the effects of RF foods and meals on subsequent nutrient intake and eating behaviour (Foltin *et al.* 1990; Caputo & Mattes, 1992). However, such studies have usually employed covert manipulations in highly controlled laboratory settings and with observational periods of only a few days detracting from their ecological validity. The question of whether the consumption of RF foods in a free-living environment would have a beneficial effect on maintenance of energy balance or, in fact, whether they might produce a meaningful reduction in total fat intake, has not been resolved. To date there has been no prospective study of the nutritional implications of extended use of commercially available RF foods by free-living consumers.

Non-obese free-living volunteers (*n 29*) were randomly allocated to either a control (CG) or an intervention group (IG). All subjects were asked to purchase their own foods from a retail supermarket and consume them *ad lib.* at home for a 6-week period. The IG subjects (*n 15*) were encouraged to purchase and consume available RF versions of common foods. These subjects were not restricted in their consumption of any other foods, but were reimbursed for the RF items. The CG subjects (*n 14*) were given no diet instruction and were repaid 10% of their food bill. Weighed diet records over 4d and anthropometric data were collected and analysed at weeks 0 (baseline), 2, 4 and 6. Data were analysed using full factorial repeated measures analysis of variance.

Compared with the CG subjects, the IG subjects immediately and consistently reduced % energy from fat from 39% to 30% (group x time $P<0.001$). In the IG subjects there was a significant increase in % energy from carbohydrate from 44% to 49% (group x time $P=0.02$), the increase in % energy from protein approached significance (group x time $P=0.06$). These increases compensated for the reduced energy density of the diet, such that there were no diet-related differences in energy intakes. However, energy intake in the first 2-week period was reduced in the IG subjects leading to a small but statistically significant reduction in body weight in this group by 1.1kg compared with CG subjects ($P<0.001$). These results indicate that, as typically purchased and used by consumers at home, use of RF foods may lead to significant reductions in fat intake. However, as an isolated dietary modification, the consumption of RF foods does not necessarily lead to a reduced energy intake.

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A comparison between the nutrient content of school lunches and recommendations. By C.H.S. RUXTON[1], T.R.KIRK[1], N.R. BELTON[3] and M.A.M. HOLMES[2], [1]Department of Dietetics and, Nutrition and [2]Department of Management and Social Sciences, Queen Margaret College, Edinburgh EH12 8TS and [3]Department of Child Life and Health, Edinburgh University, Edinburgh EH9 1UW

Since the abandonment of nutritional standards for school lunches in 1980, published data have indicated that the fat content of school meals may be too high (DoH, 1989). Recently, an expert panel has suggested that nutritional guidelines be re-introduced in order to improve the quality of the school lunch (Caroline Walker Trust, 1992) yet there is little up-to-date information on actual nutritional quality.

In this study, 146 children aged 7 to 8 years were recruited by letter from a target group of 263 attending five schools in Lothian region. School lunches were weighed by a research assistant while parents, following instruction, weighed all other food and leftovers for 7 days. Salter electronic scales weighing to 2kg in 2g increments were used in each case. Acceptable diaries were finally received from 136 families; seven were incomplete and three were discarded due to a poor standard of information. Social class was slightly biased towards groups IIIb and V. Data were analysed using COMP-EAT4 and SPSS/PC+. The following table compares the mean nutrient content of the 336 school lunches, taken by the children over the study period, with The Caroline Walker Trust recommendations (1992).

| Nutrient | Recommended values | | School meal (% recommendation) | | | |
| | | | Boys (n 65) | | Girls (n 71) | |
	Boys	Girls	Mean	%R	Mean	%R
Energy (MJ)	2.47	2.18	1.84	74	1.72	79
Protein (g)	8.5	8.5	13.2	155	12.0	141
NSP (g)	4.7	4.2	1.6	34	1.6	38
Ca (mg)	196.0	193.0	132.8	69	156.1	81
Fe (mg)	3.5	3.5	2.0	57	1.7	48
Vitamin A (μg)	150.0	150.0	105.4	70	100.3	67
Folate (μg)	60.0	60.0	28.9	48	28.8	48
Vitamin C (mg)	11.0	11.0	18.6	169	16.2	147
%Energy as fat	35.0	35.0	42.1	120	42.4	121
%Energy as CHO	50.0	50.0	45.9	92	42.1	84

NSP, non-starch polysaccharide; CHO, carbohydrate; %R, percentage of recommended value

The school lunch provided satisfactory amounts of protein and vitamin C compared to the recommendations but other micronutrients were low, as was energy. However, since mean daily intakes of energy and micronutrients were on or above Dietary Reference Values (DoH, 1991), it would seem that school lunches did not greatly influence the children's overall diet. NSP was low both in the school lunch and the overall diet (mean overall intake 8.2g boys; 7.9g girls). Percentage energy from carbohydrate was lower and percentage energy from fat higher than the recommendations, the latter reflecting the overall pattern of mean percentage energy from fat (38%).

In conclusion, the school lunches in this study did not comply with The Caroline Walker Trust recommendations. However, taking into account data on overall nutrient intake, it is suggested that the guidelines for NSP and percentage energy from fat, rather than those for micronutrient content, appear to be more relevant for the school lunches chosen by this sample of 7 to 8-year olds.

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Correction for measurement error in fat intakes estimated by a food frequency questionnaire. By R.L. THOMPSON[1], B.M. MARGETTS[2] and D.A. WOOD[1], [1]Clinical Epidemiology, National Heart and Lung Institute, London SW3 6LY and [2]Institute of Human Nutrition, University of Southampton, SO9 3TU

A self-administered food frequency questionnaire (FFQ;Yarnell *et al* 1983) has been previously validated (Thompson & Margetts, 1993) and shown to underestimate, by about 11%, the intakes by men of fat and polyunsaturated fat (PUFA), when compared with a 10 d weighed record (WR). There did not appear to be a consistent difference between the methods across the range of intakes. We have attempted to correct the FFQ for this bias. At low intakes the FFQ underestimated fat and PUFA intakes and at high intakes overestimated intake. The FFQ estimates were corrected by weighting those with high and low intakes so that on average there was no difference between the FFQ and WR. The effect of this bias was investigated by comparing FFQ estimates of fat and PUFA intakes of male smokers (S) and male non-smokers (Non-S).

The Table shows the comparisons before and after the correction factor was included.

	Before correction						After correction					
	S (*n* 78)		Non-S (*n* 181)				S (*n* 78)		Non-S (*n* 181)			
	Mean	SE	Mean	SE	MD	P†	Mean	SE	Mean	SE	MD	P†
Fat (g)	84.6	3.6	79.3	1.8	5.3	0.47	96.6	2.7	92.5	1.4	4.1	0.42
PUFA (g)	14.7	0.9	15.4	0.6	-0.7	0.12	17.5	0.8	17.9	0.5	-0.4	0.22

MD, mean difference.

† Analysis of variance after adjustment for age and occupation group.

The data show that after adjustment for age and occupation group the mean difference between smokers and non-smokers was reduced for both fat and PUFA. There was a larger effect for PUFA where the difference between the groups was reduced by almost half; however, the statistical significance was only marginally changed. This appeared to be due to a reduced standard error. After additional adjustment for energy the FFQ for PUFA gave a statistically significant result for the uncorrected FFQ (P = 0.02) but not for the corrected value (P= 0.08).

Correcting the FFQ for measurement error by this method may provide a more reliable estimate of the real differences between groups than using uncorrected estimates.

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An initial assessment of food and nutrient intake for the development of a food frequency questionnaire for use in an Afrocaribbean population sample.

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There is virtually no information available on the Afrocaribbean diet in the United Kingdom. This preliminary study aimed to determine the food and nutrient intake of this population in order to design a food frequency questionnaire (FFQ) for use in assessing the usual food intake of a larger sample. These data will be used to determine nutritional influences on the emergence of diabetes and hypertension.

A random sample of forty Afrocaribbeans was taken from ninety-five subjects who had been randomly selected from a health centre register as part of a health screening programme. They were asked to complete a 2 d food diary recording all foods and drinks consumed and the serving size. Two subjects were living outside Manchester and were therefore unavailable. The diary was completed by twenty-five subjects. Four were unable to fill out the diary and so provided a 24 h recall of foods eaten. This gave a response rate of 76 % (29/38). The completed food diaries were spread evenly throughout the days of the week. Portion weights were estimated on the basis of published (Crawley, 1988) and unpublished data. The food diaries were analysed using "Microdiet" to obtain mean daily nutrient intake.

	n	Energy kJ	Protein g	Protein % total	Fat g	Fat % total	CHO g	CHO % total	Alcohol g	Alcohol % total
Males	13	8.42	80	16	79	36	207	39	29	10
Females	16	6.05	60	17	57	35	180	47	3	1

The table shows the mean daily nutrient intake and energy profile for the sample group. The fat intake, as a percentage of energy, is lower than that for a national mainly white population (38 % men, 39 % women ; Gregory et al. 1990).

In developing the FFQ, foods which contributed at least 90 % of the macronutrient intake were listed . Foods on these lists were different from similar lists for the white population. For example, in our population the top three contributors to energy were vegetable oil, lamb and rice which provided 18.5 % of total energy intake; data from the Three Towns Study (Cade & Margetts, 1988) showed that whole milk, white bread and flour contributed an equivalent percentage to energy. Such differences emphasize the need for preliminary studies to be undertaken in order to develop specialised FFQ for use with different ethnic groups . Our FFQ has now been piloted in a further forty randomly selected Afrocaribbeans. They were also asked if they regularly ate any additional foods that were not listed. From this pilot eight additional foods, not previously listed due to seasonal variation, have been added to the original version to produce the final 120 item FFQ now being used in our large population survey.

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Development and evaluation of food photographs for use in retrospective dietary surveys. By P.J. ROBSON, M.B.E. LIVINGSTONE, E.W.A. ARCHER and P.G. McKENNA, Human Nutrition Research Group, Department of Biological and Biomedical Sciences, University of Ulster, Coleraine, BT52 1SA

Estimation of food portion size is a major source of measurement error in retrospective dietary surveys (Young *et al.* 1952). Techniques used to help subjects estimate portion size include household measures, three- and two-dimensional models and food photographs. Photographs are portable and provide direct visual representation of a wide range of foods. Despite their increasing use, little is known about the accuracy of portion sizes estimated using photographs.

Colour photographs (10cm x 15cm) of known portion weights of ninety-one foods were devised, and their ability to help subjects accurately estimate the portion sizes of foods usually consumed was assessed. Fifty-four adult volunteers (eighteen males, thirty-six females) completed food frequency questionnaires and foods eaten at least once weekly were identified. Subjects weighed these foods every time they were consumed over 14-21 consecutive days. Mean weights of usual servings were calculated for every food which each subject had weighed at least twice. One week later, subjects estimated their usual food serving size in terms of fractions or multiples of portions shown in the appropriate photographs. These were converted to weights.

Average amounts of foods weighed by each subject were compared with amounts estimated using photographs. Differences were expressed as a percentage of weighed portions. Linear regression analysis of estimated portion weight on actual portion weight was also carried out for each food (Faggiano *et al.* 1992).

Results are reported for the twenty-six foods weighed and estimated by five or more subjects. Of these twenty-six foods, 50% were under-estimated relative to weighed portions and 50% were over-estimated. Mean (SD) under-estimation of usual portion size ranged from -0.1 (38.8)% for baked beans to -39.4 (20.2)% for Rice Krispies[®]. Seven foods (carrots, boiled rice, cornflakes, Raisin Splitz[®], porridge, canned tunafish and Rice Krispies[®]) were under-estimated by more than 20%. Mean (SD) over-estimation ranged from 2.1 (36.2)% for chips to 56.5 (66.6)% for sausages. Six foods (mashed potato, milk added to breakfast cereals, home-made biscuits, cheese, wheaten bread and sausages) were over-estimated by more than 20%. The regression slope for twenty-one foods (80.8%) was less than 1.0 suggesting a tendency for smaller serving sizes to be over-estimated and larger serving sizes to be under-estimated.

It is concluded that these photographs in their present form may not be an effective instrument for accurate estimation of food portion size. Indiscriminate use of photographs should be discouraged until the impact of inaccurate portion size estimation on reported energy and nutrient intakes has been fully evaluated.

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Development of a food frequency questionnaire. By S. A. LANHAM[1] and C. BOLTON-SMITH[2], [1]Osteoporosis Screening Unit, City Hospital, Urquhart Road, Aberdeen, AB9 8AU and [2]Cardiovascular Epidemiology Unit, Ninewells Hospital, Dundee, DD1 9SY

A food frequency questionnaire (FFQ) was developed for an epidemiological investigation into the influence of diet on bone mineral density (BMD) and bone metabolism (BM). The questionnaire was loosely based on that used previously (Bolton Smith *et al.* 1991) with extension of the 0-7 d/week frequency options to include once daily, 2-3 times daily and >4 times daily for each food item. Questions on the past intake of key foods relevant to osteoporosis were integrated into the main body of the FFQ. The foods which contributed most to the nutrients of interest (including Ca, fibre, vitamin D etc.) in pre-menopausal Scottish women were identified using 7 d weighed records from twenty women aged forty-five to forty-nine years. The women were randomly selected from the Community Health Index (CHI;Garton *et al.*1992).

Stepwise multiple regression analysis (SMRA) was carried out on each of the nutrients of interest with total energy intake as the independent variable to identify the food groups which discriminate most between individuals, rather than those that contribute most to absolute intake. Standard portion sizes were assigned for each food item based on two sets of intake data: (1) From the portion sizes consumed in fifty, 7 d weighed records from the Inverurie Study on Aberdeenshire women aged forty to fifty-nine years and (2) from the 7 d weighed intake study undertaken here. The FFQ was piloted using a further seventy women selected randomly from the CHI. The response rate was 85%. Alterations to the format of the FFQ were made based upon these responses.

Comparisons of nutrient intake obtained from the FFQ were made using a further study of twenty, 7 d weighed records (WR), and the results are shown in the Table.

| | Nutrient intake/d | | | | | | Nutrient intake/d | | | | |
| | WR | | FFQ | | | | WR | | FFQ | | |
	Mean	SD	Mean	SD	r		Mean	SD	Mean	SD	r
Energy (MJ)	1806	173	1842	376	0.48*	Fibre (g)	13	5	17	4	0.59**
Fat (g)	75	13	75	18	0.45*	P (mg)	1144	253	1259	489	0.60**
Protein (g)	67	15	68	13	0.50*	Vitamin D (μg)	2.5	1.0	2.5	0.9	0.12
Ca (mg)	817	214	670	231	0.52*	Vitamin C (mg)	71	36	94.5	23	0.79***

r, Pearson correlation coefficients: * $P<0.1$, ** $P<0.05$, *** $P<0.001$.

The correlation values are well within the range of those found in previous studies (Yarnell *et al.* 1983; Bolton-Smith *et al.* 1991a). Correlations between the two methods is unlikely to be extremely high as the methods are essentially measuring two different things; an individual's intake over a short period of time or their usual intake over the previous year. Agreement within the group as shown by the mean values was good, especially in view of the fact that the numbers were small. Although further weighed intakes will be undertaken for comparison of the two methods, based upon these results, this newly designed and validated questionnaire is being used to assess the influence of diet on BMD and BM in 1000 pre-menopausal women.

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Responses to riboflavin and multinutrient supplements by Gambian school children. By C.J. BATES, P.H. EVANS and G. ALLISON, MRC Dunn Nutrition Unit, Cambridge, CB4 1XJ and Keneba, The Gambia

Micronutrient deficiencies are common in developing countries, and there is continuing need to explore the efficacy of supplements, by biochemical and functional responses, to develop monitoring procedures and to formulate new intervention policies. The present study explored responses to two types of supplement by rural West African school children. One purpose was to re-examine an observation in rural Indian school children (Prasad et al 1990).

Ninety pre-selected school children, aged 8-14 years, in subsistence farming villages in the West Kiang district of The Gambia were randomly allocated, after age- and sex-stratification, to three groups. One received a coloured lactose (placebo) tablet, one received riboflavin (B_2, 5mg), and one received a multivitamin (Protovit N; F. Hoffmann-La Roche, Basel, Switzerland, containing vitamins: A 500μg, D 6 μg, E 10 mg, C 40 mg, B_1 1 mg, B_2 1 mg, B_6 1.5 mg; B_{12} 4 μg, folate 150 μg, niacin 10 mg, biotin 100 μg and pantothenate 5 mg), on 5 d each week for 1 year. The multivitamin group also received 200 mg ferrous sulphate once weekly. Blood samples were collected at time zero and 6 weeks after starting for measurement of riboflavin status (erythrocyte glutathione reductase (EC 1.6.4.2) activation coefficient (AC)), thiamine status (erythrocyte transketolase (EC 2.2.1.1) AC) and B_6 status (plasma pyridoxal phosphate nM) :physiological tests were performed at time zero and after 6 weeks and 1 year. The Table shows that selected biochemical indices responded positively to supplementation at 6 weeks:

Groups...	Placebo (n 31)		Riboflavin (n 30)		Multinutrient (n 29)	
Index	Mean*	Change+	Mean*	Change+	Mean*	Change+
Plasma vitamin C (μM)	21.8	-5.6[a]	17.1	+0.8[a]	21.9	+18.1[b]
Riboflavin status	1.97	-0.04	1.95	-0.71[b]	2.00	-0.46[c]
Thiamine status (nM)	1.18	+0.01	1.18	+0.02	1.18	-0.05
B_6 status	12.4	+2.4[a]	13.7	+2.8[a]	13.5	+17.1[b]

* Mean value at time zero; + mean change after 6 weeks. [a,b,c] Values with unlike superscript letters are significantly different (one-way ANOVA and Scheffé test): P<0.01.

A significant improvement was seen in the arm tremor test in the boys at the 6-week time-point, in both the supplemented groups (change in contact scores, 0-6 wks: placebo (n 19) + 39 (SE 13); riboflavin (n 16) -7 (SE 11); multinutrient (n 17) -8 (SE 14); one-way ANOVA (P = 0.02)). This upholds the findings of Prasad et al 1990.

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Plasma ubiquinone (Q_{10}) concentrations in female vegetarians and omnivores.

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Coenzyme Q is a lipid soluble compound comprising a redox active quinoid nucleus and a hydrophobic side chain containing a variable number of isoprenoid units: ubiquinone-10 (Q_{10}) contains ten isoprenoid units in the side chain and is the predominant mammalian form. Besides its role in electron transport, coenzyme Q_{10} is believed to be an important antioxidant and may have a role in preventing the oxidative modification of the low-density lipoprotein (Frei *et al.* 1990). HMG-CoA reductase inhibitors, which are used therapeutically to decrease plasma cholesterol concentrations, also lead to a reduction in plasma Q_{10} concentrations in man (Folkers *et al* .1990). This raises the possibility that Q_{10} may be a conditionally essential nutrient and that dietary Q_{10} may be of importance in patients treated with these drugs, and may also explain why cardiomyopathy is sometimes observed with these drugs. It is unknown to what extent dietary Q_{10} influences plasma concentrations. We report plasma ubiquinone Q_{10} concentrations in women following vegetarian and omnivorous diets who were matched for age.

Plasma Q_{10} concentrations were determined by HPLC (Takada *et al.*1982) on samples from fasting subjects that had been stored at -20 $^{\circ}$. The results are shown in the table

| | Plasma coenzyme Q_{10} (nmol/l) | | |
	White omnivores n 8	Asian vegetarians n 8	White vegetarians n 8
Median	47	129*	97*
Range	Trace-147	18-208	17-320

* significantly different from the omnivores Wilcoxson's test : $P < 0.05$

| | Cholesterol:CoQ$_{10}$ ratio | | |
	White omnivores	Asian vegetarians	White vegetarians
Median	1.10	0.32	0.43

Plasma Q_{10} concentrations were significantly greater in both groups of vegetarian subjects compared with the omnivores. Our results suggest that the diet of vegetarians supplies more coenzyme Q_{10} than those of omnivores. Further studies are needed to identify dietary sources of Q_{10} and whether these differences in plasma concentration of Q_{10} are of physiological significance with regard to modification of LDL and atherosclerosis.

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An assessment of the iron status of a sample of normal, healthy, 1-year-old children. By V. E. FREEMAN[1], H.M.V. HOEY[2] and M.J. GIBNEY[1]. [1]Department of Clinical Medicine, Trinity College Medical School, St. James's Hospital, Dublin 8, Republic of Ireland, and [2] Department of Paediatrics, Trinity College, Dublin 2, Republic of Ireland

Those known to be primarily at risk of Fe deficiency anaemia and of Fe depletion as reflected in low Fe stores include infants and young children (Hercberg & Galan, 1992).

A recent study of twenty-two Dublin children has suggested that 50% have depleted (serum ferritin<10µg/l) Fe stores (Murphy *et al.* 1992). Other studies, many of which focus on ethnic communities, have recorded unacceptably high incidences of Fe deficiency anaemia (haemoglobin<11g/dl) and depleted Fe stores (Marder *et al.* 1990, Duggan and Steel, 1991).

The objective of the present study was to re-examine the iron status of infants in Ireland. Haematological variables were measured for forty-seven 1-year-old children. The infants were deemed to be clinically well and developmentally normal and were representative of all socio-economic classes. They have been and are involved in a prospective, longitudinal study since birth and nutritional, anthropometric and socio-economic data and illnesses have been recorded during their first year of life. A venous blood sample was taken from each infant and analysed for full blood count and serum ferritin. Of the forty-seven infants whose haemoglobin (Hb) was recorded, three (6.3%) had Hb below 11g/dl, the acceptable lower limit for this age group as defined by the World Health Organisation; mean Hb was 12.17g/dl (SD 0.8). Serum ferritin < 10µg/l, indicative of depleted Fe stores, was found in ten of forty-five infants (22%); mean serum ferritin was 26.6µg/l (SD 20.4).

This study confirms the existence of a problem with low serum ferritin in our infant population. However, only 22% have depleted Fe stores, less than half the incidence previously recorded. Using the more conservative cut off point for ferritin of 15µg/l as suggested by Cavill, Jacobs & Worwood (1986) the incidence of Fe depletion rises to 29%. This still suggests that there may be a serious problem in paediatric nutrition. However, given that the cut off point for ferritin has been extrapolated from adult data, follow-up studies are required to define more accurately the appropriate normal range for serum ferritin in this age group. It may be that the demand made on Fe stores by periods of rapid growth is a contributory factor to the low levels of storage Fe which are consistently reported in infancy.

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Antioxidant vitamin status, lipid peroxidation, and indices of nitric oxide production in patients with sepsis and secondary organ dysfunction. By HELEN F. GOODE[1], HUGH COWLEY[2], JACK P. LEEK[3] and NIGEL R. WEBSTER[1,2], [1]Clinical Oxidant Research Group, [2]Intensive Care Unit and [3]University of Leeds Department of Clinical Medicine, St James's Hospital, Leeds, LS9 7TF, UK.

Potential for free radical involvement in the manifestations of sepsis is possible through a number of mechanisms, secondary to the primary disease process (Goode & Webster, 1993). Activated neutrophils produce superoxide and nitric oxide, which can react to form peroxynitrite and the damaging hydroxyl radical (Hogg *et al*, 1992). Nitric oxide, a potent vasodilator, is also thought to play a role in the development of septic shock. Hepatic sequestration of plasma proteins and release of free iron as a consequence of inflammation will tend to potentiate free radical damage. The antioxidant vitamins A and E, and the carotenoids play a major role in the endogenous defence system. We assessed antioxidant vitamin status (plasma vitamins A and E, plasma βcarotene and lycopene), evidence of lipid peroxidation (thiobarbituric acid reactive substances; TBARS) and an indirect index of nitric oxide production (urinary nitrite excretion) in sixteen patients (seven female, nine male, age range 16-79 years) admitted onto the intensive care unit at St James's Hospital, Leeds.

All the antioxidant vitamin concentrations were significantly lower in the patients than the reference range obtained from a comparable group of healthy controls. Three patients had vitamin A levels below our detectable range (<20 μg/l) and a further eight had values below the lower limit of the reference range (<370 μg/l), with a mean concentration of 265 μg/l. Vitamin E was below the reference range (<9.0 mg/l) in all patients, with a mean value of 3.6 mg/l. Plasma β carotene and lycopene were undetectable (<15 μg/l) in eight patients and below the reference range in the rest (<101 μg/l and <154 μg/l respectively). Seven patients had evidence of lipid peroxidation as shown by markedly increased concentrations of TBARS. No relationship was found between the plasma concentrations of vitamins or carotenoids and TBARS. In the five patients from whom we were able to collect urine, the nitrite excretion was significantly increased with a mean excretion of 423 μmol/24 hours compared to 1.1 μmol/24 hours in healthy subjects.

These data show that patients with sepsis have markedly depleted concentrations of the antioxidant vitamins, either due to underlying nutritional defecit or increased utilisation, and several patients also have evidence of increased lipid peroxidation. In addition, indirect measures suggest increased nitric oxide production.These patients are likely to benefit from some form of antioxidant therapy.

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Vitamin E and Polyunsaturated Fatty Acid intake modulate the inflammatory response to cigarette smoke. By K.L. TROUGHTON, R. THOMPSON and R.F. GRIMBLE, *Department of Human Nutrition, University of Southampton, Southampton, SO9 3TU.*

Cigarette smokers are exposed to a regular influx of free radicals and inflammatory stimuli, resulting in immune cell activation, cytokine production and altered acute-phase protein synthesis (Bridges *et al.*, 1990). The peroxidative damage that ensues may be linked with coronary heart disease and atherosclerosis (Fleet *et al.*, 1992).

Antioxidant vitamins such as vitamins E, A and C are known to quench free radicals and thus reduce peroxidative damage. Conversely, polyunsaturated fatty acids (PUFA) have been associated with elevated lipid peroxidation (Harats *et al.*, 1991). Hence, the intake of these nutrients may modulate the inflammatory response to cigarette smoke.

The acute-phase proteins caeruloplasmin, α1-acid glycoprotein, α2-macroglobulin and C-reactive protein were measured in the plasma of forty-five healthy cigarette smokers (aged 35-50). A 7 d weighed dietary intake was completed and the intake ranges of vitamins E, A, C and PUFA (including supplements) were divided into thirds and results are reported for subjects according to these intake tertiles.

	Vitamin E Intake				PUFA Intake		
	Lowest	Middle	Highest		Lowest	Middle	Highest
Caeruloplasmin (U/l)	221	216	243		230	229	220
α1-Acid glycoprotein(mg/ml)	1.48	1.23	1.07*		1.36	1.21	1.33
α2-Macroglobulin (mg/ml)	2.30	2.40	2.57		2.30	2.39	2.54
C-Reactive protein (Log mg/ml)	0.53	0.53	0.18		0.47	0.49	0.88*

Thirds significantly different: * $P<0.05$.

Tertiles for vitamins A and C showed no significant differences in acute-hase protein concentrations. However, the thirds for vitamin E exhibited significant differences in α1-acid glycoprotein. There was a negative correlation between vitamin E intake and plasma α1-acid glycoprotein concentration. In addition, smokers with high PUFA intake exhibited higher C-reactive protein than the lower thirds. Thus, the intake of vitamin E and PUFA seem to modulate the inflammatory response in smokers. Troughton & Grimble (1993) found that vitamin E insufficiency in rats fed on a 100g/kg maize oil diet, enhanced the acute-phase response to endotoxin.

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Modulatory effects of olive and maize oils and butter on the response of protein synthesis in liver, lung, kidney to endotoxin in rats. By H. T. BESLER and R. F. GRIMBLE, Department of Human Nutrition, University of Southampton, Southampton, SO9 3TU

As a result of bacterial, viral, or parasitic infection, surgical injury, trauma, or some forms of cancer, the host undergoes a series of responses, which include fever and anorexia associated with loss of fat and lean tissue. The loss of the lean reflects changes in the protein metabolism of the host (Grimble, 1992), which includes an increase in the rate of amino acid output by muscle and increased transport of amino acids to liver to support increased acute-phase protein synthesis, gluconeogenesis, and support to the immune system (Grimble, 1992). We have shown that diets poor in linoleic acid and enriched with oleic acid reduced changes in the protein metabolism of rats in response to tumour necrosis factor-α (TNF-α) (Mulrooney & Grimble,1993).

A high intake of olive oil is the main characteristic of the Mediterranean diet. The diet is thought to have anti-inflammatory properties (Grimble, 1993). We therefore examined the extent to which endotoxin influenced protein synthesis in the liver, lung, kidney, tibialis muscle and spleen of rats fed olive oil, maize oil or butter at 50, 100, 200 g fat/kg diet for 4 weeks *ad lib.* All diets were adequate in all nutrients.

Male Wistar weanling rats from the Southampton University Medical School colony were injected subcutaneoussly with endotoxin (END) (Difco strain, 055:B9) at a dose of 0.8 mg/kg body weight or sterile non-pyrogenic saline(SAL) (150mmol/l NaCl). Saline injected animals were pair-fed the intakes of the endotoxin-injected animals over the 24 h period following injection. Fractional rates of protein synthesis (FSR) were measured 24 h after injection (Jepson et al. 1986).

Dietary fat...		Maize Oil			Butter			Olive Oil		
Fat (g/kg)...		50	100	200	50	100	200	50	100	200
Liver FSR	(END)	107*	99.0*	127*	81.0	73.0	102*	70.0	79.0	58.0
(%/d)	(SAL)	62.0	73.0	67.0	74.0	69.0	67.0	65.0	58.0	68.0
Lung FSR	(END)	40.0*	47.0*	56.0*	37.0	33.0	47.0*	34.0	35.0	34.0
(%/d)	(SAL)	29.0	34.0	29.0	34.0	30.0	35.0	33.0	36.0	33.0
Kidney FSR	(END)	51.0*	60.0*	66.0*	43.0	42.0	57.0*	50.0	50.0	44.0
(%/d)	(SAL)	38.0	37.0	38.0	41.0	41.0	40.0	51.0	50.0	53.0

Significantly different from saline control: * P< 0.05

Endotoxin increased FSR in the liver, lung and kidneys in the maize oil-fed animals, at all levels of intake and in the butter-fed animals, at 200 g fat/kg diet. The change in FSR in lungs and kidneys in maize oil-fed animals given endotoxin increased with dietary fat intake. Olive oil prevented increases in FSR, at all levels of intake. There were no significant effects of endotoxin on FSR in tibialis and spleens in any groups.

These results suggest that total content and proportion of linoleic acid may be an important factor for pro- or anti-inflammatory characteristics of dietary fat. However, the mechanism behind these results needs to be investigated to clarify the phenomenon.

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The influence of interleukin-6 (IL-6) and dietary lipids on total protein synthesis rates in cultured rat hepatocytes. By A. G. CLAMP and R. F. GRIMBLE, <u>Institute of Human Nutrition, University of Southampton, Southampton, SO9 3TU</u>

Tumour necrosis factor-α (TNFα) has been shown to modulate rat liver acute-phase protein production and total protein synthesis rates <u>in vivo</u>, the extent of the modulation varying according to the type of dietary fat consumed by the animal (Mulrooney & Grimble, 1993). It is probable that many of the effects of TNFα on the liver, particularly those relating to acute-phase protein production, are mediated through induction of interleukin-6 (IL-6)(Andus <u>et al.</u> 1988). It is unknown, however, whether dietary lipids can modulate the influence of IL-6 on total protein synthesis rates in rat hepatocytes <u>in vitro</u>.

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, corn oil or fish oil as the main lipid source. In all synthetic diets 10 % of the fat was provided as corn oil to prevent essential fatty acid deficiency. At the end of the 5 week period livers were removed and hepatocytes isolated.

Hepatocytes were incubated (CO_2-air; 5:95, v/v; 37°) in collagen-coated culture dishes in a medium containing 5 % foetal bovine serum, antibiotics, insulin and dexamethasone. After 24 h 3000 U of IL-6 in culture medium, or culture medium alone, was added to the dishes, which were incubated for a further 24 h. Viability of 48 h old cultures was >95 %. Hepatocyte total protein synthesis rates were measured, as uptake of tritiated leucine (counts per minute/µg protein) by liquid scintillation counting, and are shown in the table.

Diet....	Chow		Butter		Coconut		Corn		Fish	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
IL-6	7175	598	13128	3446	7442	2320	9515	1251	10488	2642
Control	7652	334	13215	3581	8752	2892	11968	2363	12838	2555
% of control	93.4	5.1	99.6	11.0	86.1	3.4	84.1	8.7	80.1	6.5

There were no significant differences in hepatocyte protein synthesis rates between the dietary groups, or between the control and IL-6 groups.

IL-6 has been shown to stimulate the synthesis of a wide range of acute-phase proteins, including α-2-macroglobulin and α-1-acid glycoprotein, and to inhibit synthesis of albumin and transferrin in hepatocyte cultures (Heinrich <u>et al.</u> 1990). Kowalski-Saunders <u>et al.</u> (1989) found that IL-6 inhibited hepatocyte albumin synthesis but not total protein synthesis. The results of the present study are consistent with these findings.

Since IL-6 induces both increases and decreases in the synthesis of acute-phase proteins, the lack of any significant change in hepatocyte protein synthesis rates in the presence of IL-6 may be due to a balance between the synthesis of 'positive' and 'negative' acute phase proteins. A better measure of the inflammatory response to IL-6 may, therefore, be the production of specific 'positive' and 'negative' acute-phase proteins, rather than total protein synthesis. This system may also be more sensitive to any possible modulation of the inflammatory response to IL-6 by dietary lipids, and may provide useful information about the underlying mechanisms.

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Relationship between antioxidant vitamins and tumour necrosis factor (TNF) in smokers and non-smokers. By P.S. TAPPIA, K.L. TROUGHTON, S. LANGLEY and R.F. GRIMBLE, <u>Department of Human Nutrition, University of Southampton, Southampton SO9 3TU</u>

Inflammatory stimuli not only bring about cytokine production but also that of free radicals (Farante *et al.* 1988). <u>In vitro</u> and <u>in vivo</u> studies have shown that free radicals enhance the production of TNF and other cytokines (Clark *et al.* 1989). However, the effects of inflammatory agents may be attenuated by the presence of antioxidants such as vitamin E (Troughton & Grimble, 1993).

Since smoking exerts an inflammatory stimulus on lung macrophages and smokers are known to have low intakes of antioxidant nutrients (Bolton-Smith *et al.* 1991), we set out to study the relationship between TNF and the antioxidant vitamin status in smokers and non-smokers. TNF was measured in whole blood stimulated with endotoxin (10 ng/ml) by means of a bioassay which utilizes the cytotoxic action of this cytokine on murine fibroblasts, L929 cells. Vitamins A, C and E were measured by HPLC. The results are tabulated below.

	TNF (nM)		Vitamin A (μM)		Vitamin C (μM)		Vitamin E (log mM)	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
Smokers (*n* 12)	0.117	0.043	4.273	1.070	44.9	16.6	0.756	-1.564
Non smokers (*n* 12)	0.083	0.035	3.87	1.507	49.5	19.7	0.856	-1.514
P value	0.05		0.49		0.55		0.04	

Vitamins A and C showed no significant difference between the two groups. The results however, indicate that the increased TNF levels in smokers may be due to a reduced vitamin E status. There was a weak inverse relationship between vitamin E (log mM) and TNF (nM; $r=-0.27$) which may reach significance with increased sample size. This enhanced TNF could be due to increased neutrophil numbers or to increased sensitivity to inflammatory stimuli, a possibility which will require further investigation.

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Sulphur amino acids ameliorate lung inflammation in response to tumour necrosis factor α in rats fed low-protein diets. By E.A.L. HUNTER and R.F. GRIMBLE, Department of Human Nutrition, University of Southampton, Southampton, SO9 3TU

Free radicals enhance production of proinflammatory cytokines such as interleukin 1 and tumour necrosis factor α (TNF). Thus, maintenance of antioxidant defences becomes important, particularly in diseases such as AIDS and cancer, where overexpression of cytokines is apparent. The inflammatory response to cytokines is modulated by dietary and endogenous antioxidant defences (Grimble et al. 1991; Troughton & Grimble, 1993). Glutathione (GSH) is an important cysteine containing antioxidant; hence we examined the efficacy of sulphur amino acid supplementation in maintaining antioxidant defences in response to TNF in rats fed low-protein diets.

Male Wistar rats (n=10) received diets containing either 200 g casein/kg with 12 g alanine/kg and 8 g cysteine/kg (Normal Protein) or isonitrogenous low-protein diets containing 80 g casein/kg supplemented with 12 g alanine/kg (Low Protein), 8 g cysteine/kg and 12 g alanine/kg (Cysteine) or 10 g methionine/kg and 12 g alanine/kg (Methionine). After 8 d half the animals from each group received 50 μg TNF/kg body weight intraperitoneally. The remaining animals were injected with sterile non-pyrogenic saline (9 g NaCl/l) on day 9 and were pair-fed the intakes of the corresponding TNF group. Fractional rates of protein synthesis (FSR), glutathione and polymorphonuclear cells (PMNs) were measured 24 h after injection.

	Normal Protein		Low Protein		Cysteine		Methionine		Pooled SEM
	Saline	TNF	Saline	TNF	Saline	TNF	Saline	TNF	
Lung PMNs (% cells)	9.1[a]	8.0[a]	10.5[ab]	13.2[b]	9.0[a]	8.6[a]	8.7[a]	8.4[a]	0.8
Lung GSH (mg/lung)	0.51[a]	0.56[ac]	0.23[c]	0.33[d]	0.39[b]	0.52[bc]	0.43[b]	0.44[b]	0.03
Lung FSR (%/d)	33	39	30	36	28	49	32	36	5
Liver GSH(mg/liver)	3.1[a]	12.6[b]	1.5[b]	2.7[d]	2.4[a]	11.7[bc]	1.7[b]	8.3[c]	0.6
Liver FSR(%/d)	76[c]	100[ad]	70[c]	131[d]	89[ab]	118[cd]	105[b]	117[bd]	7

a, b, c, d Values in the same row with unlike superscripts are significantly different (ANOVA): P<0.05.

Whilst the Normal Protein rats grew 7 g/d and the Cysteine and Methionine rats grew 3.9 and 3.7 g/d respectively, the Low Protein rats grew by 0.6 g/d. The Low Protein animals exhibited lung inflammation in response to TNF. Inflammation was not seen in the Cysteine and Methionine animals. The mechanism for this response may be via maintenance of antioxidant defences, in particular GSH. Sulphur amino acid sufficiency is central to such modulation of the inflammatory effects of TNF.

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Effects of endotoxin on metabolic responses of rats fed with olive and maize oils and butter. By
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Anorexia, fever, enhancement of hepatic protein synthesis, muscle protein loss and increases in the
concentration of plasma caeruloplasmin, depression of plasma zinc (Zn) and albumin and changes in
visceral protein and Zn metabolism are major characteristics of the acute phase response (Grimble,
1992).

Fats have been shown to modulate the acute phase response to tumour necrosis factor-α (TNF-α)
(Mulrooney & Grimble,1993). We have shown that olive oil and butter, which contain low levels of
linoleic acid, reduced a number of effects of endotoxin including anorexia, fall in rectal temperature
at 2 h after injection, elevation of caeruloplasmin and increase in liver protein and Zn concentration
(Besler & Grimble,1993). The present study examines whether the fats modulate responses to
endotoxin, which include changes in plasma Zn, albumin and alterations in lung and kidney protein
and Zn concentrations.

Weanling male Wistar rats from the Southampton University Medical School colony were fed olive
oil, maize oil or butter at 50, 100, 200 g fat/kg diet for 4 weeks *ad lib*. All synthetic diets were
adequate in all nutrients.

Rats were injected subcutaneously with endotoxin (END) (Difco strain, 055:B9) at a dose of 0.8
mg/kg body weight or sterile non-pyrogenic saline(SAL) (150mmol/l NaCl). Saline injected animals
were pair-fed the intakes of the endotoxin-injected animals over 24 h.

Dietary Fat...		<u>Maize Oil</u>				<u>Butter</u>			<u>Olive Oil</u>		
Fat (g/kg)...		50	100	200	50	100	200	50	100	200	
Plasma albumin	(END)	26.3*	26.4*	25.6*	37.4	34.3	27.9*	32.9	36.4	33.9	
(mg/ml)	(SAL)	33.6	36.6	33.5	37.3	35.4	35.4	33.4	35.9	34.3	
Plasma Zn	(END)	1.87*	1.70*	1.36*	2.25	2.17	1.67*	1.79	1.97	1.86	
(μg/ml)	(SAL)	2.65	2.65	2.84	2.23	2.29	2.34	1.77	1.93	1.89	
Lung protein	(END)	117*	137*	148*	112	106	137	103	99.0	103	
(mg/g)	(SAL)	107	104	112	105	108	104	101	109	100	
Lung Zn	(END)	117	124	144*	123	124	126	120	107	114	
(μg/ml)	(SAL)	99	108	102	123	118	101	118	110	111	
Kidney protein	(END)	175*	176*	198*	170	169	181	169	162	164	
(mg/g)	(SAL)	166	158	179	166	172	169	173	159	167	
Kidney Zn	(END)	35.3*	39.1*	43.4*	27.9	27.2	39.8*	27.4	28.2	31.7	
(μg/g)	(SAL)	29.7	30.2	30.1	28.2	29.0	30.2	27.9	28.3	31.4	

Significantly different from saline control: * P< 0.05

Overall, the olive oil, at all levels of intake, prevented alterations in protein and Zn concentration
in lung and kidney, and in the concentration of plasma Zn and albumin. Butter, at 50 and 100 g fat/kg
intake had a similar modulatory effect on metabolic responses to endotoxin. The effects of endotoxin
increased with increasing dietary maize oil content.

These results suggest that the fats with differing patterns of fatty acids, may modify the response of
endotoxin in rats in a pro- or anti-inflammatory manner. However, further studies are needed to
clarify the mechanisms by which these effects are achieved.

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Urea kinetics in free-living female vegetarians measured with a single-dose method.
By R. BUNDY, C. PERSAUD and A.A. JACKSON, <u>Department of Human Nutrition, University of Southampton, SO9 3TU.</u>

Among vegetarians, individuals have a wide range of protein intakes, in terms of quantity and quality, which make them ideally suited to study possible adaptive mechanisms. In the present study, we measured urea kinetics by using a single oral dose of 200mg [$^{15}N^{15}N$]-urea in six female vegetarians with subsequent total urine collection over 48 h. A weighed record of all food intake was maintained by each subject. We have previously shown that the single-dose method for measuring urea kinetics gives results similar to those obtained with the prime and intermittent doses given in conjunction with a controlled diet and is suitable for the non-invasive measurement of urea kinetics in free-living individuals (McClelland *et al*; 1992).

Body mass and height ranged from 55.5 to 69.7kg and from 1.61 to 1.70m respectively and all were in good health throughout the study. Energy intake ranged from 7.1 to 10.7MJ/d and N intake between 106 and 209 mg/kg per d. Urea production was between 114 and 248 mgN/kg per d and urinary urea excretion between 78 and 174 mgN/kg per d. Only 50 to 70% of the urea produced was excreted in the urine with 30 to 50% being salvaged in the colon. Between 47 and 78% of that salvaged was retained in the metabolic N pool. Urea-N production was between 80 and 211% of intake. However, when salvage of urea was considered, urea-N production was only between 58 and 103% of the 'Effective Nitrogen Intake', being that from the diet and from salvage.

The test data were compared to a control group of omnivorous females, calculated from previously published data (McClelland *et al*; 1992). N intake was between 128 and 333 mg/kg per d. Urea production was between 138 and 362 mgN/kg per d and urinary urea excretion between 90 and 209mgN/kg per d. Between 46 and 81% of the urea produced was excreted in the urine with 19 to 54% being salvaged in the colon. Urea-N production was between 81 and 149% of intake. No significant differences were found between the test and control data. The individual results for urea salvage as a proportion of production were also compared to data from 100 studies of urea kinetics in adults on mixed diets (Jackson, 1993). The individual results cover a wide range and appear to follow the overall pattern of response seen in omnivores.

From these results, it appears that salvage of urea is dependant on the quantity of protein in the diet. There is no direct evidence to suggest protein quality exerts an influence. The single-dose method can be used to measure urea kinetics by non-invasive means in free-living individuals with confidence and has potential application for the measurement of urea kinetics in large numbers of people.

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Can recorded energy intake be used to estimate energy expenditure? By M.A.TAYLOR and J.S.GARROW. Rank Department of Human Nutrition, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ

Previous studies suggest that an energy intake that is less frequent and later in the day may increase the risk of overweight (Fabry et al 1964, Kulesza 1982). Energy intake was based on intake histories and records. These were not validated. The apparent associations between meal frequency/meal timing and overeating could reflect differences in recording, not actual intake. Energy expenditure could be used to validate energy intake records. (At constant bodyweight, energy intake = energy expenditure.)

In this study, twenty-five overweight women (mean age 41 years, range 19-78 years; BMI 41 (SD 7) kg/m^2) completed a seven day record of all food and drink consumed (using household measures), completed a questionnaire and underwent two periods of thirty-six hour chamber calorimetry (A mean of the last 24 hours of each period was used).

	n	mean	SD
Measured energy expenditure (MEE;MJ/d)	25	9.3	1.5
7 day recorded energy intake (RI.7d;MJ/d)	25	5.7	1.9
Subject estimate of energy intake during RI.7d (EI.7d;MJ/d)	11	6.2	2.7
Subject estimate of energy required to maintain weight during RI.7d (EM.7d;MJ/d)	9	6.3	1.9
Mean recorded meal frequency during RI.7d (MF)	25	4.4	0.8

Twenty-four of the twenty-five subjects (96%) appeared to have under-recorded their energy intake when compared with measured energy expenditure. Under-reporting was not by a constant amount. Energy intake alone thus seemed to be a poor estimator of energy expenditure.

Weight and measured energy expenditure were associated ($r=0.72$, P< 0.01, SE 1.0 MJ/d).

No association was found between measured energy expenditure and age, mean recorded meal frequency per day, or subject estimate of energy intake during RI.7d.

Energy expenditure was associated with the mean of recorded energy intake and estimated maintenance energy requirements in 9 subjects who were prepared to estimate EM.7d ($r=0.89$, P< 0.01, SE 0.7MJ/d). For these nine subjects this gave a better estimate of MEE than weight ($r=0.89$, P< 0.01, SE 0.7 MJ/d).

This was an unexpected result and must now be tested prospectively.

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Characteristics of people recording a low energy intake for body weight in a large national survey. By G.M.PRICE[1], A.A.PAUL[1], T.J.COLE[1], W.S.HILDER[2] and M.E.J.WADSWORTH[2], [1]*MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ and* [2]*MRC National Survey of Health and Development, Department of Epidemiology and Public Health, University College London Medical School, London WC1E 6EA*

There is increasing concern about the under-recording of food intake by some subjects in dietary surveys. Such a phenomenon would seriously affect conclusions drawn about the intakes of food or nutrients by populations or sub-groups, and about the relationship between such intakes and diet-related diseases.

We have investigated this using data from a prospective national birth cohort study of people born in 1946. The body weights and 7d food intakes (described in household measures) of 959 men and 934 women were measured when they were 43 years old; none was reported to be weight-reducing. Basal metabolic rate (BMR) was calculated from body weight (Department of Health, 1991) and the ratio of each individual's mean reported daily energy intake (EI) to BMR was used to assess the validity of energy intake.

For 18.4% of the men and 22.7% of the women EI:BMR was less than 1.1, and was therefore physiologically implausible for weight-stable individuals (Goldberg et al. 1991). In an attempt to ascertain whether subjects with low EI:BMR (less than 1.1) were identifiable in other respects we used data collected on them at this and at earlier ages to explore their characteristics compared with the rest of the population. None of the following was found to be relevant: neuroticism and extroversion scores obtained at age 26 years, regular social contacts, a psychiatric symptom score, vegetarianism and reported recent changes in eating habits, all at age 43 years, region of residence at age 36 years and inconsistency over time in reporting smoking habits.

Those men and women with higher body mass index (BMI) were more likely than leaner subjects to report low EI:BMR (linear regression; $P<.0001$). Other characteristics significantly individually associated (chi-square test) with low EI:BMR, in women only, were low social class (classes IV and V more likely to have low EI:BMR, $P=.004$), low educational attainment ($P<.0001$) and smoking ($P=.033$).

Logistic regression was used to show that, after adjusting for the other variables, the most important by far was BMI ($P<.0001$); for women only, smoking ($P=.016$) and social class of origin ($P=.026$) were also significant. The shape of the relationship between BMI and the odds ratio for EI:BMR being below 1.1 differed by sex: in women the likelihood of recording a low EI:BMR increased steadily with increasing BMI, but in men this odds ratio increased substantially with BMI only once BMI was higher than 27kg/m^2.

Because high BMI is often associated with other risk factors for low health status these results confirm the need for caution when designing and interpreting studies of diet and disease.

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The diet, physical activity patterns and anthropometry of a group of free-living elderly people.
By C. FRASER and J.V.G.A. DURNIN, Institute of Physiology, University of Glasgow, Glasgow G12 8QA.

The present study has been designed to incorporate some of the standardized protocol developed by Euronut-SENECA, a study on nutrition and the elderly in Europe, as described by De Groot & van Staveren (1988). A random sample of forty-seven males and forty-eight females, all aged between 70 - 71 years, living in their own homes in the medium sized town of Paisley, Scotland was drawn from health registers. On each individual, measurements of food intake during 5 consecutive days (including the weekend) was made by the weighed individual inventory method. Calculation of the energy intake and of the main nutrients was done using the database of Paul & Southgate (1978). Physical activity was estimated using the standard diary technique during 3 consecutive days. Anthropometric measurements were made of height, weight and skinfold thicknesses, circumference of upper arm, calf, waist and buttocks.

Mean total daily energy intake for males was 8256KJ and 7306KJ for females. There are several estimates available of what constitutes desirable energy in old age. The FAO/WHO/UNU (1985) suggest adequate energy should be at least 1.4 x BMR. According to this formula our mean energy intake results for females (1.4 x BMR) equalled this value. Mean energy intake for males (1.3 x BMR) was lower. Mean intakes for principal nutrients were generally satisfactory except for selenium and vitamin D. Most of the elderly engaged in some form of physical activity during the study period; this activity appeared to be of light to medium intensity only. Few engaged in activities of high intensity. 59 % of the males and 39 % of the females considered themselves to be 'more active' to 'much more active' than others of their own age. Females were 'on average' smaller than males, weighed less, had smaller waist circumferences but had thicker bicep and tricep skinfolds and greater percentage of body fat than males. Overweight,(BMI 25 - 30), was evident in 60 % males and 69 % females.

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Muscle thickness as an index of fat-free mass. By T. WATT[1], D. WITHERS[2], R.ENGLAND[2], B. FARAGHER[3], D.F. MARTIN[2] and I.T. CAMPBELL1[1]. [1]University Dept of Anaesthesia and [2]Depts of Radiology and [3]Statistics, Withington Hospital, Manchester M20 8LR.

The fluid retention and oedema seen in multiple organ failure (Hall et al., 1991) confound the normal anthropometric techniques of assessing changes in body mass and composition. There is a need for a simple method of assessing changes in body composition in these circumstances. Most of the fluid appears to be retained within visceral and subcutaneous tissue rather than muscle (Helliwell et al., 1991), so a statistically significant relationship was established between fat-free mass (FFM) derived from body weight, and skinfold thickness (Durnin & Womersley, 1974), and muscle thickness measured on biceps (B), forearm (F), mid thigh (T), calf, abdomen and triceps using ultrasound, in 30 normal volunteers (Watt et al., 1992).

Data on 70 normal volunteers (37M, 33F; aged 18-86(median 32)yrs; height 141-189(median 167)cm; weight 40.3-98.2 (median 68.2)kg) have now been analysed to determine which muscle thicknesses are the most sensitive in predicting FFM (kg) of normal subjects.

All muscle thicknesses showed a significant correlation with FFM ($p<0.001$). However using multiple regression analysis only 3 correlated independently with FFM;

$$FFM = 6.670 + 12.463B + 2.335T + 4.054F \quad (r=0.876, p<0.001; \text{ 95% confidence interval } -10.8 \text{ to } 10.5).$$

Simple addition of these three thicknesses produced a correlation with FFM of 0.843($p<0.001$). This compares favourably with correlation coefficients, in the same group, of FFM with mid arm circumference of 0.737, arm muscle area of 0.854 and arm muscle circumference of 0.852. Coefficients of variation of repeat measurements made by one observer on 8 individuals on 5 separate occasions of the biceps, midthigh and forearm measurements were 2.95, 2.25 and 2.73% respectively.

It is concluded that in normal volunteers measuring muscle thickness over biceps, forearm and mid thigh is as good an index of fat-free mass as mid upper arm circumference and its derivatives.

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Prediction of body density from skinfold thickness in elderly subjects: are the existing equations valid? By J.J. REILLY[1], L.A. MURRAY[1], J. WILSON[2] and J.V.G.A. DURNIN[2], [1]University Department of Human Nutrition, Yorkhill Hospitals, Glasgow, G3 8SJ and [2]University Department of Physiology, Glasgow, G12 8QQ

In young and middle-aged adults body density, and hence body composition, can be accurately predicted from measurement of skinfold thickness at four sites. Relatively small numbers of subjects older than 65 years were used to generate the equations for prediction of density from skinfold thickness. Changes in body composition and fat distribution occur with advancing age: it has been suggested that the rapid loss of bone mineral which occurs in post-menopausal women (estimated at 0.6 % loss per year; Deurenberg *et al* . 1989) would lead to a marked reduction in the density of the body and the fat-free mass (FFM) of elderly women. This change appears to be less marked in men. It is therefore possible that the existing equations will overestimate body density and, hence, underestimate body fatness in elderly women. There is some evidence that this is the case (Reilly *et al*.1993).

The aim of the present study was to test the hypothesis that body density is overestimated in elderly women, but not in elderly men, by use of the existing predictive equations. In seventeen healthy elderly men (mean (SD) age 68 (3) years) and sixteen healthy women (mean (SD) age 70 (4) years) body density was measured by underwater weighing as previously described (Durnin & Womersley 1974). In all subjects four skinfolds were measured at the time of underwater weighing and body density was predicted using the appropriate equation from Durnin & Womersley (1974).

In the males measured density was higher than predicted density in twelve of seventeen cases; mean (SD) measured density was 1.045 (0.011) kg/l, mean (SD) predicted density 1.041 (0.009) kg/l. In the females measured density was lower than predicted density in eleven of sixteen cases. Mean (SD) measured density was 1.012 (0.018) kg/l, mean (SD) predicted density 1.020 (0.008) kg/l.

The existing skinfold thickness equations ,therefore, tend to overestimate body density in elderly women, but not in elderly men. As an indication of the magnitude of the difference in estimated body fatness which can result from this overestimate of body density in women, fatness (percentage of body weight) was estimated using mean measured and mean predicted body density. Fatness using predicted density (1.020 kg/l) was 35.3 % ; fatness using measured density (1.012 kg/l) was 39.1 %. The magnitude of the error may not seem substantial, but the evidence that it is systematic in nature gives cause for concern.

The error arising from overestimation of body density in elderly women is offset to some extent by the fact that Siri's (1961) equation, which permits calculation of fatness from density, assumes a constant for the density of FFM which is too high as a result of the loss of bone mineral described above. Siri 's (1961) equation therefore tends to overestimate fatness in elderly women and compensate to some degree for the underestimation of fatness inherent in the existing skinfold thickness equations.

Adjustment of the existing equations for the prediction of density in elderly women may be necessary, but a larger sample size would be desirable in order to produce revised equations.

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Different predictions of body fat from the same whole-body bio-electrical impedance measurement. By N.J. FULLER. MRC Dunn Clinical Nutrition Centre, Cambridge CB2 1QL

Whole-body bio-electrical impedance (BI) analysis may be used for the assessment of body composition in situations where other methods might be inapplicable (for example in clinical settings, and for the young or old). Various interpretations of BI are available which claim to predict reference method estimates accurately. However, comprehensive comparisons of these predictions against established reference methods or models are apparently unavailable.

Body composition was assessed in twenty eight healthy, non-obese men and women (mean body mass index 22kg/m^2, SD 3kg/m^2). Densitometry and dual-energy X-ray absorptiometry (DEXA - Lunar model DPX) for body fat and fat-free mass (FFM), and deuterium dilution for total body water (TBW) were the reference methods used. Four- and three-component models based on these reference method measurements (Fuller *et al.* 1992) were also applied. BI was measured and interpreted in eight different ways. For instruments where manufacturers' equations were unavailable, the BI measurement was reproduced by means of a variable resistor. Hydration fraction of FFM was assumed to be constant (0.72). BI predictions were compared against all reference method and model estimates using bias and 95% limits of agreement (95% LA) between methods. Results for body fat (% body weight) estimates are shown in the Table.

	4-component model		3-component model		Densitometry		Deuterium dilution		DEXA	
	Bias	95% LA	Bias	95% LA	Bias	95% LA	Bias	95% LA	Bias	95% LA
Instrument/Equation										
Bodystat-500	3.54	6.38	2.31	7.09	2.78	6.41	1.84	8.38	2.18	5.94
E-Z Comp 1500	-1.13	8.34	-2.36	8.91	-1.89	8.68	-2.83	9.73	-2.49	8.30
Holtain Equation	-5.08	8.22	-6.30	8.76	-5.84	8.53	-6.78	9.61	-6.43	8.09
Lohman Equation	3.25	7.12	2.02	7.77	2.49	7.58	1.55	8.67	1.89	6.74
Lukaski Equation	-0.19	8.48	-1.42	8.97	-0.95	8.69	-1.89	9.83	-1.55	8.36
Maltron BT-905	-1.41	8.56	-2.64	9.06	-2.17	8.80	-3.11	9.90	-2.77	8.47
RJL Equation	4.66	7.49	3.44	8.19	3.90	7.45	2.96	9.35	3.30	6.62
Valhalla Equation	3.50	7.10	2.28	7.72	2.74	7.63	1.80	8.55	2.15	6.71

The magnitude of bias between interpretations of BI and each reference method estimate was found to vary considerably (falling about equally on either side of all the reference method estimates), and the 95% limits of agreement were all in excess of 6% fat (% body weight).

It is of major concern that, despite applying the same values for BI, weight and height to these different predictions, such large differences could have been shown to exist between them. Most interpretations of BI measurements are apparently poor predictors of body fat in this group of subjects. Indeed, a total bias of >9% fat can be inferred between particular interpretations which claim to predict four-component model estimates (see Table). However, estimates of TBW and FFM are relatively more acceptable in terms of bias and 95% limits of agreement (Fuller, 1993).

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The effect of covert manipulation of dietary fat and energy density on ad libitum food intake in "free-living" humans. By R.J. STUBBS[2], P. RITZ[1], W.A. COWARD[1] and A.M. PRENTICE[1], [1]MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge, CB2 1QL and[1]Rowett Research Institute, Greenburn road, Bucksburn, Aberdeen, AB2 9SB.

In a previous study six men each studied three times (Stubbs *et al*. 1993) had *ad lib*. access to one of three covertly-manipulated diets, for 7 d while continuously resident in a whole-body indirect calorimeter. The fat, carbohydrate (CHO) and protein in each item of the diets, expressed as a proportion of the total energy content were low-fat (LF); (20:67:13); medium-fat (MF); (40:47:13); high-fat (HF); (60:27:13). Energy density increased with % fat. Diets were offered on a 3 d rotating *menu* and were fed to subjects in random order with a maintenance (MF) diet for 2 d before each dietary treatment. The present "free-living" study repeated the same protocol in seven men' mean (SEM) Age 36.86 (3.09) years; Weight 69.94 (1.80) kg; Height 1.77 (0.02) m, for 14 d per diet, while resident in -but not confined- to a hotel metabolic suite (hence "free-living"). Energy expenditure was estimated using doubly-labelled water. Energy and nutrient intakes were calculated from food tables (Paul & Southgate 1978), and dietary energy was checked by bomb calorimetry (agreement 1-2%).

Ad lib. fat intake increased with % dietary fat (F (2, 156) 1163.21; $P<0.001$). Average (SEM) daily intakes were 1.89 (0.03), 4.00 (0.06) and 7.49 (0.14) MJ fat/d on the LF, MF and HF diets respectively. CHO intakes decreased as dietary fat intake increased (F (2, 156) 115.62; $P<0.001$), producing mean intakes of 6.00 (0.12) 4.99 (0.08) and 3.82 (0.07) MJ CHO/d on the LF, MF and HF diets respectively. Energy intake, expenditure and balances are given for both studies in the Table. The effects of diet on intake and balances were significant at $P<0.001$ (ANOVA).

Diet		Energy intake (MJ/d)			Energy Expenditure (MJ/d)			Energy balance (MJ/d)		
		LF	MF	HF	LF	MF	HF	LF	MF	HF
Calorimeter	Mean	9.03	10.22	12.35	9.48	9.53	9.89	-0.45	0.69	2.46
	SEM	0.45	0.51	0.69	0.33	0.30	0.35	0.30	0.50	0.64
Free-living	Mean	9.11	10.32	12.78	12.45	12.10	11.97	-3.34	-1.77	0.80
	SEM	0.17	0.16	0.23	0.66	0.68	0.59	0.43	6.62	0.65

A relative hyperphagia occurred when the dietary energy density was covertly increased in the form of fat, as described in the calorimeter study. For each corresponding diet, energy intakes were almost identical in the calorimeter and free-living studies. However, energy expenditure was higher and energy balances were more negative on the corresponding diets of the free-living study relative to the calorimeter study. Considering these results, we suggest that in studies on appetite the full implications of energy intake data for the regulation of energy balance can only be assessed once estimates of energy expenditure are included.

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Changes in the diet of Mandinka women in The Gambia between 1978-79 and 1990-91: consequences for calcium intakes. By L.M.A. JARJOU[1], A. PRENTICE[1], Y. SAWO,[1] S. DARBOE[1], K. DAY[1] A.A. PAUL[1] AND S. FAIRWEATHER-TAIT[2], [1]MRC Dunn Nutrition Unit, Keneba, The Gambia, and Milton Road, Cambridge, CB4 1XJ, and [2]AFRC Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney Lane, Norwich NR4 7UA

A previous study in a rural area of The Gambia, based on data collected in 1978-79, demonstrated that at that time the daily Ca intakes of lactating Mandinka women averaged about 400 mg/d (Prentice et al. 1993). Ca was obtained principally from leaves, fish, cereals and groundnuts. Dried baobab leaf (Adansonia digitata), which is added to steamed millet during cooking, was an important source of Ca.

We have recently completed a second study using similar methodology in the same area of The Gambia. Sixty lactating women, 16-41 years old, parity 1-13, were studied at 3 months post-partum between June 1990 and June 1991. The results showed that there have been substantial changes in the diet of lactating Gambian women since 1978-79. The proportion of staple dishes that were made from rice increased from 57 % to 82 %, while those based on various millets declined from 34 % to 8 %. The percentage of sauces that were made from groundnuts and leaves decreased from 70 % and 24 % to 55 % and 17 % respectively, while sauces based on oil and flour increased in frequency.

The decline in popularity of millet dishes, which contain dried baobab leaf, and of sauces made from groundnuts and leaves resulted in a considerable decrease in Ca intakes. The mean(sd) intakes in 1990-91 were 283(119) mg/d (7.1(3.0) mmol/d) compared with 404(110) mg/d (10.1(2.8) mmol/d) in 1978-79; $P < 0.001$. The contribution of various foods to Ca intakes at both times are given in the Table.

Contribution to Ca intake	mg/d		mmol/d		% Total	
Food	1978-9	1990-1	1978-9	1990-1	1978-9	1990-1
Dried Baobab leaf	76	27	1.9	0.7	19	10
Fresh leaves	60	40	1.5	1.0	15	14
Fish (wet+dry)	69	32	1.7	0.8	17	11
Groundnuts	41	34	1.0	0.9	10	12
Cereals	44	22	1.1	0.6	11	8
Milk	20	25	0.5	0.6	5	9
Baobab fruit	13	3	0.3	0.1	3	1
Others	81	100	2.0	2.5	20	35

These intakes are very low compared with current FAO/WHO and UK recommendations for lactating women (1100-1200 mg/d and 1250 mg/d respectively). The implications of this for the Ca and bone metabolism of lactating Gambian women are currently under investigation.

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The effect of the CCK receptor antagonist loxiglumide, on feeding behaviour in normal-weight volunteers. By S.J. FRENCH[1], A. BERGIN[1], C.P. SEPPLE[1], N.W. READ[1] and L. ROVATI[2], [1]Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Sheffield S5 7AU and [2]Rotta Research Laboratorium S.p.a., 20052 Monza, Milan, Italy

Cholecystokinin (CCK) is thought to induce postprandial satiety at a peripheral site in the body. Specific peripheral (type-A) CCK antagonists have been shown to block the effects of exogenously-applied CCK on food intake (Lotti *et al*. 1987; Setnikar *et al*. 1987); however, the role of CCK-A receptors in satiety following normal food ingestion is less clear.

The present study investigated the effects of one such potent, specific, and competitive peripheral A-type CCK receptor antagonist, loxiglumide, on food intake, hunger and satiety over a 3 d period. Eleven healthy, normal-weight subjects (eight female, three male) recorded daily food intake using weighed dietary intake diaries (Daily Intake) whilst taking 400 mg loxiglumide or placebo capsules orally, three times daily, 15 min before meals. Additionally, subjects came into the department on the evening of the third day of drug and placebo administration and underwent a laboratory test of food intake (Lab Intake) and rated subjective feelings of hunger and fullness on visual analogue rated questionnaires before, during and after this meal. The results of the food intake data are shown in the Table.

	Placebo		Loxiglumide		P
	Mean	SEM	Mean	SEM	
Daily Intake (kJ)	7883	568	8565	635	>0.1
Lab Intake (kJ)	6157	456	6069	435	>0.1

There was no difference in mean daily food intake over the 3 d of placebo and loxiglumide administration nor at the laboratory test meal. Furthermore, there was no difference in subjects reported feelings of hunger or fullness before or after the laboratory test of food intake during placebo and loxiglumide treatments.

In conclusion, these results suggest that in normal-feeding humans CCK acting at the type-A receptor does not modulate feeding behaviour.

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The effect of covertly manipulating the dietary fat : carbohydrate ratio of isoenergetically dense diets on *ad lib*. food intake in "free-living" humans. By R.J. STUBBS[2] and A.M. PRENTICE[1], [1]MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge, CB2 1QL and [2]Rowett Research Institute, Greenburn road, Bucksburn, Aberdeen, AB2 9SB.

Hyperphagia due to ingestion of high-fat, higher-energy dense (HF, HE) diets, relative to low-fat, lower-energy dense (LF, LE) diets has been well demonstrated. It has been suggested that energy intake is primarily geared to the maintenance of carbohydrate stores and as fat displaces carbohydrate in the diet hyperphagia will ensue in order to maintain carbohydrate stores (Flatt 1987). If this were the case HF diets should also promote hyperphagia if they are isoenergetically dense (IE) relative to high-carbohydrate (HC) diets. Six men, mean (SEM) Age 37.33 (5.52) years ; Weight 73.03 (2.10) kg; Height 1.80 (0.02) m, were each studied three times during 14 d dietary treatments throughout which they had *ad lib*. access to one of 3 covertly-manipulated diets. The fat, carbohydrate (CHO) and protein in each diet, expressed as a proportion of the total energy content were low-fat (LF); (20:68:12); medium-fat (MF); (40:48:12); high-fat (HF); (60:28:12). Within each diet every item was of the same composition. The diets were isoenergetically dense. Diets were offered on a 3 d rotating menu and the order was randomised across subjects. Subjects received a maintenance MF diet for 2 d prior to each dietary treatment. Energy and nutrient intakes were calculated from food tables (Paul & Southgate 1978), and dietary energy checked by bomb calorimetry (agreement 1-2%). Subjects resided in -but were not confined to- a hotel metabolic suite throughout the study (hence "free-living"). The results of mean daily energy and nutrient intakes are given in the Table. Analysis of variance was conducted on the intakes of energy, fat and carbohydrate and protein.

Dietary treatment	Energy intake (MJ/d)		Fat intake (MJ/d)		Carbohydrate intake (MJ/d)		Protein intake (MJ/d)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LF	10.21	0.25	2.21	0.05	6.73	0.17	1.27	0.03
MF	11.37	0.28	4.42	0.12	5.46	0.14	1.49	0.04
HF	11.20	0.32	6.42	0.19	3.35	0.10	1.43	0.04

Diet significantly influenced ad libitum intakes of energy ($F_{(2, 130)}$ 10.41; $p<0.001$); fat, ($F_{(2, 130)}$ 1088.18; $P<0.001$); carbohydrate, ($F_{(2, 130)}$ 649.49; $P<0.001$); and protein ($F_{(2, 130)}$ 38.28; $P<0.001$). The smallest effect of dietary change was on energy intake. Thus the HF, HE hyperphagia previously observed at +2.46 MJ/d (Stubbs *et al*. 1993) was abolished when the diets were isoenergetically dense. The levels of energy intake and modest weight loss (-0.2 (SEM 0.09) kg by day 14) were consistent with a residual HC hypophagia. There was no evidence of carbohydrate or fat intake being tightly regulated. These data do not support a glycogenostatic model of food intake regulation.

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The effects of dietary and intraduodenal lipid on arousal, and performance in two psychological tasks. By A.S. WELLS[1], N.W. READ[1] and A. CRAIG[2], [1]Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Herries Rd, Sheffield, S5 7AU. [2]MRC/ESRC Social and Applied Psychology Unit, University of Sheffield, S10 2TN.

Ingestion of lunch is known to cause drowsiness and impair performance (Smith *et al.* 1991) but the mechanism of this effect is not known. The observation that intraduodenal lipid infusions induce sleepiness in experimental animals (Fara *et al.* 1969, Murray *et al.* 1993) suggests that fat may be the key soporific component.

The aim of the present study was to investigate these possibilities by feeding two isoenergetic lunches of similar appearance, taste, and protein content but differing in fat and carbohydrate (CHO) content in a balanced order to eight male volunteers on two non-consecutive days. Each day, data were collected hourly from a 20 min bank of psychological tasks and rating scales (20 point). At 12.45 hours, subjects ate lunch containing one third of their estimated daily energy requirements (mean = 3.66 MJ) (COMA 1991).

At 2.5 h after the high fat - low CHO meal subjects felt less alert than they did before lunch (Table), they also found the tests more mentally demanding and made more errors in a 10 min sustained attention task (Neuchterlein *et al.* 1983). There were no post-lunch differences after the low fat - high CHO meal. This is contrary to Spring's conclusion that CHO induces post-prandial calmness in male subjects (Spring *et al.* 1983).

Measure	Change (post lunch - pre lunch scores)					
Fat energy : CHO energy...	Low fat - high CHO 64:18			High Fat - low CHO 7:76		
Performance Variable	Mean	SEM	P [a]	Mean	SEM	P [a]
Alertness (scale 0-20)	-1.0	0.86	NS	-2.3	0.90	.041
Mental demand (scale 0-20)	0.3	0.70	NS	1.2	0.41	.019
Errors (no.)	1.0	0.98	NS	2.05	0.72	.021

[a] significance of change (post meal - pre meal)
P, significance level using 2-tailed t test, NS, not significant

To verify that fat caused these changes, we compared the effect of duodenal infusion of either 10 % Intralipid (Kabi Pharmacia Ltd, Bucks, U.K.) (8.36 kJ /min) or isotonic saline (9g NaCl/l) in paired studies carried out on 2 non-consecutive days in five male volunteers. Two consecutive 3 h infusions, one of lipid, the other of saline were given blind on each day using a cross-over design. Analysis of variance indicated that lipid significantly reduced alertness (F = 10.45, df 1,3, $P < .05$), and slowed the mean correct response time in a serial choice response task (F = 11.37, df 1,3, $P < .05$).

These results indicate that lipid, whether incorporated into a meal or infused into the duodenum causes drowsiness and impaired performance.

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The effect of subclinical parasitism with <u>Trichostrongylus colubriformis</u> on the diet selection of growing sheep. By I. KYRIAZAKIS[1], D. H. ANDERSON[1], S. D. B. COOPER[1], J. D. OLDHAM[1], R. L. COOP[2] and F. JACKSON[2], [1]<u>Genetics and Behavioural Sciences Department, SAC, West Mains Road, Edinburgh EH9 3JG</u> and [2]<u>Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH</u>

Subclinical gastrointestinal parasitism in sheep is associated with some impairment of N digestion and metabolism, and a reduction in voluntary feed intake (Kimambo *et al.* 1988). This could affect the diet selection of sheep given a choice between two feeds that differ in their crude protein (CP) content. To test this hypothesis, twenty-four Texel x Scottish Blackface ewe lambs growing from 28-48 kg liveweight (LWT) were given a daily dose of 2,500 larvae of the intestinal nematode *Trichostrongylus colubriformis*; twenty-four similar lambs were used as uninfected controls. Six infected and six control lambs were given a free choice between two pelleted feeds with similar energy (10 MJ metabolizable energy/kg), but different CP contents (90 (L) and 214 (H) g CP/kg fresh feed respectively). In addition, eighteen parasitized and eighteen control lambs were given *ad lib.* access to either feed L, or feed H, or their mixture M (164 g CP/kg; six per feed), in order to quantify the effects of the feeds when offered alone, and to test for any interactions between feed CP content and parasitism on the performance of the lambs. Intestinal parasitism reduced significantly ($P < 0.001$) both the rates of LWT gain and feed intake. The performance of the lambs was limited by the feed L, but there was no significant interaction between parasitic infection and the CP content of the feed.

	LWT gain (g/d)		Feed intake (g/d)	
Parasitism . . .	-	+	-	+
Feed				
L	201	142	1403	1262
M	284	207	1669	1523
H	275	212	1646	1478
Choice of L and H	288	218	1663	1485
SED	34.8		90.3	

The infection took 4 weeks to establish and develop to a significant adult worm population (as judged by the faecal egg counts and blood parameters) and until then there was no effect of parasitism on the performance of the lambs. The diet selection of the lambs given a choice between two feeds was similar between the two groups in the first sub-period (0-4 weeks) (proportion H selected was 594 v. 598 (SED 121) g feed H/kg total feed intake (TFI)), but differed significantly ($P < 0.05$) in the second sub-period (4 weeks to end) (622 v. 475 (SED 64) g feed H/kg TFI for the parasitized and control lambs respectively). Thus, while parasitized animals had a reduced rate of feed intake, by changing their diet selection they achieved a daily rate of CP intake similar to the control ones (245 v. 254 (SED 21) g CP/d respectively). However, since the parasitized lambs had a reduced rate of LWT gain, they also consumed a higher total amount of CP to reach the same liveweight (22.5 v. 18.2 (SED 2.13) kg CP respectively). We conclude that sheep are able to respond to the challenge of this intestinal parasite by adjusting their diet selection from two feeds that differ in their CP contents, to the extent that daily rate of CP intake is maintained, but not enhanced, during parasitic infection.

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Exposure to choice feeding at different ages and the subsequent choice of broiler chickens. By M. COVASA and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds, LS2 9JT*

Birds are able to select a diet that meets their requirements when given the opportunity to learn the difference between the two feeds on offer (Rose & Kyriazakis, 1991). When whole wheat is used as a choice, it is advisable for young chicks to be exposed to the grain, as experience influences their later preference (Mastika & Cumming, 1987). We have examined the role of prior exposure to whole wheat within a choice feeding paradigm in relation to age on the subsequent choice made by broiler chickens between whole wheat and a compound feed.

Forty eight, 1d-old female broiler chickens assigned to four groups were used in a 4 × 2 factorial design experiment (four periods and two learning methods). The feeds used were commercial starter crumbs from 0 to 4 weeks (235g CP/kg, 12.5 MJ/kg), grower pellets from 4 to 7 weeks (220g CP/kg, 13.1 MJ/kg), and whole wheat (140g CP/kg, 13.2 MJ/kg). For 4d chickens were offered crumbs and wheat either by giving them on alternate days (method 1) or in a choice (method 2). The chickens were introduced to the choice feeding system as follows: group 1-week 1; group 2-week 2; group 3-week 3. The fourth group received both feeds from day 1 until the end of the experiment. Before and after the 4d exposure period, chickens from the first three groups received a complete single commercial diet. From week 5 to 7 all groups were given pellets and wheat in two separate troughs.

Feed intake (g/bird) and body weight (g)

Week	Group...	1 Mean	1 SE	2 Mean	2 SE	3 Mean	3 SE	4 Mean	4 SE
5	Pellets	81.3[a]	4.2	82.7[a]	3.9	85.8[a]	4.7	93.3[a]	4.9
	Wheat	1.5[c]	0.2	8.7[ab]	1.6	7.9[b]	1.2	12.1[a]	1.5
	Body weight	673[a]	12.3	646[a]	14.4	635[a]	23.0	576[b]	40.3
6	Pellets	116.8[a]	4.6	104.4[a]	5.1	107.5[a]	5.3	103.7[a]	4.3
	Wheat	2.8[b]	1.1	12.2[a]	2.4	5.8[b]	1.4	13.8[a]	2.1
	Body weight	886[a]	42.5	943[a]	31.5	957[a]	42.9	902[a]	41.2
7	Pellets	141.7[a]	4.2	127.4[bc]	5.3	117.5[c]	5.8	132.1[ab]	4.3
	Wheat	4.4[b]	1.4	10.6[a]	2.2	8.6[ab]	2.5	13.0[a]	2.1
	Body weight	1275[a]	45.5	1209[a]	51.2	1288[a]	53.3	1215[a]	61.0

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

Both the age at which chicks were introduced to the choice feeding and the learning method significantly affected feed intake ($P<0.05$). Significantly greater amounts of wheat were eaten by group 4 followed by group 2. Chickens ate significantly larger amounts of wheat ($P<0.001$) when offered as a choice: mean (SE) g/bird; 5.1(1.2); 14.2(2.0); 12.5(2.3); 20.3(1.8), than when offered on alternate days: 0.9(0.1); 6.1(0.9); 4.1(0.7); 5.6(1.0) for groups 1, 2, 3 and 4, respectively. Body weight was significantly different ($P<0.05$) during the first four weeks, but not at the end of the experiment. Gizzard weights were significantly greater ($P<0.05$) in group 4 followed by group 2. Carcass weight and abdominal fat pad were not affected by either period or learning method. The results show that the effect of prior exposure is related to the age of birds at training and/or the interval between learning and testing and that learning is a very important process in making nutritionally appropriate choices when whole wheat is used.

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Substitution of low-protein for high-protein feed by broiler chickens. By J.M.FORBES and J.H.CATTERALL, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Given a choice between high-protein (HP) and low-protein (LP) feeds broilers, like many other animals, eat amounts of the two which meet their requirements for energy and protein (Shariatmadari & Forbes, 1990). On a single marginally protein-deficient feed, intake is increased in an attempt to redress the protein shortage (Boorman, 1979). If choice-fed birds are given restricted amounts of one of the feeds they might increase their intake of the other to maintain a constant intake of energy or protein or neither. This experiment studied such substitution by giving different amounts of HP and monitoring the intake of LP.

Thirty 2-week-old female broilers were placed into individual cages. For the first 10 d the birds were given HP (280g CP/kg; 13.9kJ ME/g) and LP (100g CP/kg; 14.3kJ ME/g) on alternate days to accustom them to both feeds. From 24 to 42 d of age six birds per treatment were restricted to amounts of HP designed to be equivalent to 0, 10, 20, 30 or 40% of their total *ad libitum* intake. The actual proportions of total intake that was HP were 0.0, 10.3, 18.3, 27.1 and 38.3% for the five treatments, respectively.

Treatment	0	10	20	30	40	SED
HP intake (g/d)	0.00[a]	12.4[ab]	27.1[bc]	33.4[bc]	46.9[c]	15.7
LP intake (g/d)	121.5[a]	108.9[ab]	93.5[ab]	89.3[ab]	75.7[b]	29.5
Protein intake (g/d)	12.1[a]	14.4[ab]	16.9[bc]	18.3[cd]	20.7[d]	1.60
ME intake (MJ/d)	1.73[a]	1.72[a]	1.71[a]	1.74[a]	1.73[a]	0.10
Abdominal fat (g)	42.1[ab]	51.0[a]	32.7[b]	36.8[b]	31.9[b]	7.6
Carcass weight (kg)	0.99[a]	1.06[ab]	1.16[bc]	1.24[c]	1.24[c]	0.07
Weight gain (kg/18 d)	0.75[a]	0.84[ab]	0.93[b]	0.967[b]	1.01[b]	0.078

ME, metabolisable energy.
[a,b,c] Mean values in the same row with unlike superscripts were significantly different ($P < 0.05$)

As the allowance of HP increased there was a progressive decrease in voluntary intake of LP such that the total intake of feed, and ME, remained almost constant. Thus, protein intake increased with amount of HP offered and this resulted in increased weight gain and carcass weight.

In the present experiment broiler chickens controlled their feed intake to maintain energy or bulk intake even if this meant a considerable reduction in protein intake below that required for optimal growth. There was no evidence, with low levels of HP, for birds increasing their LP intake to maintain protein intake. They may have a strict limit on dry matter or ME intake but this seems unlikely in view of the fact that under some other circumstances broilers increase their intake to try to maintain protein intake.

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Attitudes to fat and portion weights of fat spreads. By K. MCPHERSON and A. WISE, *The Robert Gordon University, Aberdeen AB9 2PG*

Portion weights of fat spread have been shown to differ markedly between individuals, but previous research has failed to find substantial reasons for this variability (Stephen & Wise, 1992). Other research has found some relationship between attitudes and behaviour (as measured by frequency of consumption), but no study on portion weight has been reported (Shepherd & Towler, 1992). In the present study, people were approached in a supermarket whilst they were picking up a fat spread from the display cabinet. Some (17%) refused to cooperate, but 200 successfully completed the study (equal numbers for each sex). Next to the cabinet, on the edge of a round table, were placed on colour-coded plates, in a random order, ten slices of white bread with preweighed amounts of Flora margarine (3 to 12g in 1g increments). Subjects were asked some preliminary questions about their purchase and then which slice looked most like what they would consume at home. Then they were asked which slice appeared to have the least and which the most spread. Most answered correctly (85% for 3g and 70% for 12g). Results were tallied for small (S 3-5g; n 97), medium (M 6-9g; n 50) and large (L 10-12g; n 53) amounts of spread.

They then were given an attitude questionnaire with seven-point Likert scale (strongly disagree (1) to strongly agree (7)) for self-completion in the store and results were tallied for disagreement scores (D 1-2), relatively neutral scores (N 3-5), and for agreement scores (A 6-7); they were subjected to a Chi-square test and expressed as a percentage. Significant relationships between attitudes and portion weights were found for: Q1 ($P=0.005$) belief that some foods are healthier than others; Q2 ($P=0.004$) stating that the amount of spread they use is important to health; Q3 ($P=0.003$) stating that nutrition labels are important in helping to make food choices; Q4 ($P=0.004$) stating that eating a diet low in fat is important to health; and Q5 ($P=0.005$) stating that semi-skimmed/skimmed milk tastes better than ordinary. D and N were combined for Chi-square tests on Q1, Q2 and Q4.

	Question 1			Question 2			Question 3			Question 4			Question 5		
	D	N	A	D	N	A	D	N	A	D	N	A	D	N	A
S	2	5	93	6	30	64	11	32	57	5	18	77	35	15	50
M	6	8	86	6	32	62	8	50	42	8	22	70	36	34	30
L	9	17	74	17	47	36	28	38	34	9	40	51	45	32	23

No relationships were found for nine other attitudes: that spread enhances the taste of bread, that spread should evenly cover the slice; that being the correct weight for height is important; that 'I should reduce the amount of spread I use'; that the spread bought is a low fat food; that 'I consume a healthy diet'; that excess spread should be put back; that 'I am not overweight'; and that it would be unpleasant/unacceptable to reduce the amount of spread. For Q2-Q4 females also tended to agree more strongly with the attitude and also took less spread ($P=0.002$), but for Q1 and Q5 there was no sex difference in the attitude. Females also tended to agree significantly with three other attitudes for which there was no relationship between attitude and portion weight. Further research relating attitudes to behaviour is needed to improve our understanding of factors influencing the selection of different portion sizes.

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Stephen, A. D. & Wise, A. (1992). *Nutrition and Food Science* No. 6, 17-19.

Positive and negative message constructions in nutrition education. By L. MCROBBIE, A. WISE and A. MCLEISH, *The Robert Gordon University, Aberdeen AB9 2PG*

Randall *et al.* (1992) asked members of the public to rank nutritional messages for preference and concluded that there was no difference between positive and negative statements, but Murphy *et al.* (1993) found positive statements to be more persuasive than negative ones. In the present study, 564 people at a shopping-centre cafe were asked to rank messages and 400 successfully completed the questionnaires (198 males and 202 females). Messages were either commands (C) or suggestions (S) using words like 'use' or 'you could' respectively. They also were either positive (+) or negative (-), for example 'use' or 'avoid' respectively. Other statements either put a positive idea followed by a negative (+-) or *vice versa* (-+). This gave eight different styles of writing. There were also eight different food messages, and when these were combined with the different styles of writing a total of 64 different messages were produced, for example:

+C   Use thick cut chips. They don't absorb a great deal of fat when fried.

-+S   It's sensible to avoid thin cut chips and use thick cut instead. Thin chips absorb a lot of fat when fried.

The study used a graeco-latin square design so that the same message about the eight foods appeared on a questionnaire, but with a different style. This gave eight questionnaires, each containing eight messages about different foods, written in eight different styles.

Subjects were asked to rank the eight statements for persuasiveness. The total number of subjects ranking each message type highly persuasive (1-4) or less persuasive (5-8), were calculated and expressed as a ratio, so that high ratios denote greater persuasiveness. The Chi-square test was used to calculate the probabilities. Positive commands (+C) were more persuasive than -C (1.38 *v.* 0.81; $P<.001$), but both +S and -S were less persuasive (0.72 and 0.71) and did not differ between + and -. From the double message formats, -+S were favoured over +-S (1.38 *v.* 1.02, $P=0.033$), but for commands there was no difference (-+C 1.07 and +-C 1.15). The persuasiveness ratio differed also between the foods for which messages had been written. The least favoured was for making soup rather than buying canned varieties (0.51) and the most persuasive was for changing to semi-skimmed milk (1.98). Messages about chips (0.97), bread (1.17), soft drinks (0.94), cereals (0.90), butter (1.29) and sweeteners (0.79) were less obviously highly liked or disliked.

It was concluded that positive commands and suggestions with negative ideas followed by positive ones were most persuasive, but that individual messages about foods also contained important features that influenced persuasiveness.

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Randall, L., Wise, A. & McLeish, A. (1992). *Journal of Human Nutrition and Dietetics* 5, 151-156.

Acute effect of peripheral interleukin-1β administration on macronutrient selection in the rat.
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Interleukin-1β (IL-1β) is believed to mediate the anorexia associated with infection or cancer and potently reduces food intake when administered to rats maintained on standard laboratory chow (Moldawer *et al.* 1988). However, the characteristics of this anorexia in terms of macronutrient specificity are unknown. Therefore, the effect of peripherally-administered recombinant human IL-1β on macronutrient selection was tested in free-feeding rats.

Fourteen male Wistar rats, maintained on a reversed light:dark cycle (lights off at 14:30 h) were transferred upon weaning to a dietary self-selection regimen for carbohydrate (cornstarch and sucrose mixture), protein (casein) and fat (lard), balanced for vitamins, minerals and fibre. Following an adaptation period, sterile saline (9 g/litre) and recombinant human IL-1β (1 μg/100 g body weight given intraperitoneally) were administered on successive days 30 min before the start of the dark phase. Diet intakes were measured after 2 h and 24 h.

Baseline measurements of diet intake for 4 consecutive days before injection studies revealed that each macronutrient contributed to at least 20 % of the total energy intake for each rat, with no significant individual daily variation in macronutrient consumption. Following IL-1β administration, 2 h energy intake was reduced (142 kJ (SE 17) vs 79 kJ (SE 13), $P<0.002$) and this suppression was maintained over 24 h (564 kJ (SE 29) vs 451 kJ (SE 38), $P<0.003$). However, IL-1β administration differentially affected macronutrient intake. Compared with saline injection, IL-1β suppressed protein consumption over the following 2 h (-57 (SE 9) %, $P = 0.01$) and this suppression was maintained over 24 h (-43 (SE 4) %, $P < 0.001$). Fat ingestion was similarly reduced over the same time periods (2 h, -68 (SE 6) %, $P = 0.01$; 24 h, -32 (SE 14) %, $P = 0.04$). However, 2-h carbohydrate intake was not suppressed by IL-1β and 24 h carbohydrate intake was virtually identical to that following saline treatment.

In view of the fact that standard laboratory chow provides 55-60 % of metabolizable energy as carbohydrate, anorexia observed in chow-fed animals may be a reflection of trying to avoid specific macronutrients. This being the case, further studies may reveal that avoidance of specific macronutrients may reflect some important beneficial aspect of the host-defense response to infection or cancer.

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The effect in rats of intestinal intraluminal lipid on postprandial activity determined by Doppler shift radar . By B.E.MURRAY, K.A.CLARKE and R.D.E.RUMSEY, Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN

The behavioural sequence of activity, grooming and rest following a meal has been characterized in rodents (Antin et al. 1978). The present study is concerned with the role of the lipid component of the meal in the activity response measured by Doppler shift radar in rats.

Six Sheffield strain male rats (300-325g) were surgically equipped, under Ketamine/Xylazine general anaesthesia, with chronic indwelling Silastic cannula (0.02in id, O.037in od) inserted into the lumen of the distal small intestine. The other end of the cannula was tunnelled subcutaneously to emerge anchored in the subscapular region. After a minimum 2 week recovery period and an overnight fast, the activity of each animal was determined for 30 min as 20%(20:80,v:v) Intralipid emulsion (Kabi) or a control saline (9g NaCl/l) solution was infused (0.3 ml/h) through the cannula. A second series of similar animals underwent a 30 min infusion of either Intralipid or saline which was followed by a meal of 5ml homogenized washed baked beans. After feeding, the infusion was continued for the 30 min of activity measurement. Activity was determined on individual animals using a low power output Doppler shift emitter (10.69 GHz)/receiver (RS 8960) module positioned 1m above the animal's own cage. Doppler pulses were collected in four Bins of ascending frequency (range 1.9 - 5.9 Hz), corresponding to the velocity of movement (Clarke & Parker, 1986).

The mean cumulative Doppler pulses for the unfed infusion alone experiment was 3112 (se 197) for saline and 3265 (se 271) for Intralipid. For the meal fed animals the total pulses were 3039 (se 208) for saline and 2569 (se 433) for Intralipid infusion. While these differences do not reach statistical significance, the distributions of frequency pulses within the four collection Bins are significantly different. The table gives the number of pulses in each of the frequency Bins expressed as a mean percentage with its standard error of the total number of pulses.(n 6).

Frequency Bins		4		3		2		1 (lowest)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Unfed	Saline	31.6	1.7	37.0	1.6	24.6	2.1	6.7	1.3
	Lipid	26.3*	1.5	33.7	1.7	29.2	1.8	10.7*	1.5
Fed	Saline	26.3	1.2	34.6	0.6	28.5	1.1	10.6	1.1
	Lipid	23.4	1.0	32.6*	0.9	30.0	1.1	13.8*	1.7

Significantly different from saline (Wilcoxon matched pairs signed rank test); * P < 0.05

Application of the Wilcoxon test demonstrates that in both experiments there is a significant shift from activity generating high frequency pulses to activity generating low frequency pulses. Since the total amount of activity especially in the unfed experiment is unchanged, the infusion of lipid has the effect of reducing the amplitude and/or velocity of movement. In the fed group where the total number of pulses approaches but does not reach significance, luminal fat probably reduces the amount of movement to a variable extent. Comparison of the saline infusions in the two experiments suggests that the bean meal administered by tube does not effect activity.

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Clarke K.A. and Parker A.J. (1986) IRCS Medical Sciences. 14, 843.

Effect of wheat bushel weight and enzyme supplementation on the apparent metabolisable energy content of diets and on broiler performance. By G. QUINTIN[1] and K. J. McCRACKEN[1,2], [1]Agricultural Chemistry Department, The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX and [2]Department of Agriculture for Northern Ireland.

Wheat is a major constituent of broiler diets. Recent results have suggested that different varieties can exhibit large differences in the apparent metabolizable energy (AME) content (Wiseman *et al.* 1993) while others have shown similar AME contents but large differences in performance of broilers given diets containing 750 g wheat/kg diet (Rose & Abbas, 1993). Feed enzymes have been shown to improve AME content and feed efficiency with diets containing barley, wheat and rye (Pettersson *et al.*, 1991). The present study was designed to test the AME content of three samples of wheat of different bushel weight and to study the effects of enzyme supplementation on AME content and performance.

Male, Ross broiler chicks were studied from 6-27 d of age in individual cages. The treatments were wheat (A,B,C: bushel weights 69, 67, 57 kg/hl respectively) and enzyme (Avizyme Tx, Finnfeeds; present or absent). Two replicates were done each with sixty-six birds giving twenty-two birds per treatment for the growth trial. In each replicate a 7 d balance was done on five birds per treatment from 13-20 d for determination of AME. The wheat samples contained (g/kg dry matter DM) respectively: crude protein (N × 6.25) 133, 162, 144; starch 648, 625, 634; non-starch polysaccharide 111, 99, 116. All diets contained 666 g wheat/kg and a balancer formulated to produce a good grower diet. Diets were cold-pelleted (4 mm die) and crumbled manually through a coarse screen.

Diet.....	A		B		C		P =		SEM	
Enzyme.....	+	-	+	-	+	-	Wheat	Enzyme	Wheat	Enzyme
Gain (g/d)	47.6	46.2	50.3	48.8	46.4	44.1	<0.001	0.025	0.67	0.54
Intake DM(g/d)	64.9	64.4	66.3	64.1	63.5	63.2	0.217	0.253	0.75	0.62
Gain:Intake	0.73	0.72	0.76	0.76	0.73	0.70	<0.001	0.010	0.005	0.004
AME:GE	0.76	0.75	0.76	0.76	0.75	0.75	0.117	0.793	0.004	0.003

GE = gross energy content of diet   NS = *P*>0.05

The mean AME contents (MJ/kg DM) for diets A, B, C respectively were 14.76, 14.94, 14.79 (NS; SEM 0.079) and the values with or without enzyme were 14.80 and 14.86 MJ/kg DM (NS, SEM 0.064). There was no significant effect of wheat on feed intake but gain:intake and daily gain were significantly different (*P*<0.001). There was a significant wheat × enzyme interaction (*P*<0.05) with the enzyme giving the greatest improvement in gain:intake with diet C.

The results confirm that bushel weight is a poor indicator of quality. As with Rose & Abbas (1993) the differences in AME content were too small to explain differences in FCE. Differences in crude protein content exist but calculations suggest that all diets contained sufficient essential amino acids. The wheat × enzyme interaction for FCE, coupled with the lower NSP content of the best wheat, suggests that NSP content could be an important factor.

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Protein and energy metabolism during mild zinc deficiency in adult man. By L. THOMAS[1], B. E. GOLDEN[2], M. J. JACKSON[3], P. J. AGGETT[4] and M. A. McNURLAN[1], [1]Rowett Research Institute, Aberdeen AB2 9SB, [2]Department of Child Health, University of Aberdeen, Aberdeen AB9 2ZD, [3]Department of Medicine, University of Liverpool, Liverpool L69 3BX, and [4]AFRC Institute of Food Research, Colney Lane, Norwich NR4 7UA

Impaired growth velocity is the clinical feature of mild Zn deficiency that has received most recognition. This condition may also affect the quality of growth by disturbing protein synthesis and degradation. Animal studies have shown that Zn has an effect on protein synthesis (Giugliano & Millward, 1987). In the present study we investigated the effect of mild Zn deficiency on whole-body protein turnover and substrate metabolism in adult man.

Mild Zn deficiency was induced in four healthy male volunteers, (mean (SD) age 38.5 (9.5) years and body mass index (kg/m^2) 22.4 (2.4)), by dietary means. The low Zn diet was based on that used by Taylor *et al.* (1991). The study was divided into four phases, baseline (6.5 mg Zn/d), starvation (3 d), depletion (1.5 mg Zn/d) and repletion (29.5 mg Zn/d). The calcium intake was 1.5 g/d and phytate intake 6 g/d. Plasma Zn levels were monitored throughout the study. During each of the three main phases of the study whole-body protein turnover was measured by an 8 h primed constant infusion of [1-^{13}C]leucine. The subjects fasted for the first 4 h of the infusion and received small hourly meals during the latter 4 h. Leucine flux was estimated from the isotope enrichment of plasma leucine. The rate of leucine oxidation was calculated from the enrichments of breath CO_2 and plasma α-ketoisocaproate combined with respiratory gas analysis (Melville *et al.* 1989). The nutrient utilization rates were determined from the respiratory gas analysis and leucine oxidation (Garlick *et al.* 1987).

The plasma Zn levels, (mean (SEM)), baseline 17.1 (0.8) µmol/l, depletion 8.2 (1.1) µmol/l and repletion 16.0 (1.4) µmol/l of the four subjects decreased by varying degrees. During the [1-^{13}C]leucine infusion, feeding was associated with an increase in leucine oxidation from (mean (SEM)) 11.5 (1.2) to 27.2 (2.1) µmol/kg per h ($P<0.001$) and a decrease in protein degradation from 73.5 (4.3) to 52.4 (3.2) µmol/kg per h ($P<0.001$). There was no significant change in the leucine kinetics in three of the subjects during the deficient stage. However in the fourth subject, with the lowest plasma Zn concentration (5.2 µmol/l), there were decreases in leucine oxidation (71 %), protein synthesis (31 %) and degradation (39 %).

Substrate utilization was unaffected by mild Zn deficiency. In the fasted state there was a similar contribution to energy expenditure, (mean (SEM)), by carbohydrate 43.4 (3.5) % and fat 48.6 (3.5) %, while protein contributed 8.0 (0.8) %. During the fed state a change in metabolism occurred. Carbohydrate utilization became dominant, 50.6 (3.3) %, the contribution to energy expenditure from protein increased to 21.5 (0.9) %, and from fat decreased to 27.9 (3.0) %.

In conclusion, in the subjects with the most mild Zn deficiency whole-body protein turnover and nutrient utilization were unaltered; however, with more severe Zn deficiency whole-body protein metabolism was altered.

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The influence of dietary creatine supplementation on work output and metabolism during repeated bouts of maximal isokinetic cycling in man. By R. BIRCH, D. NOBLE and P. L. GREENHAFF, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

It has recently been shown that dietary creatine (CR) supplementation can increase skeletal muscle total CR content by 20-50 % (Harris *et al.* 1991) and decrease muscle torque loss and plasma ammonia accumulation during repeated bouts of maximal voluntary unilateral isokinetic knee extensor exercise (Greenhaff *et al.* 1993). The aim of the present study was to investigate the effect of dietary CR supplementation on performance during repeated bouts of maximal isokinetic cycling.

Following a period of familiarisation, fourteen healthy male volunteers performed three bouts of maximal exercise, each lasting 30 s, on a purpose-built isokinetic cycle ergometer (McCartney *et al.* 1983). Each bout of exercise was performed at a velocity of 80 rev/min and was separated by 4 min of rest. Force production on both pedals was recorded during each crank revolution and was used to compute total work output during exercise. Blood samples, for the determination of plasma ammonia and blood lactate concentrations, were obtained from a superficial hand vein at rest before exercise and at pre-determined intervals after exercise. Two d after the first exercise test, subjects were asked to consume either 4 times 5 g of CR for 5 d (*n* 7) or an equivalent volume of placebo (glucose polymer; GP; *n* 7), after which they returned to the laboratory and repeated the three bouts of exercise and gave blood samples as before. Treatments were administered in a randomized double-blind manner. The total amount of work performed (J/kg body wt.) during each bout of exercise before and after CR and GP ingestion is shown in the Table.

	Bout 1		Bout 2		Bout 3	
	mean	SE	mean	SE	mean	SE
Pre - GP	276.0	10.8	252.7	10.4	220.3	10.2
Post - GP	279.5	10.3	256.6	9.9	224.7	10.0
Pre - CR	251.9	10.5	227.0	11.1	207.4	12.2
Post - CR	267.0*	10.3	239.6*	11.9	211.3	13.9

* significantly different from pre-supplementation value (paired *t* test) : $P<0.05$.

Total work performed during each exercise bout was similar before and after GP ingestion (<2 % change). However, after CR ingestion work output was increased by approximately 6 % in all seven subjects during exercise bouts 1 and 2 ($P<0.05$). On all occasions, peak plasma ammonia accumulation occurred 2 min after the final bout of exercise and, compared with pre-supplementation values, the peak change was lower after CR ingestion ($P<0.05$), but was unchanged after GP ingestion. There were no differences in blood lactate accumulation within or between treatments.

In agreement with previous results (Greenhaff *et al.* 1993), the present study shows that dietary CR supplementation can offset the loss of muscle force during maximal exercise. The lower peak plasma ammonia accumulation after CR ingestion suggests this ergogenic effect may be achieved by CR ingestion maintaining a higher muscle ATP turnover rate during contraction.

This study was approved by the University Medical School Ethics Committee.

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Concentrations of serum cortisol, insulin and blood glucose in dairy cows fed on diets with different amounts of concentrate and roughage. By H.A. SAMANC[1], J.A. NIKOLIC[2], Z. DAMJANOVIC[1], V. STOJIC[1], M. ANDJELKOVIC[3], and N. LEKIC[3], [1] Faculty of Veterinary Medicine, University of Beograd, 11000 Beograd, [2] INEP - Institute for the Application of Nuclear Energy, 11080 Zemun, [3] Cattle Farm, 26300 Vrsac and Stara Pazova, Yugoslavia.

High-yielding dairy cows need to adapt their metabolism rapidly *post partum* to provide the large amounts of precursors necessary for the sudden synthesis of milk lactose. Since glucose production is largely under hormone control, the concentrations of glucose, insulin and cortisol were determined 10 d before calving and 10 d *post partum* in fourteen Holstein dairy cows kept under two different nutritional regimens. All cows received hay, silage and concentrates but those in unit A were fed on a combination of maize silage and lucerne silage and concentrates at the level of 180 g/1 milk produced on the farm (roughage: concentrate dry matter ratio; 75:25w/w); while those in unit B were fed on maize silage and concentrates at 320 g/1 milk (roughage; concentrate ratio; 50;50 w/w). Milk production in each unit reached 6500 litres per lactation.

| | Pre partum | | | | Post partum | | | | LSD |
| | Unit A | | Unit B | | Unit A | | Unit B | | |
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	
Glucose (mmol/1)	2.8[a]	7.4	2.6[ab]	13.6	2.2[b]	6.6	1.7[c]	32.3	0.4
Insulin (mIU/1)	12.2[a]	30.0	22.1[b]	24.5	10.7[a]	38.2	21.9[b]	37.0	6.7
Cortisol (nmol/1)	20.3[a]	41.8	10.4[b]	38.8	14.9[ab]	53.9	8.4[b]	42.8	7.7

CV, coefficient of variation; LSD, least significant difference
[a,b,c] Values with unlike superscripts are significantly different (ANOVA)

The Table shows that the mean levels of blood glucose before calving were adequate and similar in the two groups of cows but serum insulin concentrations were significantly higher ($P < 0.01$) and serum cortisol concentrations significantly lower ($P < 0.05$) in the group of cows fed more concentrate. At 10 d *post partum* glucose concentrations were slightly but significantly lower in the cows fed on less concentrate but there were no clinical signs of ketosis. The other group of cows exhibited a marked fall in blood glucose concentrations ($P < 0.01$). Three of the seven animals had clinically apparent ketosis with urine ketone body levels above 17.8 mmol/1. Blood glucose was around 1 mmol/1 in these cows. Insulin levels and the differences between the two groups of cows were maintained *post partum*. Serum cortisol concentrations tended to be somewhat lower *post partum* in the first group of cows but the difference between the groups tended to remain.

It may be concluded that the diet used to feed the cows in unit B did not provide a hormonal balance which was able to support the mammary demands for glucose in the peripartal period. According to Stangassinger & Giesecke (1988), this is achieved by both insulin deficiency and insulin resistance, only the latter condition being met in the second group of cows.

Stangassinger, M. & Giesecke, D. (1988). Proceedings of the Nutrition Society **47**, 151A.

Influence of ethyl myristate, palmitate and oleate on plasma lipoprotein metabolism in the hamster. By T.A.B. SANDERS, SANOJA SANDARADURA AND FENELLA SLATTERY. Department of Nutrition and Dietetics, King's College, University of London, Campden Hill Road London W8 7AH.

The influence of individual saturated fatty acids on lipoprotein metabolism is poorly understood. Hepatic cholesterol concentrations are believed to play an important role in the regulation of LDL (low-density lipoprotein) receptor activity. Hamsters fed triacylglycerols rich in palmitic (16:0) and myristic (14:0) acids show a reduction in the catabolic rate of LDL but this is only evident only when the diet is supplemented with cholesterol in the range 0.6-1.2 g/kg (Woollet $et\ al$, 1992). In the present study hamsters were been fed on purified isoenergetic diets adequate in all known essential nutrients which contained 0.6 g cholesterol dissolved in 100 g fatty acid ethyl esters/kg diet. Animals received either ethyl oleate (18:1n-9) or ethyl myristate (14:0) or ethyl palmitate (16:0) for 3 weeks. Plasma lipoprotein concentrations, the fatty acid composition of adipose tissue, VLDL (very low density lipoprotein) cholesteryl ester and hepatic membranes and hepatic cholesterol content were determined.

The dominant dietary fatty acid was reflected in total fatty acid composition of adipose tissue and VLDL cholesteryl esters. However, compared with myristate, palmitate led to a higher proportion of oleic acid in adipose tissue, hepatic membrane lipids and VLDL cholesteryl esters. Plasma VLDL cholesteryl ester concentrations were lower in animals fed on myristate and palmitate and cholesterol/protein ratio in both VLDL and LDL was lower than in those fed oleate. LDL protein concentrations were greater in those fed myristate and palmitate. The most marked changes, however, were in hepatic concentrations of cholesterol (mmol/kg; Table).

Ethyl ester ...		Oleate		Myristate		Palmitate	
Cholesterol species	n	Mean	SE	Mean	SE	Mean	SE
Total cholesterol	7	28.5[a]	3.02	10.4[b]	0.76	6.8[c]	0.23
Free cholesterol	7	3.5[ab]	1.34	7.0[c]	0.643	5.7[bc]	0.20
Cholesteryl esters	7	24.9[a]	2.73	3.4[b]	0.56	1.1[c]	0.15
Cholesteryl oleate	7	17.7[a]	2.18	1.4[b]	0.33	0.3[b]	0.05

Values with unlike superscripts are significantly different $P<0.05$

Hepatic cholesteryl ester concentrations were decreased by both myristate and palmitate compared with oleate. Hepatic free cholesterol concentrations were increased by myristate compared with oleate. We suggest that these saturated fatty acids act by increasing the proportion of apoprotein-B-rich lipoproteins destined to form LDL (direct synthesis) by inhibiting cholesterol esterification within the liver.

Woollett, L.A., Spady, D.K. & Dietschy, J.M.(1992). Journal of Clinical Investigation **89**,1133-.

Methodological considerations of ^{13}C breath studies. By A.E. JONES[1], J.L. MURPHY[1], S. BROOKES[2], M. GRIFFITHS[2] and S.A. WOOTTON[1], *[1]Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU and [2]Europa Scientific Ltd, Crewe CW1 1ZA.*

Quantitative determinations of the excretion of ^{13}C-label on the breath after ingesting labelled substrate requires information on $^{13}CO_2$ enrichment and whole body CO_2 excretion. CO_2 excretion can be measured by indirect calorimetry or may be predicted from body surface area as 300 mmol CO_2/m^2 per h (Shreeve *et al.* 1976). In practice, CO_2 excretion is usually predicted and assumed to remain constant over the study period irrespective of any post-prandial increase in CO_2 excretion in response to the test meal.

The present study examined the errors associated with predicting, as opposed to measuring, CO_2 excretion in six healthy women aged 21 - 30 years following ingestion of a standardized test meal providing 46% of energy from carbohydrate, 39% from fat and 14% from protein (1660 kJ). Predicted CO_2 excretion was determined using values of body surface area estimated from height and weight (Haycock *et al.* 1976). Resting CO_2 excretion was measured by indirect calorimetry (Deltatrac; Datex Instrumentarium Corp., Helsinki, Finland) following an overnight fast before ingesting the test meal, and post-prandially at 1h intervals for 10h.

The CO_2 excretion measured before test meal consumption was 1.1-21.0% lower than predicted CO_2 excretion (median 6.7%; $P<0.05$). Peak CO_2 excretion occurred 1-3 h after test meal consumption and was 20.1-35.0% greater than pre-meal values (median 31.1%; $P<0.05$). Post-prandial CO_2 excretion returned to pre-meal values over the study period. Averaging post-prandial CO_2 excretion over the study period resulted in values higher than CO_2 excretion measured before test meal consumption by 5.6 - 14.1% (median 6.4%; $P<0.05$) but was not different from predicted CO_2 excretion (median 0.3%; -6.9 - 5.7%; NS).

In conclusion it appears that: (1) predicting CO_2 excretion is an inadequate approximation of pre-meal CO_2 excretion, (2) a single pre-meal measurement of CO_2 excretion is unrepresentative of the average post-prandial CO_2 excretion over the study period. In the absence of direct measurements of CO_2 excretion, prediction from body surface area may provide an adequate approximation of the average CO_2 excretion over the study period in healthy adults using this test meal.

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Shreeve, W.W., Shoop, J.D., Ott, D.G. & McInteer, B.B. (1976). Gastroenterology **71**, 98-101.

The bio-availability of a ^{13}C labelled dietary fatty acid in healthy adults. By J.L. MURPHY[1], S. BROOKES[2], M. GRIFFITHS[2] and S.A. WOOTTON[1], [1]*Department of Human Nutrition, Southampton, SO9 3TU and [2]Europa Scientific Ltd., Crewe, CW1 1ZA.*

Previous studies investigating the metabolic fate of ^{13}C-labelled substrates have measured the enrichment in breath $^{13}CO_2$ without accounting for recovery of ^{13}C-label in stool. Jones *et al.* (1985) have shown that the absorption of 1-[^{13}C] stearic acid is less than 1-[^{13}C] oleic and 1-[^{13}C] linoleic acid and suggested that this may influence the results from $^{13}CO_2$ breath tests. The purpose of the present study was to examine the apparent bio-availability of 1-[^{13}C] palmitic acid and the impact of correcting for faecal loss of label on recovery of ^{13}C in breath.

Following an overnight fast six healthy women aged 21-30 years ingested 1-[^{13}C] palmitic acid (10mg/kg) with a standardized test meal (1660 kJ) of low natural ^{13}C abundance. Breath samples were collected before and during a fasting period, hourly for 10h and then after 15h. All stools were collected for a 5d period between carmine markers administered simultaneously with 1-[^{13}C] palmitic acid. Enrichment of $^{13}CO_2$ in breath and ^{13}C in stool samples was analysed by mass spectrometry (ANCA system, Europa Scientific Ltd., Crewe) The results shown are for ^{13}C recoveries in stool and breath $^{13}CO_2$ as percentage administered label (% admin dose) and ^{13}C recovery in breath $^{13}CO_2$ corrected for faecal loss of ^{13}C substrate (% available dose).

[13]C Recovery	Stool [13]C	Breath $^{13}CO_2$	
	(% admin dose)	(% admin dose)	(% available dose)
Median	10.6	21.4	24.9
Min	7.2	15.7	22.4
Max	32.4	29.0	30.6

The apparent bio-availability of 1-[^{13}C] palmitic acid ranged from 67.6 to 92.8%. The proportion of ^{13}C excreted in breath could be underestimated by 5.1-30.0% (median 10.9%) without a correction for label in faeces. This study illustrates the importance of determining the factors which may influence dietary substrate bio-availability and the need to account for faecal loss of label in breath $^{13}CO_2$ studies.

Jones, P.J.H., Pencharz, P.B. & Clandinin, M.T. (1985). *Journal of Laboratory and Clinical Medicine* **105**, 647-652.

Obesity as an adaptation to a high-fat diet: evidence from a cross-sectional study. By A.V. ASTRUP[1], B. BUEMANN[1], P. WESTERN[1], S. TOUBRO[1], A. RABEN[1], and N.J. CHRISTENSEN[2], [1]Department of Human Nutrition and KVL Centre for Food Research, The Royal Veterinary and Agricultural University, Copenhagen and [2]Department of Internal Medicine and Endocrinology, Herlev Hospital, University of Copenhagen, Denmark.

Expansion of the fat stores has been proposed as a prerequisite for increasing fat oxidation in response to a high-fat diet in individuals with the predisposition to obesity (Schutz *et al.* 1992). In a cross-sectional design we measured 24 h energy expenditure and substrate oxidation in respiration chambers (Astrup *et al.* 1992) in thirty-eight overweight/obese and thirty-five non-obese women. During the 24 h they consumed a diet providing 30 % energy from fat.

Fat oxidation (g/ d) was mainly a function of total energy requirements ($r = 0.71$; $P < 0.0001$). For further analysis we, therefore, used oxidative fat energy (%), a counterpart to dietary fat energy (%). After adjustment for confounders, such as differences in fat energy of consumed food (%), age and 24 h energy balance, obese women had higher oxidative fat energy than non-obese women (40.2 % (95 % CI; 37.8-42.6) *v.* 36.0 % (33.6-38.5); $P < 0.02$) and adjusted oxidative fat energy (%) increased with increasing size of fat mass ($r = 0.31$; $P < 0.01$).

Assuming that the increment in fat oxidation achieved per kg fat mass gain is similar in normal weight and in obesity-prone subjects, the relation suggests that a 10 kg change in fat mass may be caused by a change in dietary fat energy of 1.6 % (0.4 - 2.7 %). The study supports the contention that in susceptible individuals the expansion of fat stores may be necessary to increase the oxidative fat energy to a level commensurate with their high dietary fat energy percent.

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Schutz, Y., Tremblay, A., Weinsier, R.L. & Nelson, K.M. (1992). American Journal of Clinical Nutrition **55**, 670-674

Contribution of intestinal microflora to lysine requirements in rats. By D. TORRALLARDONA[1], C.I. HARRIS[1], E. MILNE[1], V. RONAASEN[2], M.E. COATES[2] and M.F. FULLER[1], [1]Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB and [2]School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH

In a previous experiment (Torrallardona *et al.* 1993), substantial absorption of microbial lysine was measured in a rat and a pig. Those measurements were made on the assumption that the animals were not able to incorporate inorganic ^{15}N into lysine and that all labelled body lysine was, therefore, of microbial origin. In the present experiment the ability of germfree rats to incorporate inorganic N into lysine, and the absorption of microbial lysine by conventional rats, were studied.

Four germfree (GF-^{15}N) and four conventional (CV-^{15}N) rats were fed for 10 d on a protein-free diet containing fermentable carbohydrates and $^{15}NH_4Cl$; another four conventional rats (Control) were offered the same diet but with unlabelled NH_4Cl. The eviscerated carcass of each was homogenized, lysine was isolated by ion-exchange chromatography and ^{15}N-enrichment was measured by isotope ratio mass spectrometry. The results are shown in the first Table.

Rats	Carcass Lysine Enrichment (ape)	
	Mean (*n* 4)	SE
Control	0.0000[a]	0.00009
CV-^{15}N	0.0066[b]	0.00047
GF-^{15}N	0.0004[c]	0.00010

Significantly different: [a,b]$P<0.001$;[b,c]$P<0.001$;[a,c]$P<0.05$.

Although we measured a significant labelling in the GF-^{15}N group, this is very small and is within the methodological error. These results confirm the inability of germfree rats to incorporate substantial amounts (if any) of inorganic N into body lysine.

The microbial fractions of the faeces of the CV-^{15}N rats were separated by successive centrifugation and the ^{15}N-enrichment of lysine was measured. The enrichment and total amount of total body lysine were also determined.

Assuming that the bacterial sample is representative and ignoring any loss or degradation of lysine in the 10 d, the absorbed microbial lysine (mg) can be estimated as:

$$\frac{Body\ Lysine\ Content\ (mg) \times Body\ Lysine\ Enrichment\ (ape)}{Microbial\ Lysine\ Enrichment\ (ape)}$$

Measurements and estimations are shown in the following Table.

Rat	Lysine enrichment (ape)		Body lysine content (g)	Absorbed microbial lysine (mg/d)
	Body	Microbial		
CV-^{15}N-1	0.0064	0.2914	1.45	3.19
CV-^{15}N-2	0.0088	0.3135	1.00	2.81
CV-^{15}N-3	0.0077	0.2705	0.83	2.37
CV-^{15}N-4	0.0076	0.2102	0.92	3.32

These results confirm that there is a significant supply of microbial lysine in rats. The importance of coprophagy in this remains unknown.

Torrallardona, D., Harris, C.I., Milne, E. & Fuller, M.F. (1993). Proceedings of the Nutrition Society (In the Press).

Hypocaloric treatment of obese type 2 diabetics. By S. TOUBRO[1], A. ASTRUP[1], F. QUAADE[2], [1]Department of Human Nutrition, The Royal Veterinary and Agricultural University, Copenhagen, Denmark and [2]The Obesity Clinic, Fredriksberg, Denmark.

Fourteen weeks treatment of either very-low-calorie diet (VLCD,NUPO®) supplying 1.6 MJ (females) - 1.9 MJ (males) or conventional 3.8 MJ diabetes diet (C) was compared in diabetic patients. They were followed closely with medical consultations and visits to a clinical dietician 2 - 3 times weekly in the beginning, subsequently reduced to only one weekly. Randomization to the two diets was performed after discontinuation of all anti-diabetic medication. Twenty patients were included, tree dropped out (2 on VLCD and 1 on C), nine female and eight male patients completed the treatment (8 VLCD and 9 C); average (48 (29 - 61) years); BMI (38 (27-60) kg/m^2). Results after 14 weeks treatment:

	VLCD		C		P
	Mean	SEM	Mean	SEM	
Weight loss (kg)	17.5	1.9	12.6	1.5	NS
Weight loss (%)	16.2	1.6	11.7	1.2	0.04
Change in fat-free mass (kg)	7.1	1.1	4.9	0.5	NS
Change in fat-free mass (%)	10.9	1.0	7.5	0.8	0.02
Change in fat mass (kg)	10.4	1.1	7.7	1.1	NS
Change in fat mass (%)	23.6	2.7	18.4	2.1	NS
Fasting plasma glucose (mM)	10.3 → 6.7		10.4 → 6.4		NS
Hgb$_{A1c}$ (ref 3.4 - 6.1%)	7.8 → 5.7		7.3 → 5.7		NS

Estimation of compliance (actual weightloss x 100% / predicted weightloss) was approximately 60% in both groups. Oral glucose tolerance test was performed after 2 - 5 weeks on a weight maintenance diet revealed that six patients (4 VLCD and 2 C; NS) no longer had diabetes according to the World Health Organization definition. VLCD treatment had no metabolic advantages, but resulted in a larger % weight- and % fat-free mass loss. We were unable to assess whether the larger loss of fat-free mass during VLCD treatment was caused by a more marked loss of glycogen and water as body composition was assessed by bioimpedance. One year follow-up on 14 patients revealed that 5 of 7 in the C group and 2 of 7 in the VLCD group had maintained or further increased their initial weight loss.

We conclude that VLCD treatment of obese type 2 diabetics has no clear advantages over conventional diet, but can be used as a safe alternative to achieve an initial weight loss.

Faecal steroid excretion and relation to dietary intake in Indian and White vegetarians compared with white omnivores. By SHEELA REDDY[1], T.A.B. SANDERS[1] AND M.H. THOMPSON[2]. [1]Department of Nutrition and Dietetics, King's College London, Campden Hill Road, London W8 7AH and [2]Centre for Applied Microbiological Research, Pathology Division, Porton Down, Salisbury, Wiltshire SP4 0JG.

Dietary factors influencing bile steroid secretion and colonic bacterial metabolism are believed to be important in the aetiology of colon cancer and high intakes of fat, red meat and low intakes of dietary fibre increase risk (Willet *et al*,1990). Lower concentrations of faecal steroids were reported in Seventh Day Adventist vegetarians than in meat-eaters (Turjiman *et al*,1984). We measured faecal weight, pH, moisture content and concentrations of sterols and bile acids and dietary intakes in Indian vegetarian, white omnivorous and vegetarian women (aged 25-40 years) all of whom were resident in the UK. The results were as follows:

Faecal variables	Indian vegetarians (n 22)		White vegetarians (n 18)		White omnivores (n 22)	
	MEAN	95 % CI	MEAN	95 % CI	MEAN	95 % CI
Faecal wet weight (g/d)	186	143-228	160	118-201	117	89-146
Moisture (g/kg)	789	763-814	746	724-769	726	699-752
pH	6.18	5.91-6.45	6.54	6.27-6.82	6.65	6.40-6.91
Free bile acids (mg/g dry wt)	5.76	4.76-6.76	4.36	3.13-5.59	7.48	5.68-9.28
Lithocholic:deoxycholic	0.67	0.49-0.86	1.17	0.84-1.52	1.00	0.85-1.15
Animal sterol (mg/g dry wt)	16.0	13.1-19.0	15.3	11.7-18.9	28.7	22.9-34.6
Coprostanol:animal sterols	0.42	0.27-0.57	0.61	0.49-0.73	0.71	0.65-0.77

Faecal weight and moisture content were greatest and pH lowest in the Indian vegetarians. The lithocholic:deoxycholic acid (LCA:DCA) ratio and that of coprostanol:total animal sterols was lower in the Indian vegetarians than in white vegetarians and omnivores. Multivariate analysis revealed statistically significant associations between: the intakes of fibre and starch (adjusted for energy) and faecal LCA ($R^2 = 0.31$ p=0.0001)and DCA ($R^2 = 0.20$ p=0.0007) ; the intake of cholesterol and faecal neutral sterol concentrations (r=0.46 p=0.001). There was a significant positive relationship between faecal pH and LCA:DCA ratio (r=0.31 p=0.009) and that of coprostanol:total animal sterols (r=0.397 p=0.001). The lower faecal sterol and bile acid concentrations and the lower proportion of secondary metabolites of bile acids and fecal sterols in the Indian vegetarians are consistent with the lower incidence of colon cancer in this group (Marmot,1984).

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The effects of psyllium on blood lipids in hypercholesterolaemic subjects. By P. MANLEY[1], C. SUMMERBELL[2], A. LEEDS[3] and D. BARNES[4].

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Psyllium is a soluble gel forming fibre derived from the husks of blond psyllium seeds. The active fraction of psyllium is the mucilage polysaccharide which is made of 63% D-xylose, 20% I-arabinose, 6% rhamrose and 9% D-galacturonic acid. The present study was designed to investigate whether psyllium has an intrinsic hypocholesterolaemic effect on serum blood lipids. This 9 week, two-phased study was a double-blind, placebo-controlled trial in subjects with mild to moderate hypercholesterolaemia. After the first phase of 3 weeks in which subjects adhered to a low-fat diet, thirty seven subjects with serum total cholesterol levels of 5.2 to 7.8 mmol/l were randomly assigned to one of two groups for a period of 6 weeks. One group consumed daily 60 g of a high-fibre breakfast cereal containing 9.6 g soluble fibre from psyllium and the other (placebo) group consumed 60 g of a high-fibre (wheat bran based) breakfast cereal which contained negligible amounts of soluble fibre. Both maintained a low-fat diet containing 30 % of energy from fat.

During Phase I, total and low-density lipoprotein (LDL)-cholesterol levels were reduced from baseline (week 0) levels in both groups ($P < 0.001$). During Phase II, total and LDL-cholesterol levels were reduced from the low fat run in (week 3) levels in the psyllium group ($P < 0.001$), but not in the placebo group. Changes in HDL-cholesterol levels and body mass index (BMI) were not significant. Differences between the psyllium and placebo groups in total, LDL- and HDL-cholesterol levels were not significant at any stage.

Time (weeks)	Placebo 0	group 3	(n 18) 9	Psyllium 0	group 3	(n 19) 9
Total cholesterol mean	6.86	6.25[a]	6.08	6.77	6.17[a]	5.72[b]
(mmol/l) SD	0.85	0.89	0.90	0.60	0.42	0.52
LDL-cholesterol mean	4.78	4.17[a]	3.92	4.68	4.16[a]	3.72[b]
(mmol/l) SD	0.73	0.80	0.61	0.67	0.47	0.56
HDL-cholesterol mean	1.52	1.40	1.47	1.51	1.43	1.42
(mmol/l) SD	0.44	0.33	0.38	0.46	0.49	0.39
BMI mean	27.2	26.9	26.5	25.4	25.1	24.7
(kg/m²) SD	4.0	4.0	4.2	3.2	3.1	3.0

[a]Significant fall from week 0 to week 3: $P < 0.001$
[b]Significant fall from week 3 to week 9: $P < 0.001$

These results support existing evidence (Anderson et al. 1992) and suggest that psyllium-containing breakfast cereals have a total and LDL-cholesterol lowering effect which may be additional to that achieved by a low-fat diet.

Anderson, J.W., Riddell-Mason, S., Gustafson, N.J., Smith, S.F. & Mackey, M. (1992). American Journal of Clinical Nutrition 56, 93-98.

An investigation of the effects of two indigenous African foods, *Detarium microcarpum* and *Cissus rotundifolia* on rat plasma cholesterol levels. By S.BELL[1], U.A. ONYECHI[2], P.A. JUDD[2], P.R. ELLIS[1] and, S.B. ROSS-MURPHY[1]*Division of Life Sciences[1] and Department of Nutrition and Dietetics[2]*, *Kings College London, Campden Hill Rd, W8 7AH.*

Dietary water-soluble, non-starch polysaccharides (NSP) reduce post-prandial blood glucose and insulin levels and plasma cholesterol levels in normal subjects and those with diabetes mellitus (DM; Jenkins, 1979). Two previously uncharacterized foods, *Detarium microcarpum* (DT) a legume and *Cissus rotundifolia* (CR) a shrub, are traditionally used in Nigeria for thickening soups. Analysis has shown soluble NSP contents to be 428g/kg for DT and 140g/kg for CR. This study represents the initial steps in investigating the potential use of these foods in the treatment of DM in Nigeria where hypoglycaemic agents are expensive.

One weanling rat (Sprague - Dawley strain) from each of eight litters was assigned to one of four groups and fed on semisynthetic diets for 14 d, matching food intakes to the lowest intake across each litter. The control diet contained (g/kg) casein 150, maize oil 100, sucrose 100, vitamins and minerals 60, Solkafloc 50, cornstarch 530, cholesterol 10. Experimental diets contained DT, CR or guar gum (GG, as a positive control) at a level providing 80g soluble NSP/kg diet. Food intake, faecal output, weight gain and plasma cholesterol (after overnight fasting) were measured.

Rat diet	Control		GG		CR		DT	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Chol (mmol/l)	3.38[a]	0.19	2.14[b]	0.21	2.14[b]	0.16	2.25[b]	0.14
Food intake (g)	169	4	144	9	143	6	157	8
Faecal wt (g)	25.9	0.5	25.3	1.5	46.0	0.4	27.7	1.6
Wt gain (g)	42.8	3.3	30.2	2.8	15.4	2.2	37.2	3.2
Digestibility %	0.84[a]	0.003	0.82	0.007	0.67[b]	0.3	82.1	1.3

Values with unlike superscripts were significantly different $P < 0.05$ (ANOVA).

Cholesterol levels in all the experimental groups were significantly lower than the control ($P < 0.05$) using analysis of variance. For DT and GG covariates such as weight gain, food intake, faecal output and digestibility of diets had little effect but the low digestibility of the CR diet significantly affected the results.

Preliminary *in-vitro* rheological investigations suggest that NSP extracted from DT is a high molecular weight polymer with rheological properties comparable to those of GG. It has an intrinsic viscosity of 7+/-1 dl/g which is similar to a commercial GG of molecular weight 1.06×10^6 (Ellis *et al.* 1991). The similarity of effects of DT to GG suggests this indigenous food could be a useful adjunct to the treatment of DM in Africa.

Ellis, P.R., Dawoud, F.M & E.R. Morris (1991) *British Journal Nutrition*, **66**, 363-379.
Jenkins, D.J.A. (1979). *Lancet*, **ii**, 1287-1290.

Cholesterol metabolism and crypt cell proliferation rates in rats in response to rye based diets. By E. K. LUND, K. L. SALF and I. T. JOHNSON, <u>AFRC Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA</u>

Rye is a commonly consumed cereal which has received little attention as a source of dietary fibre. The effects of diets containing rye crispbread on hepatic and plasma cholesterol concentrations, cholesterol synthesis, small intestinal cell turnover (assessed using the metaphase arrest technique), fibrinogen levels and faecal output were investigated in growing rats. All the rats were made hypercholesterolaemic by being fed cholesterol (10g/Kg of fibre free diet) for 14 days before receiving the test diets. The effects of diets containing either 500 or 750 g/Kg dry weight of finely-ground rye crispbread were compared to those of a fibre-free diet, and semi-synthetic diets containing both guar gum and cellulose, designed to mimic the NSP composition of rye. Rats (20/group) were fed the test diets, of equal energy content, for 28 days using paired feeding. The table shows results from the 10 rats/group in which cholesterol supplementation was continued throughout the test period.

Diet	Faecal output (g dry wt/week) Mean	SEM	Cell turnover (cell divisions/crypt per h) Mean	SD	Serum cholesterol (mmol/l) Mean	SEM
Control: Fibre free	6.4[a]	0.2	10.1[a]	0.6	5.3[a]	0.7
Control: (58.5g/Kg NSP)	13.3[b]	0.4	---	---	5.1[ab]	0.2
Control: (88g/Kg NSP)	15.1[b]	0.4	21.5[b]	1.8	4.5[ab]	0.2
Rye: (500g/Kg)	17.6[d]	0.5	7.2[a]	2.0	4.4[ab]	0.4
Rye: (750g/Kg)	22.7[c]	0.8	10.2[a]	2.5	4.2[b]	0.3

a,b,c,d values with unlike superscripts are significantly different (ANOVA): $P < 0.05$.

Rye caused a marked increase in faecal output, greater than that seen in the positive control groups. However crypt cell proliferation at the midpoint of the small intestine was less than that seen in the guar/cellulose group. Rye reduced total plasma cholesterol only in those rats which continued on the high-cholesterol diet. Dietary cholesterol decreased clotting time in response to thrombin from 0.346 (SEM 0.025) min to 0.243 (SEM 0.028) min. in the absence of fibre, and to 0.200 (SEM 0.006) min when 750g rye/Kg was fed ($P < 0.05$). Rats fed the 750 g/Kg rye diet containing no cholesterol had the longest mean clotting times, 0.370 (SEM 0.08) min. Liver cholesterol concentrations in the fibre-free control group, 221 (SEM 6) mg/g dry weight of liver, were significantly higher ($P < 0.05$) than those of the groups fed rye, 135 (SEM 5) mg/g dry weight. Rye also caused an increase in hepatic cholesterol synthesis to 70 (SEM 16.7) pmol/μg per min compared to 26 (SEM 6; $P<0.05$) pmol/μg per min in the rats fed on a fibre-free diet. These data imply that rye can cause a reduction in plasma cholesterol with associated effects on haemostasis, probably due to malabsorption of bile acids and cholesterol.

Effect of cholesterol feeding on DNA damage in hamster heart cells. By E. TURLEY, J.J. STRAIN, N.C. ARMSTRONG, J.M. ALLEN and V.J. MC KELVEY-MARTIN, Human Nutrition Research Group, University of Ulster, Coleraine, BT52 1SA.

Dietary cholesterol and oxidized cholesterol derivatives have been implicated in atherosclerosis. We have previously shown that cholesterol feeding decreases hepatic antioxidant enzyme activities (Armstrong *et al.* 1993). An alternative view is that the oxidized cholesterol derivatives may represent past interception of blood and tissue oxidants *in vivo* and thus cholesterol could be acting as an antioxidant (Smith, 1991). In the current study we investigated the influence of dietary cholesterol on DNA strand breakage, as assessed by the comet assay (McKelvey-Martin *et al.* 1993).

Male and female Syrian hamsters were fed on either a control diet (150g maize oil/kg diet) or a cholesterol-supplemented diet (5g cholesterol and 150g maize oil/kg diet) for 10 weeks. Hearts were excised, and small (μg) samples taken, treated with collagenase, Type H (*E.C.* no. 3.4.24.3.), washed and mounted in agarose for lysing, electrophoresis, staining and subsequent viewing using a Nikon Optiphot epifluorescence microscope (McKelvey-Martin *et al.* 1993).

The results of the comet assay for cholesterol-fed and control animals are shown in the Table.

Diet Group		Physical area(μm^2)		Tail Length(μm)		Tail moments(ratio)	
		Mean	SE	Mean	SE	Mean	SE
Female:	Control (*n* 4)	1382	232	139	32	20.6	10.7
	Cholesterol-fed (*n* 4)	959	261	87	37	7.9	6.3
Male:	Control (*n* 3)	1496	321	136	44	18.1	10.8
	Cholesterol-fed (*n* 6)	1399	407	121	43	12.4	8.8

Two-way ANOVA indicated that there was no significant difference between sexes in the three indices generally accepted as being the most representative of cellular damage i.e the physical area, tail length and tail moments. There was, however, a trend ($P=0.054$ for tail length) towards less DNA damage in heart cells from cholesterol-fed hamsters than in those of controls.

These data indicate a trend towards a protective effect of cholesterol feeding at this level in hamster heart cells. This apparent protective effect may be related to antioxidant properties of cholesterol.

Armstrong, N.C., Allen, J.M. & Strain, J.J. (1993). In Trace Elements in Health and Disease with Special Emphasis on Atherosclerosis [M.F. Reis, editor]. London: Smith-Gordon & Co. (In the Press)
McKelvey-Martin, V.J., Green, M., Schmezer, P., Pool-Zobel, B.L., DeMeo, M.P. & Collins, A.M. (1993). Mutation Research **288**, 47-64.
Smith, L.L. (1991). Free Radicals in Biology and Medicine **11**, 47-61.

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

It has been suggested that polyunsaturated fatty acids (PUFA) possess immunosuppressive properties and so may provide potential for dietary therapy of autoimmune and inflammatory diseases. *In vitro* studies have shown that fatty acids, particularly the n-6 and n-3 PUFA, have profound effects on lymphocyte composition and functions. Few studies have made systematic comparisons between a number of different dietary lipids. In the present study the effects of a variety of dietary lipids on the proliferation of lymphocytes from lymph nodes, spleen and thymus were investigated.

Weanling Lewis rats were fed for 10 weeks on a low-fat (LF; 20 g/kg) diet or on high-fat diets containing 200 g/kg of either hydrogenated coconut oil (HCO; rich in saturated fatty acids), olive oil (OO; rich in the n-9 monounsaturated fatty acid oleic acid), safflower oil (SO; rich in n-6 PUFA), evening primrose oil (EPO; containing the n-6 PUFA γ-linolenic acid) or menhaden oil (MO; rich in n-3 PUFAs). All diets contained a further 10 g corn oil/kg to prevent essential fatty acid deficiency, 1.2 g vitamin E/kg as antioxidant and adequate amounts of vitamins, minerals and fibre. Lymphocytes were prepared from spleen, thymus and cervical and mesenteric lymph nodes. They were cultured with various concentrations (1-20 µg/ml) of the T-cell mitogen concanavalin A (Con A) and either foetal calf serum (FCS) or autologous serum. Proliferation was measured as incorporation of [^{3}H]thymidine, and is expressed as stimulation index (incorporation with mitogen : incorporation without mitogen).

Figure 1. Effect of dietary lipid manipulation on the proliferation of mesenteric lymph node lymphocytes stimulated by 5 µg/ml Con A
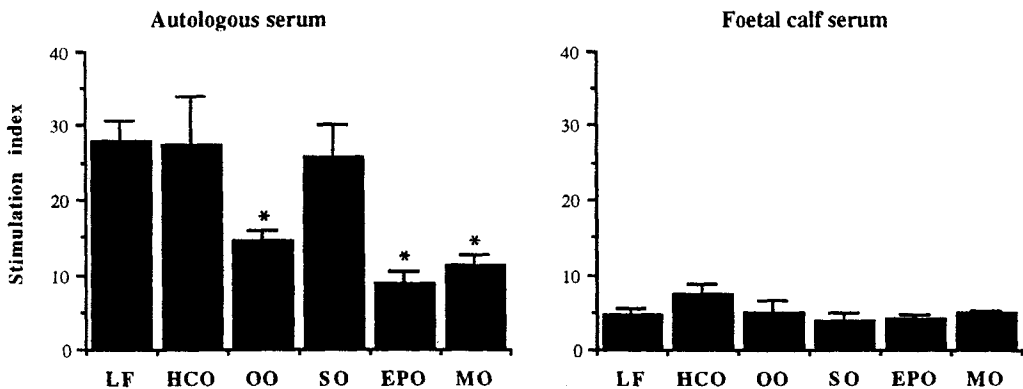
Data are mean (SEM); n 5; *significantly different (p<0.05) from LF (Student's t-test).

Proliferation of lymphocytes from mesenteric lymph nodes was suppressed by the OO, EPO and MO diets compared with the LF, HCO and SO diets; these effects were totally masked by culture in the presence of FCS (see Figure 1). All five high-fat diets inhibited the proliferation of splenic lymphocytes compared with the LF diet, but only the OO diet had a suppressive effect on thymic lymphocytes. In all cases culturing with FCS partially or totally masked the effects of dietary lipid manipulation. These results indicate that dietary lipid manipulation can affect lymphocyte proliferation and that both the serum and the composition of the lymphocyte itself contribute to this suppression.

Factors influencing caecal butyrate in rats fed on raw potato starch. By J.C. MATHERS and H. SMITH, <u>Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU</u>

Starch resistant to pancreatic α-amylase [EC 3.2.1.1] may make a significant contribution to the substrates flowing from the terminal ileum into the large bowel (LB). The objective of the present study to was investigate the effects of replacing maize starch (MS; well digested in the small intestine (SI)) with raw potato starch (RPS; expected to resist SI digestion) on LB fermentation in the rat.

Male Wistar rats initially weighing approximately 100 g were fed on 15 g/d of one of four semi-purified diets (five rats/diet) containing 0, 80, 160 or 240 g RPS and 240, 160, 80 or 0 g MS/kg diet respectively. The remainder of each diet was constant and contained (g/kg) casein 200, sucrose 410, vitamin premix 15, mineral premix 33, cellulose 50, maize oil 50 and Cr_2O_3 (an indigestible marker) 2. After 10 d adaptation, there was a balance period of 5 d and 8-10 d later the animals were killed.

Dietary RPS (g/kg)	0	80	160	240	SEM	Statistical Effects
Ileal starch digestibility	0.99	0.72	0.63	0.28	0.057	L***
Caecal mass (g)	1.9	4.2	6.2	9.5	0.45	L***
Caecal SCFA (mmol/mol total SCFA)						
Acetate	611	591	638	686	23.8	L*
Propionate	194	149	156	145	16.2	NS
Butyrate	123	208	175	146	19.9	Q*
Caecal transit time (d)	0.34	0.76	1.13	1.35	0.122	L***

SCFA, short chain fatty acids; L, Q, linear and quadratic effects of dietary RPS.
NS, not significant   * $P<0.05$ *** $P<0.001$

As expected, virtually all the MS was digested in the SI but 0.72 of the RPS flowed to the LB. This extra substrate was associated with a substantial increase in caecal mass and altered caecal fermentation pattern. With 80 g RPS/kg diet, there was a 69% increase in the molar proportion of butyrate but further increases in starch supply to the LB were accompanied by reductions in the proportion of caecal butyrate.

This study demonstrates that RPS, a rich source of RS, has the capacity to provoke marked changes in LB fermentation but the pattern of SCFA is not proportional to the change in substrate supply. It is of particular interest that the fall in molar proportion of butyrate with the higher dietary RPS inclusion rates occurred with an increase in caecal transit time (TT) confirming the potential importance of TT in controlling caecal butyrate (Mathers & Dawson, 1991).

Mathers J.C. & Dawson, L.D. (1991). *British Journal of Nutrition* 66, 313-329.

The effect of two Nigerian foods containing non-starch polysaccharides on postprandial blood glucose and insulin levels in healthy subjects. By U.A. ONYECHI[1], P.A. JUDD[1] and P.R. ELLIS[2], [1]*Department of Nutrition and Dietetics and* [2]*Division of Life Sciences, King's College London, Campden Hill Road, London W8 7AH.*

Studies have shown that addition of water-soluble non-starch polysaccharides (s-NSP) to carbohydrate-rich meals or drinks reduces the post-prandial rise in plasma glucose and insulin in healthy and diabetic subjects (Jenkins *et al.* 1976; Ellis *et al.* 1988).

Two unexploited Nigerian plant foods, *Detarium microcarpum* (DT), a legume, and *Cissus rotundifolia* (CR), a shrub, were investigated to determine their effect on post-prandial blood glucose and insulin concentrations in five healthy non-diabetic subjects. Preliminary analysis of these foods has indicated that they contain significant amounts of s-NSP, with rheological properties similar to those of guar gum.

Soup meals containing meat and vegetables (433 g cooked weight, served with boiled white rice; total available carbohydrate content 50 g) were fed to subjects in a randomized order after an overnight fast. Each subject consumed three soup meals on separate occasions: a control, soup containing *Detarium microcarpum* (DTSM) or *Cissus rotundifolia* (CRSM). The test meals contained about 8 g total NSP from the addition of DT and CR. Venous blood samples were taken at fasting and at 30, 60, 90, 120 and 150 min post-prandially and analysed for glucose and insulin. Repeated measures analysis of variance was carried out on the incremental rises of blood glucose and insulin relative to fasting.

Post-prandial plasma glucose levels were significantly decreased after consumption of DTSM and CRSM compared to the control at the post-prandial times shown in the Table. Similar reductions in plasma insulin were seen after the DTSM and CRSM compared to the control, but these were not statistically significant.

	Incremental glucose levels (mmol/l; n 5)						Incremental insulin levels (mU/l; n 5)					
	Control		DTSM		CRSM		Control		DTSM		CRSM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
30	2.6[a]	0.3	1.3[b]	0.5	1.1[b]	0.5	37.0	14.4	28.2	17.1	27.6	16.2
60	3.1[a]	0.5	1.6[b]	0.5	1.4[b]	0.5	26.6	12.0	18.4	8.3	20.9	10.4
90	2.9[a]	0.7	1.5[b]	0.6	1.6[b]	0.5	19.8	10.0	14.9	7.2	14.3	6.5
120	2.5[a]	0.6	0.6[b]	0.5	1.0[b]	0.3	14.1	7.2	11.4	5.1	11.6	6.6
150	1.6[a]	0.5	0.4[b]	0.7	0.6[b]	0.4	10.2	7.7	7.7	3.8	8.6	5.4

a,b Values with unlike superscript are significantly different (ANOVA): P < 0.05.

It was concluded that DT and CR showed marked beneficial effects in reducing post-prandial blood glucose levels and are worthy of further investigation as therapeutic aids in the management of diabetes mellitus.

Ellis, P.R., Burley, V.J., Leeds, A.R. & Peterson, D.B. (1988). *Journal of Human Nutrition and Dietetics* 1, 77-84.

Jenkins, D.J.A., Leeds, A.R., Gassull, M.A., Wolever, T.M.S., Goff, D.V., Alberti, K.G.M.M. & Hockaday, T.D.R. (1976). *Lancet* ii, 172-174.

The effect of two types of guar gum in solid and liquid foods on postprandial blood glucose, plasma insulin and C-peptide in obese subjects. By R.M. FAIRCHILD[1], P.R.ELLIS[2], R.M.F. KWAN[3], A.J.BYRNE[3] and M.A.MIR[3], *[1]School of Home Economics, University of Wales College of Cardiff, CF1 3AS, [2]Division of Life Sciences, King's College London, W8 7AH and [3]Department of Medicine, University Hospital of Wales, Cardiff, CF4 4WX*

Impaired glucose tolerance and hyperinsulinaemia are common metabolic disturbances amongst the obese population. The incorporation of low doses of guar gum into foodstuffs significantly improves postprandial glucose control of non-obese subjects in response to a carbohydrate-containing meal (Fairchild *et al.* 1990). We have investigated, therefore, the effects of guar gum flour (RG30, Hercules Ltd) and granulate (Guarem, Rybar Laboratories Ltd), in solid and liquid foods, on carbohydrate tolerance in obese subjects.

After overnight fasts, thirteen obese subjects (body mass index 39.4 (SE ± 1.7)) consumed breakfast meals (one control, three supplemented with guar gum) on separate days in random order. Each meal consisted of orange juice, toasted white bread (wheat) with butter and jam, cornflakes with whole milk and drinking water (available carbohydrate 106g, protein 17g, fat 30g, energy 3095kJ). Guarem (5.1g) was either mixed into orange juice (immediately before consumption) or incorporated into bread; guar gum flour was incorporated into bread only. Venous blood samples, taken whilst fasting and at 15, 30, 45, 60, 90, 120 and 150 min after the start of each meal, were analysed for glucose, insulin and C-peptide.

All the guar-containing meals substantially reduced the postprandial plasma glucose, insulin and C-peptide responses compared with the control. Significant reductions in plasma glucose were seen following all the guar meals. The guar flour in bread meal tended to reduce the glucose level at the latter part of the postprandial period compared with the Guarem bread and juice meals (see Table). Only the guar flour bread and Guarem bread (not Guarem in juice) meals significantly reduced the plasma insulin responses, the guar flour bread meal at 120 and 150 minutes ($P<0.05$) and the Guarem bread meal at 15 ($P<0.005$) and 90 ($P<0.05$) minutes. The guar flour bread meal also significantly reduced the postprandial C-peptide response ($P<0.05$) at 120 minutes. The Guarem in juice meal reduced the C-peptide response at 30 minutes ($P<0.05$).

Change in plasma glucose (mmol/l) from fasting values; n 13

Postprandial time (min)	15		30		45		60		90		120		150	
Meal Type	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	1.1*a*	0.3	3.1*a*	0.3	3.4*a*	0.4	3.2*a*	0.6	3.4*a*	0.6	2.7*a*	0.4	1.8	0.3
Guar gum flour in bread	0.8	0.3	2.9	0.2	3.1	0.3	2.7	0.4	2.0*b*	0.5	1.6*d*	0.4	1.3	0.3
Guarem in bread	0.4*c*	0.1	2.2*c*	0.3	2.8	0.3	2.6	0.4	2.1*b*	0.5	1.9	0.4	1.8	0.4
Guarem in juice	0.8	0.2	1.9*b*	0.4	2.5*b*	0.3	2.3*b*	0.4	2.4	0.4	1.8	0.4	1.3	0.4

Significant differences within columns, using repeated measures ANOVA: *a, b* $P<0.05$; *a,c* $P<0.001$; *a,d* $P<0.005$.

In contrast to our previous study (Fairchild *et al.* 1990) Guarem administered as a pre-meal drink is effective in reducing postprandial hyperglycaemia in obese subjects.

Fairchild, R.M., Daniels, C.E.J., Ellis, P.R., Naqvi, S.H.M., Kwan, R.M.F. & Mir, M.A. (1990). *Proceedings of the Nutrition Society* 49, 54A.

Variations in the formulation of commercially-available oral rehydration solutions intended for use in the treatment of diarrhoea. By J.B. LEIPER and R.J. MAUGHAN, Department of Environmental and Occupational Medicine, University Medical School, Aberdeen, AB9 2ZD .

The WHO and UNICEF have recommended two formulations of oral rehydration solutions (ORS) for world-wide use in the treatment of diarrhoea (glucose 111, Na^+ 90, Cl^- 80, K^+ 20, and either bicarbonate 30 or citrate 10 mmol/l). Industrialized countries, however, have not accepted a standard oral rehydration solution composition and the formulation of commercially-available solutions varies markedly. We have analysed fifty-six ORS obtained from twenty-one countries which included African, American, EEC, Mediterranean, Middle Eastern, Pacific and Scandinavian countries. The sample, although not intended to include all or even the recommended formulations from each country, was purchased from pharmacies as over-the-counter products and as such was considered representative of the commercial ORS readily available for home use. Of the fifty-six ORS examined, fifteen (27%) duplicated the WHO/UNICEF formulation and a further three (5%) differed only in the glucose concentration (100 mmol/l). Fifty-five of the solutions (98%) contained a median (range) glucose concentration of 111(9-326) mmol/l, while one had no identifiable carbohydrate source. Additional carbohydrate was present in eleven (20%) solutions (27.0(19.7-49.0) g total carbohydrate/l); this was mainly sucrose and/or fructose. The median (range) Na^+ concentration was 51 (21-90) mmol/l and Cl^- concentration was 45 (17-80) mmol/l. The median Na^+:glucose ratio of the glucose-containing solutions was 1:1.8 (1:1-1:9.3), with twenty-five (45%) solutions having ratios >1:2. All solutions contained K^+ (20(8-25) mmol/l) and a base. In fifteen (27%) solutions bicarbonate was the only base (18(10-30) mmol/l). Citrate was the only base in thirty-four (61%) of the solutions (10(7-30) mmol/l); five (9%) solutions contained both bicarbonate and citrate, five (9%) solutions contained lactate (13(4-30) mmol/l) with bicarbonate and/or citrate, and one solution contained acetate (27 mmol/l). The median osmolality (mosmol/kg) of the solutions was 304(167-442); twenty-two (39%) solutions were isotonic (306(276-310)), sixteen (29%) solutions were moderately hypotonic (236(167-266)), thirteen (23%) were marginally hypertonic (319(311-324)) and five (9%) were markedly hypertonic (435(344-442)). The pH of eighteen (32%) of the solutions was acidic (4.6(3.6-5.9)), while ten (18%) solutions were alkaline (8.2(8.0-8.9)).

Recommendations of the European Society of Paediatric Gastroenterology and Nutrition working group for the composition of ORS to be used for the treatment of European children suffering from diarrhoea suggested that moderately hypotonic (200-250 mosmol/kg) glucose-electrolyte ORS containing (mmol/l): glucose 74-111, Na^+ 60, K^+ 20 and citrate 10 would maximize water absorption and redress electrolyte imbalance (Walker-Smith, 1992). Based on these criteria, many of the ORS examined would appear to be unsuitable for the treatment of diarrhoea in children.

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The effect of food deprivation, time of exposure and type of feed on diet selection of broiler chickens. By M. COVASA and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds, LS2 9JT*

It is widely accepted that birds, when offered a free choice of energy feeds (grains) and protein concentrates, learn to balance their diets (Hughes, 1984), and that diet selection is affected by preference, experience and social interactions (Chapple & Lynch, 1986). Undernutrition, poor performance and death can result from the failure of chickens to eat a novel feed, even when it is freely available (Rogers, 1989). In order to increase the amount of wheat eaten by chickens from an early stage, we examined whether choice feeding could be influenced by the level of feed deprivation of chickens and by the length of time to which they have been previously exposed to wheat, when wheat is offered on its own or mixed with a compound feed.

Forty eight 2-week-old female broiler chickens assigned to eight groups were used for 5 weeks in a 2 x 2 x 2 factorial experiment (2 feed deprivation levels, 2 periods of feeding time, 2 types of feed: whole wheat or a mixture (whole wheat-starter crumbs; 1:1, w/w)). In the first part of the experiment, from week 2 to 4, chickens were either deprived of feed for 2h (D) or not deprived (N) and they received either whole wheat (W) or the mixture (M) for 2 or 6h. For the rest of the period chickens were allowed *ad lib* access to commercial starter crumbs. In the second part of the experiment, from week 5 to 7, all groups were offered both wheat and commercial pelleted feed in two separate troughs.

In the first part, chickens from group D ate significantly ($P<0.001$) more wheat (33.1g (SE 9.5) than group N (16.7 g (SE 6.7)). Also, chickens which received wheat for 6h had a greater intake (34.0g (SE 11.2)) than those which had wheat for 2h (15.9g (SE 8.5); $P<0.001$). The feed restriction imposed in the first part did not have any significant effect on the subsequent choice made by birds in the second part ($P>0.05$). The length of feeding time with feed W or M did not affect feed intake in the second part ($P>0.05$). The type of feed significantly affected choice feeding. Chickens previously exposed to wheat ate 13.9 (SE 7.2) g/bird of wheat compared with 4.0g (SE 1.8) eaten by those exposed to the mixture ($P<0.001$). There were no significant differences in body weight, body weight gain and feed conversion throughout the experiment ($P>0.05$). Carcass weight and abdominal fat pad were not affected by any of the treatments. The weights of the full proventriculus plus gizzard were significantly ($P<0.05$) affected by the type of feed: 42.1g (SE 3.3) for W group and 34.6g (SE 3.5) for M group. The results showed that the selection of feed by chickens is greatly influenced by the type of feed to which they have been previously exposed. When wheat is offered alone, its subsequent intake in the choice feeding paradigm is substantially greater. Thus, the consumption of wheat could be manipulated through suitable learning without affecting the growth performance.

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The effects of fibre in the breakfast upon short-term appetite control: a comparison of soluble and insoluble fibre. By H.J.DELARGY[1], V.J.BURLEY[1], K.R. 0'SULLIVAN[2], R.J.FLETCHER[2], and J.E.BLUNDELL[1] *[1]BioPsychology Group, Department of Psychology, University of Leeds, Leeds, LS2 9JT and [2]The Kellogg Company, The Kellogg Building, Talbot Road, Manchester, M16 0PU*

Meals high in dietary fibre have been shown to exert a restraining effect on the expression of appetite (Blundell & Burley, 1987). This effect can be observed as a late-acting intensification of satiety (Burley & Blundell, 1990) and for this reason dietary fibre may help meal-to-meal control over appetite. However the amount of fibre contained in a meal needs to be large in order to demonstrate these effects under scientifically controlled conditions. Although some studies have compared soluble and insoluble fibre (Stevens *et al.* 1987) little is known about the respective modes of action upon dietary patterns or the profile of hunger.

An initial study compared the effects of a minimal breakfast (juice and hot beverage) of approximately 420kJ, a low-fibre breakfast (2100kJ), and a high-fibre breakfast (2100kJ) upon energy intake at an *ad lib.* lunch 4 h later. The fibre was incorporated into a breakfast cereal, the low-fibre cereal containing 4g of fibre (soluble-insoluble, 50:50, w/w) and the high-fibre cereal containing 20g of fibre (soluble-insoluble, 50:50, w/w). The three experimental conditions were presented in a systematically counterbalanced order. Twelve healthy males (BMI range 19-26 kg/m²) were randomly assigned to each order. ANOVA revealed that there was a significant effect of breakfast type on lunchtime energy intake (F 3.65, df 2, $P < 0.05$). Energy intake at lunch was significantly suppressed by the high-fibre breakfast compared with the minimal breakfast (t 3.51, $P < 0.01$), but the low-fibre breakfast failed to produce such a suppression (t 1.15, NS). There was no significant effect of breakfast-type on total energy intake on the test days (F 0.79, df 2, NS).

A second study investigated the effects of varying the soluble:insoluble fibre ratio incorporated into a breakfast cereal upon energy intake, and subjective ratings of motivation to eat, assessed over the rest of the day. The soluble fibre used was psyllium gum and the insoluble fibre was wheat bran. The four breakfasts were equi-energetic (2100kJ) and all contained a total of 20g fibre (soluble:insoluble ratios were 8:2, 2:8, 6:4, 4:6). The study was double-blind. The breakfasts were presented in a systematically counterbalanced order. Sixteen healthy males (BMI range 19-26 kg/m²) were randomly assigned to each order. The soluble:insoluble fibre ratio consumed at breakfast produced no significant effect on energy intake at lunchtime 3.5 h later (F 0.54, df 3, NS) or on energy intake for the rest of the day (F 1.29, df 3, NS). There was a significant effect of breakfast-type on ratings of hunger (F 3.29, df 3, $P < 0.03$) and desire to eat (F 3.31, df 3, $P < 0.03$) up to 2 h after the start of breakfast: the high insoluble:soluble fibre breakfast suppressed these sensations more than the other breakfasts. At the end of the test day the subjects rated their stomachs as significantly more empty after the high insoluble:soluble breakfast than after the those containing a higher proportion of soluble than insoluble fibre (t -2.24, $P < 0.04$ and t -2.39, $P < 0.03$), and awoke the following morning having a significantly greater desire to eat (F 5.58, df 3, $P < 0.01$).

These results indicate that the total amount and type of dietary fibre can alter the pattern of food intake across the day but do not confirm an effect on total energy intake. This could have implications for the management of appetite.

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Exercise-induced anorexia in lean men. By N. A. KING[1,], V. J. BURLEY[1] and J.E. BLUNDELL[1],
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The relationship between exercise and appetite is particularly important, at a time when there is a serious interest in the theoretical and practical issues concerned with the problem of weight loss and maintenance. In two studies we have investigated the existence of exercise-induced anorexia (suppression of appetite) and examined the relationship between exercise and appetite following bouts of exercise differing in intensity, duration and overall energy expenditure.

In the first study eleven lean males were randomly assigned to each of the three trials (control, low-intensity and high-intensity treatments). After a standard breakfast subjects returned mid-morning for the appropriate treatment session (rest period, low-intensity exercise (30% $\dot{V}O_2$ max, 60 min) and high-intensity exercise (70% $\dot{V}O_2$ max, 30 min). Modulation of appetite was monitored using visual analogue scales and food intake using a post-exercise test meal. Results revealed that intense exercise induced a significant suppression of hunger during (t 3.3, df 10, $p < 0.005$) and immediately after (t 2.6, df 10, $p < 0.05$) exercise when compared with the control treatment rest period. However, this strong suppression of hunger was short lived; 15 min post-exercise there was no longer a significant difference between the intense exercise and control treatments. Although there was a tendency for a slight increase in absolute energy intake during the test meal following the low-intensity exercise session (7410 kJ), analysis of the post-exercise (and rest) test meal data revealed that there was no significant difference in either absolute energy intake or macronutrient intake. Therefore, there was no suppression of absolute food intake following the intense exercise (5949 kJ) session compared with the control treatment (5652 kJ). Even when energy expenditure is accounted for there was no significant difference between the 'relative' energy intakes. i.e the difference between absolute energy intake and the energy expenditure during the treatment sessions.

The second study used a design similar to the first except the low-intensity exercise session was replaced by a long-duration, high-intensity (70% $\dot{V}O_2$ max, 52 min) exercise session. Both the short and long intense exercise bouts induced a significant suppression of hunger during (t 3.59, df 11, $p < 0.005$; t 5.31, df 11, $p < 0.001$ respectively), and immediately after exercise (t 3.36, df 11, $p < 0.01$; t 9.83, df 11, $p < 0.001$ respectively) when compared with the control treatment. Although there was no significant difference in feeling of hunger between these two intense exercise treatments during the exercise sessions, there was a significantly greater suppression of hunger immediately after the long duration exercise session than after the short duration exercise session (t 2.99, df 11, $p < 0.05$). This reduction is short-lived and dissipates rapidly (15 min) and again fails to effect absolute energy intake in the post-exercise test meal. Analysis of the onset of eating times revealed that there was a significant delay in subjects beginning to eat following both the long-duration (t 2.95, df 11, $p < 0.05$) and short duration (t 3.51, df 11, $p < 0.01$) exercise sessions when compared with the control treatment. Despite the absence of an absolute reduction in energy intake further calculations revealed that relative to the energy expended the long-duration, high-intensity exercise session did produce a significant short-term negative energy balance. Therefore, once energy expenditure was accounted for the remaining 'relative' energy intake during the long-duration, high-intensity exercise treatment was significantly lower than the control (t 4.55, df 11, $p < 0.001$) and the short duration exercise (t 3.42, df 11, $p < 0.01$) treatments.

In summary, exercise-induced anorexia does exist and it can be characterized by a suppression of the feeling of hunger (followed by a rapid return), a delay to onset of eating and a relative suppression of food intake, at least in the short-term. These features are influenced by both the duration and intensity of exercise. It is also necessary to make the distinction between absolute and 'relative' energy intake when referring to exercise-induced anorexia.

The prevalence of overweight and obesity in different fat and sugar consumption groups. By C. BOLTON-SMITH and M. WOODWARD[a], Cardiovascular Epidemiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY. [a]Honorary Research Fellow.

A reduction in total fat intake to around 30 % energy is the most uniformly recommended nutritional advice for the improvement of health and the prevention of disease. Numerous data suggest that this is unlikely to be achieved in conjunction with a low dietary sugar intake (Gibney, 1990). A high-fat diet has been suggested to predispose to overweight and obesity based on differential energy utilisation in controlled metabolic studies (Flatt, 1987).

The fat:sugar ratios of the diets of 5,768 men and 5858 women aged 25-65 years from the cross-sectional Scottish Heart Health and MONICA studies were examined with respect to the prevalence of overweight (BMI > 25) and obesity (men BMI > 30; women BMI > 28.6). Diet was assessed by food frequency questionnaire, as reported previously (Bolton-Smith *et al.* 1991). Food sugars were assigned to intrinsic or extrinsic categories based on MAFF guidelines: namely, intrinsic sugar was that present naturally in whole fresh fruits, vegetables and grains, plus 50 % of the analysed sugars present in the same cooked, pulped or otherwise processed foods, extrinsic sugar (non-milk) was that sugar not naturally occurring within the intra-cellular structure of foods, plus 50 % of the sugar in refined or processed fruits, vegetables and grains. The Table shows the prevalence (%) of obesity by fifths of nutrient intakes expressed as percentage of total energy intake.

Intake 5ths..	Men					Women				
	1	2	3	4	5	1	2	3	4	5
ES	18.5	12.9	11.7	8.1	4.5‡	25.2	21.0	20.7	19.0	12.8‡
IS	10.0	11.3	10.6	11.0	13.8*	20.3	17.7	20.3	20.2	21.9
Fat	9.1	9.3	8.9	13.6	18.0‡	16.0	18.5	22.1	23.4	25.5‡
Starch	11.7	12.5	12.8	10.5	11.1;	21.1	20.4	22.2	20.9	19.7
F:TS	5.8	7.9	10.1	13.8	19.1‡	13.8	17.1	20.6	21.3	27.4‡

Intake fifths: 1, lowest; 5, highest. ES, extrinsic sugars; IS, intrinsic sugars; F:TS, fat:total sugars ratio;
Prevalence of obesity differs significantly between intake fifths (*Chi-squared test*): • P < 0.05; ‡ P< 0.001

The greater prevalence of obesity, and similar trends for overweight, with lower dietary total carbohydrate and higher fat:total carbohydrate ratio (data not shown) are primarily due to the extrinsic sugar component of the diet as shown by the far steeper changes in prevalence of obesity between intake fifths of the extrinsic sugar variables. The fat:sugar ratio was a significant independent predictor of BMI in analysis of variance with adjustment for age, total energy intake, smoking and alcohol consumption for men and for women.

These data support the hypothesis that dietary fat intake predisposes to obesity independently of total energy intake. The strong inverse association between obesity and extrinsic sugar intake may be due to the reported difficulties of the UK population to exchange fat energy for the bulkier starch-rich foods, with the result that a low-fat, low prevalent obesity, diet is relatively high in sugars. Health messages to reduce both fat and sugars in the diet may thus be difficult to achieve in this population.

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Nutritional composition (by chemical analysis) of home-prepared weaning foods for infants. By JANE B. MORGAN, ALISON M. REDFERN and B. JACQUELINE STORDY, <u>School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH</u>

One of the nutritional aims of introducing solid food to the diet of infants is to increase nutrient density thereby providing adequate supplies of energy and protein without including excess Na. To this end compositional (and labelling) standards have been established for processed cereal-based foods and baby foods for infants and young children (European Community (EC) draft directive, 1991). Little is known about the nutritional composition (by chemical analysis) of infant foods prepared in the home.

A study of weaning among 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 savoury meal. The meals were analysed (by NAMAS accredited methods) for fat, protein (N x 6.25), total carbohydrate and Na at Campden Food and Drink Association. Total energy was calculated from protein, fat and carbohydrate using the energy conversion factors used in food labelling. (Holland *et al.* 1991). We present the results of our analyses of 108 savoury meals classified according to the age of the infant (Table). Of the meals, 48 % had an energy density less than human milk (290 kJ/100 ml), 9.6% exceeded the EC standard for fat content (1.1 g/100 kJ), 17.5 % failed to meet the EC standard for protein (0.7 g/100 kJ) and 23.8 % exceeded the EC standard for Na (200 mg/100 g).

| Age (months) | 4-6 | 7-9 | 10-12 | Proposed EC |
Nutrient	Mean	Mean	Mean	Directive
Energy (kJ/100 g)	300	320	455	
Fat (g/100 kJ)	0.37	0.51	0.73	1.1
Protein (g/100 kJ)	1.3	1.32	1.21	0.7
Na (mg/100 g)	79.6	141.7	203.0	200

These data reveal that the energy density and protein content of many home-prepared meals is surprisingly low. There was some indication that energy density and Na content increased in the meals for older infants compared with the younger ones. However, the Na content of some of the meals for younger infants was high. Later estimations of the 24 h records of total energy and nutrient intake of which the meal was a part, will reveal whether these findings reflect overall nutrient supply.

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The nutrient contribution of weekday lunch in 7 to 8-year-old children. By C.H.S. RUXTON[1], T.R.KIRK[1], N.R. BELTON[3] and M.A.M. HOLMES[2], [1]Department of Dietetics and Nutrition and [2]Department of Management and Social Sciences, Queen Margaret College, Edinburgh EH12 8TS and [3]Department of Child Life and Health, Edinburgh University, Edinburgh EH9 1UW

Little has been published on the contribution of school and other weekday lunches to the diets of primary school children since Nelson & Paul (1983) reported work carried out before the removal of school lunch nutritional standards. The present study reports a comparison between the mean nutrient content of school lunches (SL, n 336), home prepared packed lunches (PL, n 256) and home lunches (HL, n 85) eaten by 136 7 to 8-year-olds and shows the percentage contribution of each to overall mean nutrient intake (%CON). The methodology has been described elsewhere (Ruxton et al, 1993).

Nutrient	SL	%CON	PL	%CON	HL	%CON
Energy (MJ)	1.78	22.5	2.12	32.7	1.86	26.2
Protein (g)	12.5	23.8	12.8	29.3	13.9	28.5
Total (g)	51.2	22.8	70.4	33.7	56.0	24.4
Total CHO (g)	22.3	24.4	31.7	33.3	26.6	23.7
Total sugar (g)	24.8	20.8	37.4	33.7	25.1	22.6
NSP (g)	1.6	22.3	2.6	36.4	1.8	23.8
Ca (mg)	145.8	17.0	196.8	31.8	211.4	21.9
Fe (mg)	1.8	18.8	2.2	25.9	2.1	27.2
Vitamin A (μg)	102.5	17.4	143.5	26.1	122.4	21.0
Vitamin B6 (mg)	0.3	22.5	0.2	24.4	0.2	17.2
Nicotinic acid eq. (mg)	5.3	23.0	5.6	27.5	4.9	22.1
Vitamin C (mg)	17.3	19.5	24.3	31.4	6.0	19.9
%Energy as fat	42.3		36.4		39.0	
%Energy as CHO	45.4		53.2		48.2	

CHO, carbohydrate; NSP, non-starch polysaccharide

Using a Sign test, PL were found to be higher in energy ($p < 0.01$), total carbohydrate ($P < 0.0001$), starch ($P < 0.0001$), total sugars ($P < 0.001$), non starch polysaccharide ($P < 0.0001$) and iron ($P < 0.01$) than SL. HL were lower in vitamin C ($P < 0.001$) and B6 ($P < 0.01$) than SL and lower in vitamin C ($P < 0.01$) than PL. SL had a higher percentage energy (%E) from fat ($P < 0.01$) and a lower percentage energy from carbohydrate ($P < 0.05$) than PL, probably due to a more frequent inclusion of bread products in the PL. PL contributed more energy ($P < 0.01$), protein ($P < 0.05$), total carbohydrate ($P < 0.0001$), starch ($P < 0.0001$), total sugar ($P < 0.001$), NSP ($P < 0.0001$), Ca ($P < 0.001$), Fe ($P < 0.01$), vitamin A ($P < 0.01$) and nicotinic acid equivalent ($P < 0.01$) to the overall diet than SL and more vitamin C ($P < 0.001$) than HL. SL contributed more vitamin B6 ($P < 0.01$) and less vitamin C ($P < 0.001$) than HL.

Nelson & Paul (1983) concluded that school lunches contributed less to mean daily nutrient intake than other mid-day meals. This statement is also true for the data shown here. Packed lunches made the greatest contribution to total nutrient intake for most nutrients while %E as fat and %E as CHO in the packed lunches were closest to the Department of Health (1991) recommendations of 35% and 50% respectively.

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Fat substitutes: their potential effect on the fat content of the diet. By H. J. LIGHTOWLER and G. J. DAVIES, School of Hospitality, Food and Product Management, South Bank University, Borough Road, London SE1 0AA

Concern has been expressed regarding the amount of fat in the diet, and in particular saturated fat (Department of Health, 1991). A small quantitative survey was undertaken to assess the potential use of foods incorporating low-fat substitutes in their ingredients, as a means of reducing the percentage of energy from fat and especially saturated fat.

The subjects in this study comprised ten females and ten males, matched for age and occupational category. All food and drink consumed over a period of 3 consecutive days, to include 2 weekdays and 1 weekend day, was weighed and a record kept of the amount eaten. The fat content of the diets was analysed using a nutritional analysis programme. A modelling exercise was then undertaken whereby fats used as a spread, yogurt and ice cream were replaced using three food products that incorporated a low-fat substitute, Simplesse, a fat alternative made from milk whey protein, in their ingredients. The fat content of the diets was re-analysed and comparisons made between before and after substitution. The percentage of energy from fat before and after substitution is shown in the Table.

	Before		After	
	Mean	SEM	Mean	SEM
Energy (MJ)	12.5	0.9	11.9	0.9
Percentage of total energy from:				
Total fat	33.2	1.1	28.9	1.0
Saturated fat	11.7	0.8	9.4	0.5
Polyunsaturated fat	3.8	0.5	2.9	0.3
Monounsaturated fat	9.0	0.5	7.7	0.4

The modelling exercise demonstrated that foods containing fat substitutes have potential for reducing the fat content of the diet, with little impact on total energy intake. It should be noted, however, that the fat intakes of the sample studied were already within dietary recommendations and not representative of the population (Gregory et al, 1990). The inherent assumption of the modelling, namely that diets would remain the same except for the substitution, may not be valid for consumers with unrestricted choice of foods and diets.

Further work is necessary to evaluate the food products with specific reference to acceptability and availability.

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