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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at the University of Aberdeen on Monday–Friday, 10–14 July 1995, at which the following papers were presented.

Food consumption patterns and misclassification of total fat intake of individuals. By A.D. CARVER and A.E. DE LOOY. Department of Dietetics and Nutrition, Queen Margaret College, Edinburgh, EH12 8TS

The objective of the present study was to develop and validate a shortened method of assessment of dietary fat intake that can be used in Primary Health Care settings by staff with little nutritional expertise to identify individuals with high fat intakes, advise them regarding change and track changes in diet. Diaries of 7-d weighed intakes of 69-middle aged men were examined to derive a scoring system based on the ratio of consumption of complex carbohydrates : high-fat foods. One *'grain'* point was scored each time bread, rice, pasta or potato was consumed. Meat, meat products, fish, egg, cheese and alternatives were categorised as either high, medium or low in fat and scored three, two or one point respectively with the sum giving the *fatscore*. Dividing the total *grain* by the *fatscore* gave *grainbyfat*. The relationships between scores and calculated macronutrient intakes were examined by the Pearson Correlation coefficient. Comparison was made between scores of high, medium and low fat consumers (%energy as fat) using the Student's *t* test. Misclassification was examined by cross-tabulation of tertiles and calculation of the kappa statistic.

The results showed that despite a good relationship between scores and calculated intakes (r 0.45, $P<0.001$) and highly significant differences between scores of high and low fat consumers there were still high levels (12%) of misclassification with kappa < 0.4 indicating poor agreement. This may be related to the relatively low correlation (r 0.42, $P<0.001$) between absolute fat intake (g/d) and % food energy from fat.

	High *grainbyfat* score	Medium *grainbyfat* score	Low *grainbyfat* score
High fat (>41.2% energy as fat)	4	7	12
Medium fat	7	9	7
Low fat (<37.2% energy as fat)	12	7	4

These errors in classification could be clinically significant resulting in reassurance being given to high fat consumers. We have further examined diaries to see how patterns of food choice of individuals who are misclassified can be identified to modify the tool. The subjects who had high *grnbyfat* scores and high percentage energy as fat had high intakes of cakes, puddings and chocolate biscuits. The subjects who had low *grainbyfat* scores and low percentage energy as fat had high intakes of sugar, soft drinks and confectionery.

This tool, together with others published, identifies group characteristics but fails at the individual level in an important proportion of subjects.

Investigation of the action of a preload of incorporated or added fat on appetite ratings and food consumption. By A. SANTANGELO and N. W. READ, *Centre for Human Nutrition, Northern General Hospital, Sheffield S5 7AU*

Studies in human volunteers have suggested that the interaction of fat with gastrointestinal receptors may suppress hunger and induce satiety. This action of meals containing fat, however, may be affected by the learned expectation of satiation as well as by the extent to which the fat is bound within the food.

The aim of the present study was to compare the effect on appetite ratings and food consumption of two preloads of sponge cake of identical energy and macronutrient composition (energy value 3238 KJ; fat content 43.6 g; carbohydrate content 84.4 g; protein content 11.1 g; total weight 200 g), one in which the fat was incorporated in the sponge matrix (51 % of energy as incorporated fat), the other in which the fat was added as butter icing (41 % of energy as added fat and 10 % of energy from the cake). Paired studies (separated by one week) were carried out on six healthy volunteers (three male and three female, 23-28 years old, normal weight, non smokers and not restrained eaters) to investigate the effect of the preload (served at 08.30 hours with 300 ml water) on self-reported appetite ratings (100 mm visual analogue scales, VAS) and on the intake of a test meal 4 h later (direct measurement). The two preloads had similar sensory properties and did not differ in the taste (72.0 (SE 1.8) *v.* 64.0 (SE 3.7); P=0.114), expected energy content (77.0 (SE 5.0) *v.* 78.3 (SE 2.1); P=0.759) and expected fat content (70.0 (SE 5.6) *v.* 75.7 (SE 3.3); P=0.283) recorded on VAS by the subjects during their consumption. The test meal was a buffet style, self-selection meal that allowed *ad libitum* consumption of a variety of thirteen different foods and two different drinks.

Analysis of appetite ratings 240 min after each preload revealed a significant difference between the incorporated and added fat preloads as shown in the table. The energy intake at lunch-time was higher after the preload with incorporated fat (3987 (SE 498) KJ) compared with the preload with added fat (3552 (SE 393) KJ) but this difference was not significant.

	Fullness (mm)		Hunger (mm)		Desire to eat (mm)		Prospective consumption (mm)	
Preload	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Incorporated fat	11.7	5.8	69.2	9.5	68.7	8.6	68.2	8.4
Added fat	29.8	6.0	55.3	8.7	57.3	7.5	61.5	8.4
P value	0.010		0.008		0.022		0.050	

In conclusion, added fat preload reduces hunger, desire to eat and prospective consumption, increases fullness and tends to reduce food intake compared with the incorporated fat preload. These results are compatible with the hypothesis that added fat may have more contact with intestinal receptors and increases the duration of satiety.

Macronutrients, appetite and day-to-day food intake in humans. By A.M. JOHNSTONE, R.J. STUBBS and C.G. HARBRON, *Rowett Research Institute and SASS, Bucksburn, Aberdeen AB2 9SB*

Work on free-living subjects suggests that protein is more satiating than carbohydrate (CHO) which is more satiating than fat. A 7-day calorimeter study (Stubbs *et al.* 1993) suggested that protein and CHO balance and stores showed a negative relationship to the subsequent day's intake, whereas fat oxidation, but not stores, exhibited such a relationship. The aims of the present study were (i) to test whether overfeeding isoenergetically dense high-protein, high-CHO or high-fat diets would differentially influence appetite on the same day, and (ii) to determine whether changes in nutrient balance would influence the subsequent day's food intake.

Six men (mean weight 74 (SD 4) kg, BMI 24 (SD 2) kg/m^2) were each studied three times on a 5-d protocol. On days 1 and 2 they were given a medium fat (MF) maintenance diet (comprising 40:47:13% fat, CHO and protein by energy) calculated at 1.6 x resting metabolic rate (RMR). On day 3, they ate a MF diet at 1.5 x RMR with an additional 0.6 x RMR as protein, fat or CHO, giving a total of 2.1 x RMR. On days 4 and 5 (outcome days), subjects had *ad libitum* access to isoenergetically dense MF (40:47:13) meals (550 kJ/100 g). Nutrient composition was calculated using the values from Holland *et al.* (1991). Subjects entered the calorimeter at 08.00 hours on day 3 for 48 h. Fat and CHO oxidation rates were calculated from non-protein gaseous exchange, using the coefficients of Livesey & Elia (1988), protein oxidation was estimated from urinary N excretion. Intakes were monitored throughout day 5 on leaving the chamber. Subjective sensations of hunger, appetite and satiety were tracked hourly during waking hours. Results are shown in the table below.

	High protein (HP)				High carbohydrate (HC)				High fat (HF) [MEAN]			
	Energy (MJ)	Protein (MJ)	CHO (MJ)	Fat (MJ)	Energy (MJ)	Protein (MJ)	CHO (MJ)	Fat (MJ)	Energy (MJ)	Protein (MJ)	CHO (MJ)	Fat (MJ)
Day 3	Manipulation day											
Intake	15.3	5.7	5.2	4.4	15.1	1.5	9.2	4.4	15.3	1.6	5.2	8.8
Oxidation	11.4	2.9	3.9	4.5	11.0	1.3	6.2	3.5	11.3	1.3	4.4	5.6
Balance	3.8	2.7	1.2	-0.2	4.1	0.3	3.0	0.8	4.0	0.3	0.8	3.2
Day 4	*Ad libitum* day											
Intake	13.8	1.7	6.2	5.8	12.7	1.6	5.7	5.4	12.1	1.5	5.5	5.0
Oxidation	11.0	2.4	4.5	4.2	11.3	1.1	5.9	4.3	11.1	1.2	4.6	5.4
Balance	2.7	-0.7	1.7	1.6	1.4	0.5	-0.2	1.0	0.9	0.4	0.9	-0.4

Analysis of variance confirmed that, by the end of day 3 there was a significant positive balance of each overfed nutrient v. the other two diets ($P<0.01$). Throughout day 3, subjects felt significantly less hungry and more full on the HP diet relative to the other two diets ($P=0.002$). On this day there was a clear suppression of hunger on the HP diet which was partially related to accelerated protein oxidation. These changes in nutrient oxidation and balance were not of significant duration to influence subsequent day's energy intake. Increased oxidation of the overfed nutrient continued on day 4 which was greater for protein ($P<0.001$) than CHO ($P=0.07$) or fat ($P=0.1$).

This data suggests that HP diets are more satiating than isoenergetically dense HC or HF diets. Protein appeared to exert an acute within-day effect that did not influence intakes on the subsequent day. Large changes in nutrient balance produced on 1 d appear to be poorly compensated for by changes of energy intake on a subsequent day in men.

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The gastrointestinal handling and metabolic disposal of [1-^{13}C]palmitic acid in patients with cystic fibrosis. By J.L. MURPHY[1], A.E. JONES[1], R. H. TRENEER[1], A. HOUNSLOW[1], B. ZORICH[2] and S.A. WOOTTON[1], *[1]Institute of Human Nutrition, University of Southampton and [2]Department of Child Health, Southampton General Hospital, Southampton SO16 6YD*

Balance studies have shown that gross faecal lipid and energy losses in patients with cystic fibrosis (CF) on their habitual pancreatic enzyme replacement treatment (PERT) remain substantially elevated. This may contribute towards an energy deficit sufficient to limit growth or cause weight loss (Murphy *et al.* 1991). Whilst attention has been directed towards understanding and correcting pancreatic insufficiency, little is known of the extent to which the absorption of dietary lipids and their metabolic handling may be altered in CF. The purpose of the present study was to employ stable-isotope tracer methodologies to examine the gastrointestinal handling and metabolic disposal of [1-^{13}C]palmitic acid in CF.

Following an overnight fast, thirteen patients with CF (six males and seven females; aged 5-22 years) and sixteen healthy controls (eight males and eight females; aged 5-16 years) ingested [1-^{13}C]palmitic acid (10 mg/kg) with a standardized test meal (1660 kJ) of low natural ^{13}C abundance. Breath samples were collected before and during a postprandial period (fasting) for at least 6 h and then after 10 h and 24 h. Whole-body breath CO_2 excretion was measured by indirect calorimetry (Deltatrac, Datex Instrumentarium Corp., Helsinki, Finland) before and at hourly intervals for at least 6 h after label administration. A baseline stool sample and all stools passed were collected for a 3 d period. Enrichment of $^{13}CO_2$ on breath and ^{13}C in stool samples was analysed by mass spectrometry (ABCA and ANCA systems, Europa Scientific Ltd., Crewe). The results shown are for ^{13}C excretion in stool as a percentage of the administered label (% admin dose) and ^{13}C excretion on breath $^{13}CO_2$ corrected for faecal loss of ^{13}C substrate (% absorbed dose).

	Stool ^{13}C (% admin dose)		Breath $^{13}CO_2$ (% absorbed dose)	
	CF	Controls	CF	Controls
Median	3.2***	21.1	33.2*	51.3
Min	1.2	10.7	9.4	28.4
Max	11.6	64.9	83.1	82.2

Significantly different from controls (Mann-Whitney U); *$P<0.05$, *** $P<0.0001$

In CF significantly less of the ^{13}C label was excreted in stool than in controls ($P<0.0001$) with a smaller difference observed between individuals. The proportion of absorbed ^{13}C label excreted on breath $^{13}CO_2$ differed markedly in both groups although it was reduced in CF compared with controls ($P<0.05$). This study suggests that in CF there may be preferential absorption of [1-^{13}C]palmitic acid (when ingested as a free acid). Whilst the postprandial oxidation of absorbed [1-^{13}C]palmitic acid varied substantially within the CF group, it was less than that observed in controls.The assumptions underlying these observations need to be examined by characterizing the nature of ^{13}C-label in stool.

The support of The Cystic Fibrosis Trust and Scientific Hospital Supplies Ltd is gratefully acknowledged.

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The gastrointestinal handling and metabolic disposal of [1-^{13}C]palmitic acid in normal, healthy children. By A.E. JONES, R.H. TRENEER, J.L. MURPHY and S.A. WOOTTON, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

The current dietary recommendations for adults to reduce total lipid and saturated fatty acid (SFA) intake have been indiscriminately applied to children from the age of 5 years (Department of Health, 1994).This assumes that the metabolic handling of SFA is similar in both children and adults although there have been no studies which directly examine this assumption. The present study utilized stable isotope tracer methodology to examine the gastrointestinal handling and metabolic disposal of [1-^{13}C]palmitic acid in normal, healthy children.

Following an overnight fast twelve children (five boys and seven girls; 5-10 years) ingested [1-^{13}C]palmitic acid (10 mg/kg) incorporated within a standardized test meal (1660 kJ) of low natural ^{13}C abundance. Breath samples were collected for measurement of ^{13}C enrichment before and at hourly intervals for 10 h following label administration and again at 24 h. Whole-body breath CO_2 excretion was measured by indirect calorimetry (Deltatrac, Datex Instrumentarium Corp., Helsinki, Finland) at the same time points. A baseline stool sample and all stools passed over a 3 d period marked by carmine administered with the test meal and on rising on day 4 were collected. ^{13}C enrichment was analysed by mass spectrometry (ABCA and ANCA systems, Europa Scientific Ltd., Crewe). The excretion of ^{13}C within stool expressed as a percentage of administered label and within breath as $^{13}CO_2$, expressed as a percentage of administered and absorbed label, which takes into account stool losses, were compared with results obtained in a group of six healthy, lean women (Murphy *et al.* 1995) and are shown in the Table as the median and range.

	Stool (% administered)		Breath (% administered)		Breath (% absorbed)	
	Median	Range	Median	Range	Median	Range
Boys	17.3	11.6 - 36.1	52.6	27.6 - 70.7	67.4	36.1 - 82.2
Girls	15.2	10.7 - 25.9	43.4	26.1 - 62.9	54.1	30.7 - 80.3
Children	16.3	10.7 - 36.1	47.0*	26.1 - 70.7	55.5*	30.7 - 82.2
Lean women	10.7	7.5 - 33.7	21.3	15.4 - 28.9	24.9	22.0 - 31.1

*Significantly different from lean women (Mann-Whitney U); $P<0.05$.

There was no difference in ^{13}C excretion within stool or breath between boys and girls. Compared with the lean women there was no significant difference in the excretion of ^{13}C within stool but the excretion of ^{13}C within breath was significantly greater in children when expressed both as a percentage of administered and absorbed label. There was also a difference in the breath ^{13}C-excretion time course profile. In the women one peak occurred 4-9 h after label administration. In five out of twelve children a double peak occurred, the first at 2-3 h and the second at 5-6 h after label administration. These results would suggest that the gastrointestinal handling of [1-^{13}C]palmitic acid is similar in normal, healthy children and lean women but that the metabolic disposal of absorbed [1-^{13}C]palmitic acid is different, with a greater proportion being oxidized rather than retained in children compared with lean women. The greater oxidation of the [1-^{13}C]palmitic acid probably reflects the greater relative metabolic demand for energy in children than adults.

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COMPARISONS OF METABOLIC RESPONSES TO DIGESTIBLE AND PARTLY INDIGESTIBLE STARCHES IN HEALTHY HUMANS, *By L. ACHOUR, B. FLOURIÉ, F. BRIET, C. FRANCHISSEUR, F. BORNET, J.C. RAMBAUD and B. MESSING, INSERM U290, hôpital saint-Lazare, 75010 et Éridania Béghin-Say, 75008, Paris, France*

Starch is the main energetic fuel in the human diet. Most starches are extensively digested in the human small intestine, and only a small fraction reaches the colon. The digestion and absorption are dependent on the starch treatment (Englyst & Cummings, 1987). It is now technologically possible to modify starch in order to slow down its digestion in the small intestine. The digestion of technologically modified (retrograded) starch will start in the small intestine and continue in the colon, where its fermentation releases short-chain fatty acids (mainly acetate) and gases (H_2, CO_2). The aim of the present work was to study the metabolic consequences of this shift in starch digestion. For this purpose we measured certain metabolic indices in healthy humans consuming a highly digestible maize starch, and the same maize starch after retrogradation, after which it is 50% digested in the human small intestine and completely fermented in the colon (Molis *et al.* 1992).

Eight healthy volunteers were studied during two periods separated by 1 week. In each period, fasting volunteers consumed, at 08.00 hours, the test meal containing either the digestible or retrograded maize starch; blood and breath were sampled in the absorptive period hourly for 8 h. The same meal was given again on the same day at 22.00 hours; and at 08.00 hours on the next morning, i.e. 10 h after the ingestion of the test meal, blood and breath were sampled in the fasting subjects hourly for 3 h i.e. in the post-absorptive period.

In the absorptive period (0 – 8 h), the areas under the curves of blood glucose (12.3 (SE 0.6) *v.* 11.0 (SE 0.7) mmol/l) and insulin (65.7 (SE 6.7) *v.* 38.6 (SE 3.9) mU/l) were higher ($P<0.05$), but blood acetate and free fatty acid (FFA) concentrations, and hydrogene excretion were not significantly different after the ingestion of digestible starch compared with retrograded starch. In the post-absorptive period (10 – 13 h after meal ingestion), blood glucose, insulin and FFA concentrations were not significantly different, but blood acetate concentrations (474 (SE 43) *v.* 297 (SE 54) μmol/l), breath H_2 excretion (39 (SE 7) *v.* 1 (SE 1) delta ppm) and $^{13}CO_2$ expired (16.9 (SE 1.1) *v.* 9.1 (SE 1.6) % of ingested ^{13}C amount) were significantly higher ($P<0.05$) after the ingestion of retrograded starch than after ingestion of digestible starch, whereas blood glycerol concentrations were significantly higher (0.51 (SE 0.08) *v.* 0.38 (SE 0.05) mmol/l; $P<0.05$) after the ingestion of digestible starch than after retrograded starch.

It is concluded that in healthy humans, the digestion of retrograded maize starch is slow in the small intestine and its colonic fermentation continues 10 – 13 h after its ingestion. Compared with the highly digestible maize starch, the shift in starch digestion induced by retrogradation leads to changes in metabolic responses: retrograded maize starch reduces the glycaemic and insulineamic responses in the absorptive period, and lipolysis in the post-absorptive period. This last effect could be related to an inhibitory action on the lipolysis of short- chain fatty acids produced during the colonic fermentation of unabsorbed starch.

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Measurement of unabsorbed dietary carbohydrates and their fermentation products following the ingestion of ^{13}C carbohydrates in patients with malabsorption syndrome. By F. BRIET, B. FLOURIE, L. ACHOUR, M. MAUREL, J.C. RAMBAUD and B. MESSING, *Unité de recherche sur les Fonctions Intestinales, le Métabolisme et la Nutrition, INSERM U. 290, Hôpital S' Lazare, 75010 Paris, France*

Dietary carbohydrates (CHO) which are not absorbed in the small intestine are fermented in the colon, producing gases (CO_2, H_2), short-chain fatty acids and bacterial mass. Currently, there is no available method to determine precisely the amount of unabsorbed dietary CHO and their fermentation products excreted in stools. In the present work, we developed a clinical test for assessing the malabsorption of dietary CHO together with their fermentation products through the digestive tract. For this purpose, patients with a malabsorption syndrome and normal subjects were given orally CHO naturally enriched with ^{13}C and we measured the excretion of ^{13}C in stools and $^{13}CO_2$ in breath. Nine patients with short bowel syndrome (SBS) (length 73 (SD 40) cm) with (*n* 7) or without (*n* 2) remaining colon in continuity and 8 healthy volunteers (HV) ingested a test meal after an overnight fast (3318 KJ, protein-fat-CHO, 17:26:57 % energy) containing naturally ^{13}C enriched CHO in the form of 50 g maize starch (257 μmol ^{13}C) and 50 g sugar cane (233 μmol ^{13}C) and carmine red as a recovery marker. Patients consumed a low-^{13}C diet and stools were collected 1 d before and 3 d after the test-meal. Breath samples were collected and VCO_2 was measured using indirect calorimetry hourly for 6 h after test-meal ingestion. In breath, in the pre -meal and red post-meal stools, ^{13}C enrichment was measured by an isotope ratio mass spectrometer. Faecal and breath ^{13}C enrichment was corrected for the basal pre-meal value.

	Excess faecal output of ^{13}C (μmol)		% of ingested dose recovered in stools		% of ingested dose exhaled in breath /6 h	
	Mean	SD	Mean	SD	Meam	SD
SBS	122	88	25	18	29	9
(range)	(6-245)		(1-50)		(16-40)	
HV	6	7	1	1	25	2
(range)	(0-16)		(0-3)		(20-29)	

The excess faecal output of ^{13}C in SBS was inversely related to the $^{13}CO_2$ exhaled in breath for 6 h following the ingestion of the test meal (Spearman correlation Rho=-0.9, $P<0.01$).

It is conclued that a faecal measurement of ^{13}C from dietary CHO is a useful tool for assessing together the fate of CHO and their fermentation products through the digestive tract of patients with malabsorption syndrome. In addition, our preliminary results show that a 6 h breath $^{13}CO_2$ test could avoid stool collection.

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Hyperosmolality of apple juice is not the main factor which promotes water secretion in the intact human jejunum. By J.B. LEIPER[1], F. BROUNS[2] and R.J. MAUGHAN[1]. *[1]Department of Environmental and Occupational Medicine, Aberdeen University, AB9 2ZD and [2]Nutritional Research Centre, Limburg University, The Netherlands*

Consumption of apple juice is now recognized as being potentially cathartic (Hyams & Leichner, 1985). At present it is not clear what factor produces the diarrhoea in susceptible people. The osmolality of a solution has a marked effect on net water movements across the intestine: carbohydrate solutions which are moderately hypo-osmotic or iso-osmotic to normal human serum promote net water absorption, while solutions which have a high osmolality tend to produce net water efflux into the intestinal lumen. We have previously demonstrated that hyper-osmotic soft drinks, including apple juice, promote net water efflux in the perfused human jejunum (Leiper & Maughan, 1992). However, when one of the soft drinks was diluted 50:50 with water, the resulting iso-osmotic jejunal mixing segment aspirates (mean osmolality 301 mosmol/kg) reversed the median (4.6 (range -24.4 to 8.9) ml/cm per h) net water efflux produced by the mixing segment aspirates of the undiluted drink (mean osmolality 496 mosmol/kg) to a net median absorption (1.5 (range -6.0 to 9.7) ml/cm per h).

The present study investigated whether dilute apple juice with an osmolality similar to that of human serum would promote net water absorption in the human jejunum. Water transport from a sports drink (A), mineral water (B) and apple juice diluted 50:50 with mineral water (C) was examined in nine healthy men using a steady-state jejunal perfusion technique (Leiper & Maughan, 1987). After passage through the mixing segment, the mean osmolality of both solutions A (279 (SD 15) mosmol/kg) and C (296 (SD 26) mosmol/kg) was essentially the same as that of human serum while solution B was markedly hypo-osmotic (64 (SD 46) mosmol/kg). Following *in vitro* acid hydrolysis, the mean glucose content of solution A (151 (SD 29) mmol/l) was greater than C (67 (SD 25) mmol/l), while the mean Na concentrations of solutions A (46 (SD 13)), C (37 (15)) and B (34 (19) mmol/l) were similar. Whereas net water absorption occurred when the iso-osmotic sports drink was perfused (median 3.4 (range 1.2 to 19.3) ml/cm per h), the hypo-osmotic mineral water produced no net movement of water across the mucosal wall (median 0.1 (range -6.9 to 2.1) ml/cm per h). Aspirates of the apple juice diluted with mineral water, although iso-osmotic to serum, produced a median efflux of water (6.6 (range -34.9 to 2.2) ml/cm per h).

This study suggests that the hyperosmolality of apple juice is not the only factor producing the net efflux of water into the intestine. A solution which was iso-osmotic to human serum, with glucose and Na contents similar to those of the mixing segment aspirates of the dilute apple juice used in this study, would be expected to promote a reasonable rate of water absorption in this model. The non-absorbable carbohydrates contained in apple juice do not appear to have a major influence in producing diarrhoea in susceptible people (Kneepkens *et al.* 1986). The cathartic response to apple juice is therefore not mainly due to an osmotic effect but is presumably due to a secretagogue. The identity of this secretagogue and whether it is present in other commonly ingested fruit juices remains to be determined.

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An epidemiological investigation into the influence of diet on bone mass. By SUSAN A. NEW[1], CAROLINE BOLTON-SMITH[2], DAVID A. GRUBB[3] and DAVID M. REID[1], [1]*Osteoporosis Research Unit, Victoria Pavilion, Woolmanhill Hospital, Aberdeen AB9 1GS,* [2]*Cardiovascular Epidemiology Unit, Ninewells Hospital, Dundee DD1 9SY,* [3]*Computing Department, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Dietary influences (including present and past intakes) on bone mineral density (BMD) were investigated in pre-menopausal women aged 45-49 years who attended a screening programme for osteoporosis during 1991-1993. Preliminary results of the winter (New *et al.* 1995) and summer (New *et al.* 1994) studies have been reported. BMD was measured using dual-energy X-ray absorptiometry. Dietary intake was assessed using a postal food frequency questionnaire (FFQs) which had been developed and validated against 7 d weighed records (Lanham *et al.* 1993). Past intakes of milk products and fruit during childhood and early adulthood were recorded and answers categorized into low, medium and high intakes. From 1230 FFQ sent, 1008 were returned (82% response rate with one reminder letter). Results are presented for 995 women as fourteen FFQ were excluded due to incorrect completion.

Nutrient intakes (expressed as 'energy adjusted' by calculating the residual from regression analysis) were grouped into quartiles and the mean BMD at the lumbar spine (LS), femoral neck (FN) and greater trochanter (GT) were calculated. Significant differences were found between the lowest and highest quartile for K intake at the LS, FN and GT BMD sites ($P<0.005$; $P<0.05$; $P<0.006$ respectively), and at the LS BMD for Ca, Mg, fibre, vitamin C and alcohol intake ($P<0.01$ to $P<0.05$ respectively) as shown in the Table below. These differences remained significant after adjustment for age, weight, height, smoking social class and physical activity. Differences were most pronounced in the lowest quartile of nutrient intake suggesting a possible threshold effect. Differences were also found in the LS and GT BMD for women who reported low intakes of milk and fruit in their early adulthood compared with those women reporting medium or high intakes ($P<0.01$).

		Lowest (L) and highest (H) quartiles for nutrient intake									
		Potassium		Magnesium		Calcium		Fibre		Alcohol	
		L	H	L	H	L	H	L	H	L	H
LS BMD (g/cm^2)	Mean	1.039[a]	1.081[b]	1.043[a]	1.080[b]	1.042[a]	1.074[b]	1.042[a]	1.076[b]	1.047[a]	1.087[b]
	SD	0.166	0.161	0.166	0.154	0.151	0.162	0.157	0.153	0.146	0.163
FN BMD (g/cm^2)	Mean	0.874[a]	0.898[b]	0.878	0.895	0.885	0.896	0.876	0.891	0.889	0.891
	SD	0.136	0.128	0.131	0.127	0.123	0.124	0.129	0.121	0.127	0.119

a,b Values with unlike superscripts within a category were significantly different $P<0.05$.

Positive effects on acid-base balance may explain some of the benefits of K (Sebastian *et al*, 1994) Mg, vitamin C and fibre intake, which is further supported by differences in bone mass with past intake of foods rich in these nutrients. The results provide further evidence that moderate consumption of alcohol is not harmful to bone health.

SAN is grateful to the Nutritional Consultative Panel of the UK Dairy Industry for financial support.

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A study of bone growth in adolescent girls: the effect of an 18-month, milk-based dietary intervention. By J. CADOGAN[1], R. EASTELL[2], N. JONES[1] and M. BARKER[1], [1]*Centre for Human Nutrition,* [2]*Department of Human Metabolism and Clinical Biochemistry, University of Sheffield, Sheffield S5 7AU*

Osteoporosis is a major public health problem in the UK. It is generally accepted that the amount of bone achieved at skeletal maturity, termed the peak bone mass (PBM), is a major determinant of the risk of fracture in later life. A critical time for bone mineral accretion is puberty, since by the end of longitudinal growth approximately 90-95% of PBM has been attained. PBM is determined by a number of factors, including nutrition. Previous studies have investigated the effect of a Ca supplement on bone acquisition in childhood and adolescence (Johnston *et al.* 1992; Lloyd *et al.* 1993). The aim of the present study was to evaluate the effect of a food intervention, in the form of milk, on bone growth in adolescent girls.

The study design was a randomized, longitudinal intervention trial of the effect of 18 months of milk supplementation on bone mineral accretion. The study sample comprised eighty-five healthy Caucasian girls, aged 11-12 years, recruited from four schools in Sheffield, UK. Subjects were randomly assigned (stratified according to pubertal stage) into either an intervention group who were requested to consume 568 ml milk daily, or a control group who were asked to continue with their habitual diet. Dietary intake was evaluated at baseline and at 18 months by the 7 d weighed intake method. All other measurements were carried out at baseline, and at 6, 12 and 18 months. Total body bone mineral density (TBBMD) and bone mineral content (TBBMC) were measured by dual energy x-ray absorptiometry using a Hologic QDR 1000/W densitometer. Height and weight were measured in light clothing. Pubertal stage was ascertained by self-assessment.

Eighty subjects successfully completed the study. Mean daily milk intakes in the intervention and control groups were, respectively, 170 (SD 122) ml and 142 (SD 127) ml at baseline, and 486 (SD 186) ml and 160 (SD 113) ml at 18 months. For the milk group, the mean daily Ca intake was 739 (SD 218) mg at baseline, and 1125 (SD 294) mg at 18 months (P=0.001); for the control group, the Ca intake was 753 (SD 199) mg at baseline, and 703 (SD 205) mg at 18 months (NS). The intervention group compared with the control group had greater increases of TBBMD (9.6 *v.* 8.5%; P=0.04) and TBBMC (27.0 *v.* 24.1%; P=0.03) (Student's t test). Analysis of covariance was performed to control for baseline values: the intervention group had greater increases of TBBMD (0.09 *v.* 0.08 g/cm^2, P=0.04) and TBBMC (428 *v.* 391 g, P=0.08). Similarly, in percentage terms, the increase in TBBMD was 9.6 *v.* 8.6% (P=0.05), and for TBBMC was 26.6 *v.* 24.5% (P=0.07). There were no significant differences in increments in heights and weights between the two groups over the 18 months. In addition, there were no significant differences in the proportion of subjects in each pubertal stage throughout the whole study period (Chi-squared test).

In conclusion, the level of milk supplementation achieved in our trial (approximately half a pint a day) resulted in small but significant increases in bone mineral density and bone mineral content in adolescent girls, compared with girls who were not supplemented. The increases observed, if sustained throughout the pubertal growth period, may enhance peak bone mass.

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Effects of dietary and non-dietary factors on bone metabolism in women. By SUSAN A. NEW[1], SIMON P. ROBINS[2], MARK J. GARTON[1], JAMES C. MARTIN[1], DAVID A. GRUBB[3], CAROLINE BOLTON-SMITH[4], SUSAN J. LEE[5] and DAVID M. REID[1], [1]*Osteoporosis Research Unit, Woolmanhill Hospital, Aberdeen AB9 1GS,* [2]*Biochemistry Division, Rowett Research Institute, Aberdeen AB2 9SB,* [3]*Computing Department, Rowett Research Institute, Aberdeen AB9 8AU,* [4]*Cardiovascular Epidemiology Unit, Ninewells Hospital, Dundee DD1 9SY,* [5]*Biomedical Research Unit, Jessop Hospital, Sheffield S3 7RE*

The effects of dietary and non-dietary factors on bone mineral density (BMD) and bone metabolism (BM) were assessed in sixty-two healthy peri-menopausal women aged 45-55 years. Women were invited for a BMD scan of their lumbar spine (LS) and femoral neck (FN) using dual-energy X-ray absorptiometry and of their forearm total (pQCTTOT), trabecular (pQCTTRAB) and cortical (pQCTCORT) using peripheral quantitative computed tomography (pQCT). Bone resorption was determined by urinary excretion of pyridinoline (Pyd) and deoxypyridinoline (Dpd) using reversed-phase HPLC (Pratt *et al.* 1992), and bone formation by serum osteocalcin using an in-house ELISA technique similar to that described by Egsmose *et al.* (1989). Dietary intake was assessed using a food-frequency questionnaire (Lanham *et al.* 1993).

K and Mg intakes (adjusted for energy intake by calculating residuals from regression analysis) were positively correlated to pQCTTOT and pQCTCORT and negatively correlated to Pyd and Dpd excretion ($P<0.05$). Differences were found between the lowest and highest quartile for K, Mg, Ca, fibre and alcohol intake in pQCTTOT and pQCTCORT ($P<0.01$) and between the lowest and highest quartile for K, Mg, fibre and vitamin C in Pyd and Dpd excretion as shown in the Table below. These differences remained significant after adjustment for age, weight, height, smoking, social class, physical activty, age of menarche and menopausal status.

		Lowest (L) and highest (H) quartiles for nutrient intake									
		Potassium		Magnesium		Calcium		Fibre		Vitamin C	
		L	H	L	H	L	H	L	H	L	H
pQCTTOT (mg/cm^2)	Mean	366.8^a	401.2^b	362.7^a	401.7^b	370.6^a	390.5^b	375.7^a	405.7^b	386.4	397.0
	SD	44.7	46.7	42.0	44.0	42.5	41.6	46.2	44.1	52.3	36.6
pQCTCORT (mg/cm^2)	Mean	541.2^a	587.5^b	531.7^a	579.8^b	548.9	547.8	537.9	576.1	550.7	579.6
	SD	50.5	46.7	42.6	71.1	60.3	60.5	44.3	64.3	56.9	43.1
Pyd/Cr (nmol/mmol)	Mean	55.0^a	41.6^b	53.3^a	43.9^b	49.3	43.7	51.7^a	46.6^b	52.4^a	46.6^b
	SD	11.3	10.4	12.4	10.0	10.3	9.8	15.2	10.5	12.5	13.2
Dpd/Cr (nmol/mmol)	Mean	15.6^a	11.1^b	15.0^a	11.5^b	12.7	11.2	14.4^a	12.0^b	14.8^a	11.1^b
	SD	5.1	3.7	5.5	3.1	4.1	2.8	5.8	3.3	4.8	3.9

a,b Values with unlike superscripts within a category were significantly different $P<0.05$.

The results indicate increased bone resorption and decreased bone mass in women with low intakes of K, Mg, fibre and vitamin C independent of other important factors. These findings highlight the influence of nutrition on bone health.

SAN is grateful to the Nutritional Consultative Panel of the UK Dairy Industry for financial support.

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Sources of iodine in the vegan diet. By H.J. LIGHTOWLER and G.J. DAVIES, *Nutrition Research Centre, South Bank University, 103 Borough Road, London SE1 0AA*

Iodine is an essential trace element required for normal growth and development and intakes, whether deficient or excessive, can lead to thyroid dysfunction.

The major food sources of I in the UK diet are milk and milk products. As vegans do not consume these foods, or sea fish which is a rich source of I, they are considered to be a potentially 'at risk' group for low intakes of dietary I. A limited number of studies have indicated that vegans are at risk from low I intakes (Abdulla *et al.* 1981; Draper *et al.* 1993; Rauma *et al.* 1994). One study has also indicated that vegans may be at risk from excessive intakes if taking an I supplement (Key *et al.* 1992).

A study has been undertaken to assess the current sources of I in the vegan diet. Vegans (*n* 373), recruited through the Vegan Society, completed a diet questionnaire. The number of vegans consuming I from different sources is shown in the table.

Sources of I	*n*	%
Seaweed & products	169	45
Iodized salt	14	4
Iodine supplements	21	6
Seaweed & products/Iodized salt	26	7
Seaweed & products/Iodine supplements	36	10
Iodized salt/Iodine supplements	5	1
Seaweed & products/Iodized salt/Iodine supplements	4	1
None	98	26

Of the vegans who responded, over 1 in 4 (26%) were not taking any special precautions about their I intake (see Table) and over a half (54%) were consuming only one source of I. The I content of the different sources is of paramount importance. There is a misconception that seaweed products are a rich source of I and the study shows that 1 in 5 (20%) of the vegans were entirely dependent on only seaweed products which are normally eaten in small amounts and may contain variable quantities of I. Thus, the findings indicate that a number of vegans may be at risk for low I intakes.

The findings also show that some vegans may be consuming I in excess of the reference nutrient intake (RNI) of 140 μg/d: 19% of vegans were consuming at least two of the three identified sources. Vegans who are aware of the consequences of low I intakes, and compensate by taking I supplements, may be at risk of overdosing with I. Nearly 1 in 5 (18%) of our vegans were taking I supplements which alone, and certainly with other dietary sources, could lead to a risk of excessive intakes.

The results from the questionnaire suggest that some vegans are at risk of either low or excessive intakes of I. A thorough investigation into the I content of vegan diets and the consequences, both short and long term, of I deficiency and toxicity for this group merits further investigation.

The support of Dr Alan Long from VEGA is gratefully acknowledged.

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Adjustment of the expression of digestive enzymes to casein level in food in *Penaeus vannamei* (Crustacea, Decapoda). By ALAIN VAN-WORMHOUDT[1], GILLES LE MOULLAC[2], BIRGIT KLEIN[1] and DANIEL SELLOS[1], [1] *Laboratoire de Biologie Marine du Collège de France, BP 225, 29182, Concarneau,* [2] *IFREMER, Centre du Pacifique, French Polynesia, Tahiti.*

Penaeus vannamei is one of the most important shrimp for crustacean aquaculture. Adjustment of food level to digestive enzyme activity is an important step in the understanding of regulation of enzyme expression and to formulate diets with optimum performance and low economical cost.

A 21 d feeding experiment was conducted during which dietary casein level varied between 25 and 48% and starch remained constant. Trypsin (EC 3.4.21.4) and amylase (EC 3.2.1.1) activities were measured and isoenzymes revealed after electrophoresis.

Trypsic activity was measured by hydrolysis of the substrate L-Benzoyl-Arginine p-nitroAnilide (BAPNA), 50 μM. One unit of trypsic activity was defined as 1 μmole of p-nitroanilide liberated per mn per mg protein.Trypsin specific activities were enhanced by the level of casein in the diet with no apparent change in isoenzyme pattern. Specific activities ranged between 0.9 and 1.3 units. Amylase was measured using glycogen as a substrate and specific activity corresponded to mg maltose liberated per 10 mn per mg protein. Amylase activities decreased as dietary casein increased ; specific activities ranged between 55 and 40 units. For this enzyme two major isoenzymes were determined for 25% casein in the diet as only one was seen for 40% .

For precision of these changes at a molecular level, a hepatopancreas cDNA library (Sellos and Van-Wormhoudt, 1992) was screened and cDNA for amylases and trypsins were cloned and sequenced. Concerning trypsin, five cDNA were characterized encoding mature proteins of 237 AA and the existence of a particular zymogen sequence of 14 AA was reported. Concerning amylase, three cDNA were characterized encoding three mature proteins of 496 AA. Total RNA of digestive gland at different intermolt stages and after different dietary treatments of *P. vannamei* adults were extracted. The samples were denaturated by deionized glyoxal and serial dilutions were dotted on sheets of Nylon (Hybond N^+, Amersham). A [α^{32}P]ATP-labeled EcoRI-ClaI-fragment of 425 bp of a trypsin encoding cDNA was used as a probe for the revelation of trypsin mRNA by filter hybridization. Filter-bound radioactivity was determined by counting in a scintillation spectrophotometer. A correlation between the mRNA expression (cpm/μg RNA) and trypsin activity was determined, suggesting a transcriptional regulation of the trypsin biosynthesis.

In order to establish the level of polymorphism of the amylase cDNA expressed during the feeding of the animals with the two different diets, fragments of 348 bp were obtained by RT-PCR using Pfu DNA polymerase (Stratagene). The amplification products were cloned in PCR-Script SK(+) vector (Stratagene) and sequenced. Eight variable positions were detected between sequences from animals fed with the 25% casein diet and sequences from animals fed with the 40% casein diet. A discriminating position showed, in the case of the 25% casein diet, that adenosine was present in 33% of the sequences (giving Asparagine) and guanosine was present in 66% of the cDNA sequences, giving an Aspartic acid in the protein. In the case of the 40% casein diet, the adenosine was found in 100% of the cDNAs. This difference in the electric charge could explain the migration of the two isoformes observed in the case of the 25% casein diet protein equipement.

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Production by the intestinal cell line HT29 cl.19A of arachidonic acid 5-lipoxygenase metabolites and their effect on electrogenic chloride secretion. By S. BATTU, G. CLEMENT, J.M. WAL, J. COOK-MOREAU, J.L. BENEYTOUT and J.F. DESJEUX, *URA-CNRS 1485, Limoges, INRA-CEA, Saclay, and INSERM U290, Hôpital Saint-Lazare, 75010 Paris, France*

The arachidonic acid 5-lipoxygenase products may be important in the pathogenesis of diarrhoea associated with intestinal inflammation, because they may stimulate electrogenic chloride secretion in the epithelium. However, the role of the enterocyte as a producer or target of 5-lipoxygenase products remains controversial (Stenson, 1990). Therefore, on the same clone of a chloride-secreting intestinal cell line of human origin (HT29 cl.19A) (Nath *et al.* 1994), we measured (1) the expression of mRNA for 5-lipoxygenase, LTA4 hydrolase and five-lipoxygenase-acting-protein (FLAP) after total RNA extraction, reverse transcription and cDNA amplification by polymerase chain reaction (PCR), using specific primers, (2) the formation of arachidonic acid metabolites, by HPLC and (3) the effect of such metabolites on short-circuit current (Isc) stimulation, an index of chloride secretion, in HT29 cl.19A filter-grown cells mounted in Ussing chambers.

We obtained the following results : the mRNA from 5-lipoxygenase and LTA_4 hydrolase were equally expressed in HT29 cl.19A cells at different stages of culture, but they were not expressed for FLAP. When dispersed HT29 cl.19A cells were incubated in the presence of [1-^{14}C] arachidonic acid, calcium ionophore A23187, Mg^{++} and Ca^{++}, they produced 5-hydroxyeicosatetraenoic acid (5-HETE) and leukotriene B_4 (LTB_4). No peptidoleukotrienes were detected. 5-HETE alone induced a small increase in Isc (1.2 (SE 0.2) $\mu A/cm^2$), and LTB_4 had no effect. However, several hydroperoxyeicosatetraenoic acids (HPETE) produced by autoperoxidation of arachidonic acid (5-, 9-, 11-, 12- and 15- HPETE) as well as 9-, 12-, and 15-HETE, induced significant increases in Isc ranging from 3.2 (SE 0.4) to 12.1 (SE 3.7) $\mu A/cm^2$.

The present results, all obtained on the same intestinal epithelial cell line, indicate that the 5-lipoxygenase is present and functional in the enterocyte ; however the two main metabolites produced, 5-HETE and LTB_4, have barely detectable effects on electrogenic chloride secretion. Thus, the metabolites produced by the enterocytes do not seem to act as secretory agents, but might rather be the mediators acting on lamina propria cells, such as neutrophils and T lymphocytes. Once triggered, these cells may participate in the inflammatory response, including electrogenic chloride secretion by the enterocytes.

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Expression of fatty-acid-binding-proteins (FABP) along the small intestine in mice: effects of a high-fat diet and fatty acids. By H. POIRIER, I. NIOT, A. BERNARD, H. CARLIER and P. BESNARD, *Laboratoire de Physiologie de la Nutrition, EA DRED 580, ENSBANA, Université de Bourgogne, 1 Esplanade Erasme, 21000 Dijon, France.*

Cytosolic fatty-acid-binding-proteins (FABP) are abundant 14-15 kDa proteins that have a great affinity for saturated and unsaturated long-chain fatty acids (LCFA). FABP are thought to play a significant function in cell fatty acid transport and metabolism. Most mammalian fatty acid metabolizing tissues contain a single FABP, but the small intestine contains two FABP, the intestinal and liver FABP (I-FABP and L-FABP). Despite a lot of literature, little is known about the regulation of the I-FABP and L-FABP expressions. The aim of the present work was to study the effects of dietary fats and fatty acids on the regulation of FABP expressed in the small intestine.

In a first experiment, mice were fed *ad libitum* a commercial diet (UAR-A04, usine d'alimenation rationnelle) and daily force-fed with 0.2 ml sunflower-seed oil for 7 d. Controls were given 9 g/L NaCl in the same way. In the controls, the L-FABP mRNA level, low in the proximal duodenum, reached a plateau between 100 and 250 mm after the pylorus then declined progressively towards the caecum. L-FABP was apparently absent in the last 50 mm of ileum. In contrast, the higher I-FABP mRNA level found in the distal part of the small intestine. Chronic sunflower-seed oil ingestion induced a large increase of both I-FABP and L-FABP expression in duodenum and jejunum. A similar induction occured in mice force-fed with 31 μmol linoleic acid (18:2 *n*-6), the main LCFA found in the sunflower-seed oil. Since we have recently demonstrated in mice that the L-FABP gene can be switched on or off in the terminal ileum by drugs (Mallordy *et al.* 1995), we have used this intestinal model to study the time-course of linoleate action. When this LCFA was directly infused in the ileum, L-FABP mRNA was first detected 4 h after infusion, then reached a maximum between 16 and 24 h.

In a second experiment, the effects of various fatty acids, a non-metabolizable fatty acid (α-bromopalmitate) and a dioic acid (hexadecanedioic acid) were studied using our terminal ileum model. FABP mRNA levels were determined 24 h after infusion of 31 μmol of each molecule. The controls were given 9 g/L NaCl in the same way. Capric acid (10:0), palmitic acid (16:0) and hexadecanedioic (18:3 *n*-3), arachidonic acid (20:4 *n*-6) and α-bromopalmitate triggered a high induction of the L-FABP mRNA level. The apparence of L-FABP mRNA after sunflower-seed oil treatment or fatty acids infusion also led to the appearence of L-FABP protein in the cytosol attesting to the physiological redevance of our mRNA findings. In contrast to the L-FABP, infused sunflower-seed oil or fatty acids only produced weak changes in I-FABP mRNA in the terminal ileum of mice.

These results constitute the first evidence that the L-FABP expression is submitted to regulation by LCFA. Since we have recently shown the transcriptional origin of the L-FABP induction by sunflower-seed oil, the regulations reported here probably take place via a transcriptional mechanism. What molecular route could follow the nutrients to affect gene transcription ?

The hypothesis that B-oxidation of LCFA is required cannot be ruled out since α-bromopalmitate, which cannot be directly oxidized, is a potent stimulator in our experiments. LCFA have been shown to activate the expression of the nuclear transproteins : the peroxisome proliferator activated receptor (PPAR). Possibilities include the implication of transregulators of this family in the regulation of FABP expression.

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Effect of glucocorticoids on the fatty-acid-binding protein (FABP) expression in rat liver. By L. FOUCAUD[1], I. NIOT[1], T. KANDA[2] A. MALLORDY[1], H. CARLIER and P. BESNARD[1],[1] *Laboratoire de physiologie de la Nutrition, EA DRED 580, Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation, Université de Bourgogne, 1 Esplanade Erasme, 21000 Dijon, France,*[2] *Department of Biochemistry and Surgery, School of Medicine, Niigata University, Niigata, Japan*

Liver fatty-acid-binding protein (L-FABP) is a cytosolic 14 kDa protein abundantly expressed in the liver. It binds with a high affinity, especially with long-chain fatty acids (LCFA). In some situations, such as pre and postnatal development in fetuses and newborns or pregnancy and lactation in dams, physiological variations of the cytosolic L-FABP occur in the liver. These observations suggest the possibility of hormonally-mediated effects. Since plasma glucocorticoid level is relatively high in pregnant and lactating rats, the influence of this hormone on the L-FABP production was investigated in the liver of virgin female rats. After L-FABP purification and antiserum obtention, a sensitive competitive enzyme-linked immuno sorbant assay (ELISA) of the L-FABPc was carried out and used to determine accurately the amount of cytosolic L-FABP in our experiment. One group of animals was adrenalectomized (ADX) and another was adrenalectomized then daily subcutaneously supplemented for 5 d with 1.5 mg dexamethasone/kg of body weight (ADX + DEX). Controls were sham-operated (SO) females. The cytosolic level of L-FABP, unchanged after adrenalectomy as compared with controls, was greatly decreased after dexamethasone treatment (Fig. 1A). The pre-translational origin of these modifications was established since the L-FABPc mRNA levels were similarly modified (Fig. 1B). The fact that the β-actin mRNA levels were unchanged proves the specificity of the data found.

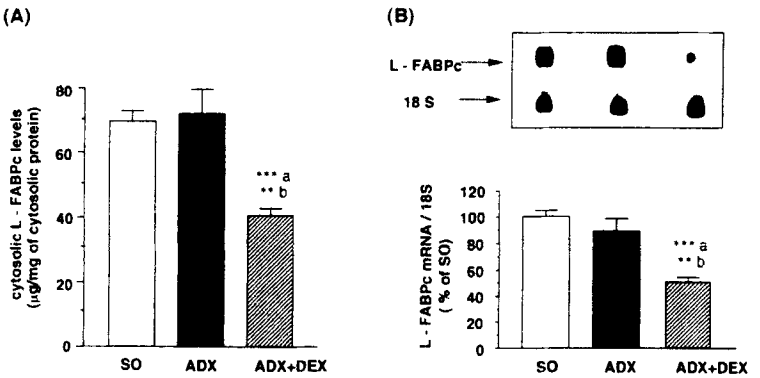

Fig. 1 : effect of adrenalectomy (ADX) and dexamethasone supplementation (ADX + DEX) on the L-FABP expression in the liver of virgin female rats. Controls were sham operated (SO).(A) Cytosolic L-FABP amounts assayed by a competitive ELISA.(B) L-FABP mRNA levels. Values are means with their standard errors. ADX + DEX *v.* SO (a) or *v.* ADX (b). ** $p <$ 0.01; *** $p <$ 0.001.

The decrease in the L-FABP expression may appear paradoxical since it is established that dexamethasone treatment triggers an increase in the plasma free fatty acid level (0.82 (SD 0.17) *v.* 0.69 (SD 0.27) mmol/l in SO controls, $p<0.01$) associated with a liver steatosis (83.7 (SD 4.2) *v.* 62.0 (SD 3.4) mg of total lipids/g of liver in the SO controls, $p<0.001$). However, this down-regulation of the L-FABP expression might have occurred via the high plasma insulin levels found in the ADX + DEX group (64.3 (SD 5.0) *v.* 7.7 (SD 1.4) µU/ml in SO controls, $p<0.001$). *In vitro* studies are under way to explore the respective roles of glucocorticoids and insulin on L-FABP expression in the liver.

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Lipoprotein lipase activity and gene expression in heart and skeletal muscles in the preruminant calf. By B. GRAULET, J.F. HOCQUETTE and D. BAUCHART, *INRA, Laboratoire Croissance et Métabolismes des Herbivores, Centre de Clermont-Ferrand/Theix, 63122 Saint-Genès Champanelle, France*

The rate-limiting step for delivery of long-chain fatty acids from triacylglycerol-rich lipoproteins to peripheral tissues (muscles, adipose tissues) is usually lipoprotein lipase (LPL; *EC* 3.1.1.34) activity. LPL was shown to be regulated by various mechanisms including transcription, translation or posttranslational processing (Enerbäck & Gimble, 1993) that have rarely been investigated in muscles. Moreover, LPL has never been studied in bovine muscle although calves raised for veal production are maintained on fat-rich milk replacers (approximately 35% of metabolizable energy). The aim of the present work was to quantify LPL activity and LPL mRNA levels in heart and various oxidative and glycolytic skeletal muscles from preruminant calves to assess the potential for fat utilization by these tissues.

Samples of heart (H) and of seven skeletal muscles (*masseter* (MA), *diaphragma* (D), *rectus abdominis* (RA), *semitendinosus* (ST), *tensor fasciae latae* (TFL), *longissimus thoraci* (LT) and *cutaneus trunci* (CT)) were taken from six preruminant Montbéliard male calves fed on a conventional milk diet and slaughtered at 5 months of age. Isocitrate dehydrogenase (ICDH; *EC* 1.1.1.42) activity (characteristic of oxidative metabolism) was measured spectrophotometrically. LPL assay was performed at 25° with Intralipid® as substrate into which [^{3}H]triolein had been incorporated by sonification. RNA was isolated, analysed by Northern-blot and hybridized with a bovine LPL cDNA probe (Senda *et al.* 1987). Quantification of LPL transcripts was performed by scanning densitometry. Values were corrected for variations of RNA loaded on the gel using results of hybridization with an 18S rRNA probe.

On the basis of ICDH activity, H, MA and D were classified as oxidative muscles. H exhibited the highest ICDH activity: 2.73 (SE 0.17) nkat/g wet tissue *v.* 0.94 (SE 0.11) and 1.19 (SE 0.16) nkat/g in MA and D respectively, and 0.14-0.22 nkat/g in other muscles ($P < 0.05$). LPL activity was positively related to ICDH activity, except for D. The highest values were observed in H and MA: 1026 (SE 100) and 625 (SE 124) mU/g wet tissue respectively, *v.* 0-286 mU/g in D and other muscles ($P < 0.05$). The LPL probe hybridized to two major transcripts (~2.7-3.4 kb and ~3.2-3.6 kb) and one minor transcript (1.7 kb) in calf muscular tissues as previously reported in the mammary gland of the dairy cow (Senda *et al.* 1987). LPL mRNA levels were much higher in H and MA (1.00 (SE 0.34), 0.79 (SE 0.26) densitometric arbitrary units (DAU) per mg total RNA respectively) than in D or in other muscles (0-0.27 DAU/mg; $P < 0.05$).

In conclusion, LPL activities and LPL mRNA levels were much higher in H and MA than in D (oxidative muscles) or in oxido-glycolytic skeletal muscles. The differences observed in levels of LPL mRNA paralleled the differences in LPL activities among calf muscles as previously described in rat muscles (Ong *et al.* 1994). Theses results suggest that the difference in LPL activity among muscles probably results, at least in part, from differences in gene transcription or mRNA stabilization as in the rat (Ong *et al.* 1994). The pretranslational mechanism by which LPL expression is controlled in such a different manner among muscles remains to be investigated.

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Effects of fasting and refeeding on lipoprotein lipase and GLUT4 gene expression in tissues of lean and genetically obese *(ob/ob)* mice. By A. NESTOR and P. TRAYHURN, *Division of Biochemical Sciences, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The enzyme lipoprotein lipase (LPL; *EC* 3.1.1.34) plays a key role in the metabolism of lipids, providing tissues with free fatty acids by hydrolysing triacylglycerols in chylomicrons and very-low-density lipoproteins. Previous work has documented a tissue-specific regulation of the expression of the LPL gene in response to the increase in fatty acid utilization induced by stimulating energy expenditure through acute exposure to cold (Nestor & Trayhurn, 1994). In normal mice, acute cold-exposure results in increases in LPL mRNA in both brown adipose tissue (BAT) and the heart, but there is a decrease in white adipose tissue (WAT); these responses are not, however, evident in genetically obese (*ob/ob*) mice (Nestor & Trayhurn, 1994). In the present study, the tissue-specific regulation of LPL gene expression was examined in lean and obese (*ob/ob*) mice in response to fasting and subsequent refeeding. Levels of LPL mRNA were measured in BAT, heart, and WAT, together with the mRNA encoding the insulin-sensitive glucose transporter, GLUT4.

Eight-week-old male lean and obese *(ob/ob)* mice of the 'Aston' strain were housed individually in cages with wire-mesh flooring, and fed on a high-carbohydrate—low-fat diet. Food was withdrawn for 24 h, and half of the mice were killed (fasted group) with the remaining animals being refed for 24 h (refed group). A control group of mice had access to food at all times. Tissues were removed, frozen in liquid N_2, and stored at -80°. Total RNA was extracted from the tissues, fractionated by agarose gel electrophoresis, and blotted onto a charged nylon membrane (Trayhurn *et al.* 1994). Each mRNA was detected by a chemiluminescence-based method utilizing antisense oligonucleotides end-labelled with digoxigenin (Trayhurn *et al.* 1994). A 30-mer oligonucleotide was used to detect LPL mRNA, and a 32-mer oligonucleotide to probe for GLUT4 mRNA (Trayhurn *et al.* 1994). Hybridization conditions and the chemiluminescence detection procedure were as described previously (Trayhurn *et al.* 1994). Signals were visualized by exposure of membranes to film and quantified by densitometry.

Results are expressed relative to controls (controls assigned an arbitrary value of 1), for six lean and four obese mice in each group. In BAT of lean mice, fasting resulted in a substantial increase in the level of LPL mRNA (7.4 times; $P<0.01$); this returned to control values after refeeding for 24 h. In WAT, however, there was a decrease in LPL mRNA level in fasted mice, but this effect was not statistically significant (0.21 times; $P>0.05$). In contrast to lean mice, there was no effect of fasting on LPL mRNA level in BAT of obese animals (0.92 times; $P>0.05$). There was also no significant change in LPL mRNA level in WAT of the obese on fasting (0.46 times; $P>0.05$). GLUT4 mRNA level decreased significantly in BAT of lean mice after a 24 h fast (0.44 times; $P<0.05$), and increased significantly following refeeding (2.0 times; $P<0.05$). A similar trend was seen in the level of GLUT4 mRNA in WAT of lean animals. In BAT of obese animals, GLUT4 mRNA level fell with fasting (0.33 times; $P>0.05$), increasing significantly after refeeding (3.5 times; $P<0.01$). There was no significant effect ($P>0.05$) of fasting, or refeeding, on LPL or GLUT4 mRNA levels in the heart in lean or obese animals.

These results indicate that there is a tissue-specific regulation of the expression of the LPL gene in lean mice in response to the major changes in nutrient flux induced by fasting and refeeding. The increase in LPL mRNA, and concomitant fall in GLUT4 mRNA, in BAT on fasting are consistent with a sparing of glucose by peripheral tissues during food deprivation. The results also indicate that there is an abnormality in the regulation of LPL gene expression in BAT of *ob/ob* mice; neither fasting nor cold-exposure (Nestor & Trayhurn, 1994) lead to the increases in LPL mRNA level that occur in lean mice.

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Effects of exercise training with food selection on GLUT4 glucose transporter expression in insulin-sensitive tissues in the rat. By N. RIETH[1], M. GUERRE-MILLO[2] and C. LARUE-ACHAGIOTIS[1], [1] *CNRS, URA 1294* and [2] *INSERM, U177, 75006 PARIS, FRANCE.*

It has been shown previously that long-term exercise training modifies GLUT4 expression in skeletal muscles in rats placed on a standard diet (approximately 70 % of total energy intake as carbohydrate). The present study was designed to reassess this question in rats provided with separate sources of the three macronutrients (protein, fat and carbohydrate) (Larue-Achagiotis *et al.* 1994). After 10d adaptation to the self-selection regimen, age-matched male Wistar rats were randomly assigned to trained (T), sedentary (S) and control (C) groups. Trained rats were given treadmill-exercise for 2h at 20m/min, every day at the beginning of the night period, for 30d. Sedentary rats were food-deprived during the time of the running period while control rats had access to food at all times.

The rats were killed by decapitation immediatly after the last bout of exercise. Two depots of white adipose tissue (inguinal and epididymal pads), heart and mixed muscles from posterior leg were rapidly dissected out and flash frozen in liquid N_2.

Expression of the insulin-sensitive glucose transporter isoform GLUT4 was assessed by Western blotting of post-nuclear total membranes, using a C-terminal peptide antibody.

Body weight gain (T: 80.2 (SD 6.5) *v.* S: 117.3 (SD 7.6) *v.* C: 127 (SD 7.5) g), glycaemia (T: 1.3 (SD 0.1) *v.* S: 1.55 (SD 0.08) *v.* C: 1.28 (SD 0.07) g/l), insulinaemia (T: 18.1 (SD 2) *v.* S: 33 (SD 7) *v.* C: 37.4 (SD 4.4) U/ml) and triacylglycerolaemia (T: 0.60 (SD 0.06) *v.* S: 1.3 (SD 0.2) *v.* C: 0.89 (SD 0.12) g/l) were lower in Trained *v.* Sedentary and Control rats.

The total energy intake, measured on the last 5 d of the experiment, was slightly (but not significantly) lower in trained *v.* sedentary and control rats (T: 71.9 (SD 2.8) *v.* S: 80 (SD 2.9) *v.* C: 81.1 (SD 2.3) kcal).

In Control rats, the ratio Fat/Carbohydrate intake (F/CHO) varied from 0.5 to 4. However, in Trained rats, F/CHO ranged from 1 to 30 indicating large interindividual variations macronutrient self-selection intake in response to exercise.

In muscle tissue, GLUT4 concentration per mg membrane protein was negatively correlated with F/CHO in Control rats ($r = -0.93$ $p<0.05$); in contrast, the two parameters were positively correlated in Trained rats ($r = +0.82$ $p<0.01$) suggesting a specific effect of exercise increasing in muscle GLUT4 expression.

In adipose tissue, no relationship was observed between F/CHO and GLUT4 concentration in inguinal tissue. But, in epididymal adipose tissue, these parameters were negatively correlated, whatever the group.

These results indicate a marked effect of long-term exercise training on macronutrient self-selection and on GLUT4 expression in muscle tissue.

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Variations in GLUT4 protein content among bovine adipose tissues. By J.F. HOCQUETTE[1], C. CASTIGLIA[1], P. FERRE[2] and M. VERMOREL[1], *[1]INRA, Laboratoire Croissance et Métabolismes des Herbivores, Theix, 63122 Saint-Genès Champanelle and [2]INSERM U342, Hôpital Saint Vincent de Paul, 75014 Paris, France*

The rate-limiting step in the metabolism of blood glucose by adipocytes is transport across the plasma membrane, which is carried out by facilitative glucose transporters. The presence of insulin-sensitive glucose transporters (GLUT4) has recently been demonstrated in adipocytes from ruminants (Trayhurn *et al.* 1994; Hocquette *et al.* 1995). The aim of the present study was to further characterize GLUT4 in bovine adipose tissues by quantifying GLUT4 protein content in perirenal (PAT), omental (OAT), intermuscular (IMAT) and subcutaneous (SCAT) adipose tissues.

Samples of PAT, OAT, SCAT and IMAT were taken at slaughter from six 10-month-old Belgian Blue calves. GLUT4 amounts were measured in crude membranes by Western-blot analysis using a polyclonal antibody against rat GLUT4 (Hocquette *et al.* 1995). Results were expressed in densitometric arbitrary units (DAU) per mg protein in crude membranes. Then, results were expressed in different ways to take into account variations among tissues in biochemical variables and in protein yields from crude membrane preparations (0.61 (SE 0.05), 1.48 (SE 0.25), 1.13 (SE 0.14) and 0.94 (SE 0.07) mg protein/g tissue wet weight in SCAT, IMAT, OAT and PAT respectively). Comparisons between tissues were made using a non-parametric test (the sign test). The results, normalized to the value of GLUT4 content in OAT of each animal, are shown in the Table.

	SCAT		IMAT		OAT	PAT	
	Mean	SEM	Mean	SEM	Mean	Mean	SEM
GLUT4 (DAU/mg protein in crude membranes)	0.51^{a}	0.15	0.84^{a}	0.25	1.00^{a}	1.73^{b}	0.49
GLUT4 (DAU/mg protein in tissue homogenate)	0.26^{a}	0.08	1.18^{abc}	0.51	1.00^{b}	2.15^{c}	0.58
GLUT4 (DAU/g wet tissue)	0.29^{a}	0.09	1.01^{b}	0.21	1.00^{b}	1.43^{b}	0.30
GLUT4 (DAU/mg DNA)	0.30^{a}	0.10	1.45^{b}	0.55	1.00^{bc}	2.91^{c}	0.66

Mean values within a row with different superscript letters were significantly different ($P<0.05$).

Regardless of the expression of the results, GLUT4 content was the highest in PAT and the lowest in SCAT. Indeed, GLUT4 was barely detectable in SCAT. Differences were particularly significant when results were expressed per mg DNA, i.e. per unit number of cells. These differences among bovine adipose tissues are in concordance with results in sheep demonstrating lower activities of hexokinase, phosphofructokinase and pyruvate dehydrogenase in SCAT than in PAT (Vernon *et al.* 1987). Moreover, the low amount of GLUT4 in bovine SCAT suggests a low insulin responsiveness of glucose transport in adipocytes from SCAT as previously described in sheep (Sasaki, 1990).

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Inhibition of glucose transport and lipogenesis in rat adipose tissue by activation of β3-adrenergic receptors. By D. LANGIN[1], G. ENRIQUE-TARANCON[2], E. LOPEZ-SORIANO[2], X. TESTAR[2], M. LAFONTAN[1] and C. CARPÉNÉ[1], [1] *INSERM U317, Bat L3 CHU Rangueil, Toulouse, France, and* [2] *Facultat de Biologia, Barcelona, Spain.*

Adipose tissue deposition is reduced by β3-adrenergic agonists which are known to possess thermogenic and lipolytic effects, demonstrated both *in vivo* and *in vitro*, at least in rodents (Arch & Kaumann, 1993). In addition to their lipolytic action on white fat cells, β3-adrenergic agonists counteract the insulin activation of glucose transport (Carpéné *et al.* 1993). In the present study the effects of a variety of β-agonists on lipogenesis were investigated in the rat adipocyte. Human and guinea pig adipocytes were also studied in parallel because they do not exhibit β3-adrenergic lipolytic responses (Carpéné *et al.* 1994).

Using BRL 37344 and CL 316243 as specific agonists for β3-adrenoceptors, or dobutamine and procaterol for the respective stimulation of β1-and β2-adrenoceptors, we compared the adrenergic effects on [^{3}H]2-deoxyglucose uptake and on [^{3}H]glucose incorporation into cell lipids. When insulin was used to stimulate rat fat cell metabolism the two dose-response curves (glucose uptake and incorporation into lipids) were superimposed: basal values were increased tenfold and half-maximal stimulations were obtained at sub-nanomolar concentrations. Both maximally stimulated responses obtained in the presence of 10 nM insulin were inhibited by the β3-agonists. However, the inhibitions were not total and were only seen in the presence of adenosine deaminase (ADA, EC 3.5.4.4), as shown in the Table. Isoproterenol and norepinephrine, which are non-selective on the three subtypes of β-adrenoceptors, also reduced insulin-stimulated glucose transport and incorporation into lipids, whereas dobutamine and procaterol, acting only on β1-and β2-adrenoceptors, were without inhibitory effect.

β-Agonist (1μM) added to:	Percentage of corresponding control							
	Dobutamine		Procaterol		BRL 37344		CL 316243	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Insulin 10 nM								
glucose transport (n = 4)	108	3	107	8	91	7	87	12
lipogenesis (n = 3)	114	10	92	6	83	23	95	2
Insulin 10 nM + ADA 2 IU/ml								
glucose transport (n = 4)	102	12	105	20	47	9 *	48	10*
lipogenesis (n = 3)	121	14	106	8	45	12*	41	5 *

* Significantly different from control, P <0.05.

All the β-agonists stimulated to the same maximal level the lipolytic activity of the rat fat cells exposed to insulin plus ADA. Moreover, dobutamine, procaterol and isoproterenol were able to stimulate lipolysis in human fat cells and guinea pig adipocytes, but did not counteract the insulin-stimulated glucose transport. In the same cells, BRL 37344 and CL 316243 neither stimulated lipolysis at a similar level as the above agonists nor inhibited the glucose transport. Thus, the adrenergic inhibition of insulin-stimulated glucose transport seems to be specifically mediated by β3-adrenergic receptors.

Acute exposure of rat fat cells to BRL 37344 did not modify the insulin-activated GLUT4 translocation from low density microsomes to plasma membrane. This observation suggests that the counter-regulation of insulin-sensitive glucose transport by β3-agonists does not affect early post-receptor events but alters transporter function/insertion at the cell surface level.

The fat depletion of adipose tissue induced by chronic exposure to β3-adrenergic agonists could result not only from their lipolytic activity but also from their capacity to inhibit the insulin-dependent incorporation of glucose into fat stores.

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Administration of an anti-adipocyte monoclonal antibody to piglets: effects on adipose tissue development and lipid content. By L. DE CLERCQ[1], J. MOUROT[2], C. GENART[1], C. BOONE[1], G. MINEUR[1] and C. REMACLE[1], *[1]Laboratoire de Biologie Cellulaire (BANI/CELL) Université Catholique de Louvain, 1348 Louvain-la-neuve, Belgium; [2]Station de Recherches Porcines, INRA, 35590 Saint-Gilles, France*

An anti-porcine-adipocyte monoclonal antibody was produced by fusion of the murine myeloma cell line SP2O with splenocytes of Balb/c mice immunized with whole porcine adipocytes. The selected antibody 4G7, of the IgG2b subclass, is specific to porcine adipocytes and preadipocytes and does not present any cross-reactivity with rat or human adipocytes. In the presence of complement, it is cytotoxic for cultured porcine preadipocytes.

Large White piglets were injected twice by the intraperitoneal route with 0, 0.1 or 1 mg/kg body weight of purified 4G7 antibody on day 3 and day 6 of life. The piglets were killed on day 35 after the start of the experiment. The monoclonal antibody had no influence on their body-weight gain (controls: 7.281 (SD 1.007) kg, 0.1 mg/kg : 7.043 (SD 1.109) kg, 1 mg/kg: 6.454 (SD 0.971) kg). After partial dissection of carcass halves, all compartments were weighed. No difference was noted in the weights of muscles, bones, heart, liver or spleen relative to carcass weight. All the subcutaneous adipose tissues analysed were however less abundant after treatment with the maximal dose. This weight decrease was significant at the $P<0.05$ level for the subcutaneous dorsal adipose tissue. Estimation of dry matter of second carcass halves and subcutaneous dorsal and retroperitoneal fat pads confirmed the results of the partial dissections: the piglets treated with the highest dose of antibody had significantly lower amounts of dry matter ($P<0.05$) than the control ones, the 0.1 mg/kg group presenting intermediate values. Total lipid content of the crushed carcass halves was also depressed by administration of the monoclonal antibody, (1mg/kg: 8.85 (SD 0.61) % *v.* 0.1 mg/kg: 9.58 (SD 1.05) % *v.* controls: 10.88 (SD 1.11) %, $P<0.02$) while the total lipid content of the *Longissimus dorsi* remained unaffected.

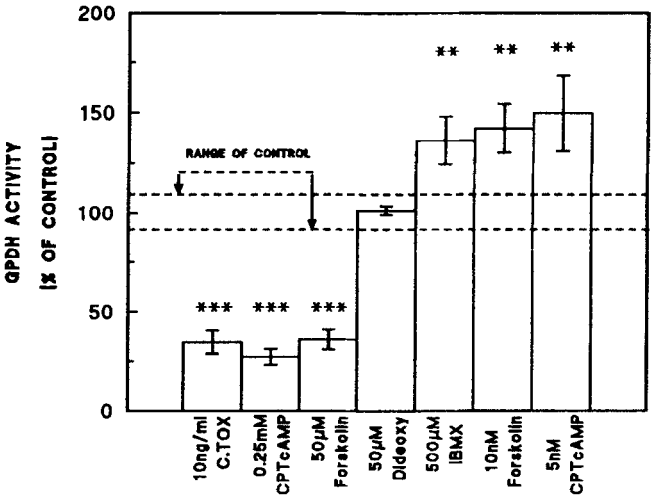
The size distributions of the subcutaneous dorsal adipocytes, studied by planimetry after collagenase digestion and osmication showed a small but significant shift to smaller sizes for the treated piglets compared with controls.

Hence, administration to piglets of the 4G7 anti-adipocyte monoclonal antibody during the first week of life, restricts the development of the adipose tissue without affecting the muscular tissue at least until 5 weeks of age. Further work has to be done to study the long-term effects of such treatment as well as the effects of administration of a mixture of specific monoclonal antibodies.

Cyclic AMP modulates adipogenesis in 3T3-F442A cells. By STEPHEN J. YARWOOD, NEIL G. ANDERSON and ELAINE KILGOUR, *Department of Biochemistry and Molecular Biology, Hannah Research Institute, Ayr KA6 5HL.*

The molecular mechanisms which control the terminal differentiation of mature fat cells remain to be fully elucidated. Numerous studies have demonstrated that the intracellular signalling molecule cyclic AMP (cAMP) can modulate the growth and development of various cell types. In the present study we have investigated the effects of manipulation of intracellular cAMP levels on the differentiation of 3T3-F442A preadipocytes in serum-free medium.

3T3-F442A fibroblasts were grown to confluence in Dulbecco's modified Eagle's medium containing 100ml/l calf serum. Confluent cultures were induced to differentiate by replacing the growth medium with a defined differentiation medium (DDM) containing growth hormone (2 nM), insulin (1.8 μM), triiodothyronine (0.1 ng/ml), epidermal growth factor (50 ng/ml) and other factors as previously described (Kilgour & Anderson, 1993) along with the drugs being tested. After 3 d the drugs were removed and after a further 6 d the activity of α-glycerophosphate dehydrogenase (EC 1.1.1.8, GPDH), a marker for adipocyte differentiation, was measured. Cellular cAMP concentration was measured useing a modification of the competitive binding assay for cAMP (Brown *et al,* 1971).



Values are means with their standard errors, *n* 4-9. Significantly different from control group: $^{**}P<0.01$, $^{***}P<0.001$.

The Figure shows that elevating intracellular cAMP levels during the differentiation had profound effects upon the subsequent terminal differentiation of the cells. Raising intracellular cAMP levels with 50 μM-forskolin, cholera toxin (C.TOX) or 0.25 mM-8-(4-chlorothio)cAMP (CPT-cAMP) attenuated GPDH activity. Dideoxyforskolin, an inactive analogue, had no effect upon differentiation. In contrast, inclusion of 10 nM-forskolin, 5 nM CPT-cAMP or the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX) promoted differentiation. These results demonstrate that the increases in intracellular cAMP levels as achieved with IBMX, 10nM-forskolin and 5nM-CPT-cAMP, promote differentiation while the increases in cAMP levels induced with 50 μM-forskolin, 0.25 mM-CPT-cAMP and C.TOX treatments inhibit differentiation. The molecular mechanisms responsible for the differential effects of cAMP on the differentiation process are currently under investigation.

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Regulation of ovine preadipocyte differentiation *in vitro*. By K.S. ADAMS[1], D.J. FLINT[1], A. CRYER[2] and R.G. VERNON[1], [1]*Hannah Research Institute, Ayr KA6 5HL, and* [2]*School of Molecular and Medical Biosciences, University of Wales, PO Box 911, Cardiff CF1 3UB*

Adipose tissue depots develop asynchronously in sheep with abdominal depots in general developing earlier than carcass depots (Flint & Vernon, 1993). To obtain further insight into the factors and mechanisms responsible, we have developed a system for the proliferation and differentiation of ovine stromal-vascular preadipocytes in culture. We have used this to compare the ability of preadipocytes from different depots to develop in response to defined stimuli.

The stromal-vascular fraction cells were isolated from samples of omental (abdominal), popliteal (intermuscular) and subcutaneous adipose tissue from young sheep and were plated out (2.5×10^4 cells/ml) and maintained in Medium 199 containing Earles salts and supplemented with 2 mM-acetate, 4.8 mM-L-glutamine, antibiotics and 200 ml/l fetal calf serum as described previously (Adams *et al.* 1992). Once cells reached confluence (about 10 d), the medium was changed to a serum-free medium (DMEM-F12) supplemented with 1.6 μg/ml insulin, 2 nM-tri-iodothyronine (T_3) and 10 μl/ml excyte (a lipid supplement) to allow differentiation, which was monitored visually by the appearance of lipid droplets in cells and by measurement of a marker enzyme glycerol 3-phosphate dehydrogenase (GPDH; EC 1.1.1.8).

Differentiation was apparent after 3 d and virtually complete by 7-9 d of culture in the serum-free medium. In the absence of insulin, T_3 and excyte, mean GPDH activity was 1.6 nmol/min per mg protein for cells from all three depots, but in the presence of the supplements, GPDH activity was 4.5, 24.6 and 24.0 nmol/min per mg protein for omental, subcutaneous and popliteal respectively; values are means of four observations, SED 5.7 from ANOVA. Activity in the two carcass depots was significantly greater ($P<0.05$) than for omental tissue. Thus under the same, defined stimulus, carcass preadipocytes from young lambs have a greater propensity to differentiate than those from the abdominal depot selected.

	Supplement to DMEM-12 T_3 + excyte				
	None	None	Insulin	GH	GH + insulin
GPDH	1.2	1.5	13.5	0.8	3.0

GPDH nmol/min per mg protein, SED 0.8; means of three observations

Further studies tested the roles of insulin and growth hormone (GH) in promoting differentiation. Results for subcutaneous preadipocytes are given in the Table which shows that omission of insulin prevents differentiation while addition of GH inhibits (this contrasts with findings for some preadipocyte cell lines) (Flint & Vernon, 1993).

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Regulation of the antilipolytic effect of adenosine in sheep adipose tissue by growth hormone. By R. DORIS[1], E. KILGOUR[1], M.D. HOUSLAY[2] and R.G. VERNON, [1]*Hannah Research Institute, Ayr KA6 5HL and* [2]*Institute of Biomedical and Life Sciences, University of Glasgow G12 8QQ*

Growth hormone (GH) enhances the responsiveness of adipocytes to acutely acting lipolytic agents by several mechanisms. One of the more important mechanisms appears to be to decrease the ability of anti-lipolytic agents (e.g. adenosine, prostaglandin E) to inhibit lipolysis. These agents have their own receptors which activate inhibitory GTP-binding proteins (Gi) which in turn inhibit adenylate cyclase activity. We have found that treatment of sheep *in vivo* with GH or culture of sheep adipose tissue explants with GH leads to a diminished response to an adenosine analogue N^6-phenylisopropyl adenosine (PIA) but with no apparent change in the number of adenosine receptors or amounts of Gi isoforms (Doris *et al.* 1994). This suggested a more subtle change, perhaps due to phosphorylation of Gi, altering its ability to interact with either its receptor or adenylate cyclase.

Explants of sheep adipose tissue were maintained in culture for 24 h without hormones and then for a further 24 h plus or minus 100 ng/ml ovine GH. Subsequently, adipocytes were isolated, disrupted and a membrane fraction prepared (Vernon *et al.* 1995). The ability of pertussis toxin to stimulate NAD-ribosylation of the membranes was assessed (Wong *et al.* 1985).

The ability of pertussis toxin to stimulate NAD-ribosylation of the adipocytes was unchanged by GH; as pertussis toxin stimulates ribosylation of the undissociated (heterotrimeric) form of Gi, this suggests that the dissociated:undissociated ratio was unchanged by GH.

Pertussis toxin stimulated NAD-ribosylation of sheep adipocyte membranes (cpm/mg protein)

- GH		+ GH		
- PIA	+ PIA	- PIA	+ PIA	SED (*n*)
3154	1965	3472	2169	377(5)

As shown in the Table, preincubation of membranes with 1 μM PIA for 2 h at 37° decreased the amount of NAD-ribosylation due to receptor-induced dissociation of Gi but this was not altered by culture with GH. This suggests that GH does not alter the ability of Gi to interact with the adenosine receptor; the attenuation of the antilipolytic effect of adenosine by GH may thus be due to changes in the interaction of Gi with adenylate cyclase.

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Nutritional regulation of the expression of the *ob* (obese) gene in white adipose tissue of mice

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The genetically obese (*ob/ob*) mouse is widely used as an animal model in obesity research. Despite the elucidation of a number of abnormalities by conventional physiological and biochemical approaches, the fundamental defect in *ob/ob* mice has remained unidentified. The recent application of molecular genetics has, however, now resulted in the cloning and sequencing of the *ob* gene (Zhang *et al.* 1994). The gene appears to be expressed only in adipose tissue, and codes for an 18 000 Mr protein; it is predicted that the *ob* protein is secreted from adipocytes (Zhang *et al.* 1994). Given the critical role that the protein must play in the regulation of energy balance, it would be expected that expression of the *ob* gene should be sensitive to nutritional status. In the present study we have examined the effects of fasting and fasting—refeeding on the level of the mRNA for *ob* in white adipose tissue of mice. This has been done using an antisense oligonucleotide designed as a probe for *ob* mRNA.

Ten-week-old male lean mice of the "Aston" strain were used. Tissues were removed following cervical dislocation, frozen in liquid N_2, and stored at -80°. To investigate the effects of fasting and fasting—refeeding, mice housed at 22° were divided into three groups (six mice per group) and caged individually. Two groups were fasted for 24 h, one of which was subsequently refed for 6 h. The control group had continuous access to food. Total RNA was extracted from tissues, fractionated by agarose gel electrophoresis, and blotted onto a charged nylon membrane (Trayhurn *et al.* 1994). The mRNA for *ob* was detected by a chemiluminescence-based procedure (Trayhurn *et al.* 1994) utilizing a 33-mer antisense oligonucleotide probe end-labelled with digoxigenin (Boehringer Mannheim). The oligonucleotide (5'-GGTCTGAGGCAGGGAGCAGCTCTTGGAGAAGGC-3'), was designed from the sequence of mouse *ob* mRNA (Zhang *et al.* 1994), and synthesized commercially (R & D Systems Europe). Hybridization conditions and the chemiluminescence detection procedure were as described previously (Trayhurn *et al.* 1994). Both CSPD and CDP-*Star* (Tropix) were used as chemiluminescence substrates. Signals were visualized by exposure of membranes to film, and quantified with a molecular imager using phosphor storage screens sensitive to chemiluminescence (Bio-Rad).

The antisense oligonucleotide probe was based on a region of the *ob* mRNA downstream from the site of the primary mutation in *ob/ob* mice; it contains 63.6% G+C residues. The oligonucleotide detected a single band (about 4.1 kbases) on Northern blots of total RNA from epididymal white adipose tissue within a few minutes of exposure to film. This was also the case with two other antisense oligonucleotides designed and tested as probes for *ob* mRNA. The mRNA encoding *ob* was detected in white adipose tissue, but not in any of the other tissues examined (liver, brain, heart, skeletal muscle, lung, kidney, gut, spleen, testis). A weak signal was sometimes obtained with brown adipose tissue (interscapular), which may reflect contamination with white adipocytes. *ob* mRNA was detected in each of the major white adipose tissue depots, including the subcutaneous, with the highest levels being present in the epididymal tissue and the lowest in the omental and pericardial fat. Fasting for 24 h led to a substantial fall in the level of *ob* mRNA in the epididymal fat pads (28% of controls; $P<0.002$). Following refeeding for 6 h, the mRNA level increased rapidly and there was no longer any significant difference from the control levels (refed 69% of controls; $P>0.05$).

The present report demonstrates that the mRNA encoding *ob* can be rapidly detected in white adipose tissue with a chemiluminescence-based procedure utilizing a 33-mer antisense oligonucleotide probe, and that the *ob* gene is expressed throughout the main white fat depots in mice. The level of the mRNA is influenced by nutritional status, declining on fasting and rising on refeeding, responses that are consistent with the putative major role of *ob* protein in the regulation of energy balance.

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Elevated expression of the *ob* (obese) gene in white adipose tissue of diabetic (*db/db*) mice

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The mutant gene in genetically obese (*ob/ob*) mice has recently been identified and sequenced (Zhang *et al.* 1994). The primary lesion results in the introduction of a stop codon towards the 3' end of the translated region of *ob* mRNA. The production of an abnormal gene product in *ob/ob* mice leads to increased expression of the *ob* gene through putative feedback signals to adipose tissue, the organ in which the gene is expressed (Zhang *et al.* 1994). On the basis of parabiosis experiments, it is suggested that the primary defect in a closely related mutant, the diabetic (*db/db*) mouse, lies in the receptor for the *ob* protein (Coleman, 1978). If this is the case, then over-expression of the *ob* gene in *db/db* mice would be anticipated. In the present study we have examined *ob* gene expression in adipose tissues of normal and diabetic (*db/db*) mice, through measurement of the level of the mRNA.

Male *db/db* mice, aged 8-9 weeks, with the *db* gene on the C57Bl/Ks background were purchased, together with normal (*db/+*) siblings, from Harland-Olac (Bicester). They were housed at 22° for 1 week after receipt, before being killed by cervical dislocation. The epididymal fat pads and a sample of subcutaneous white adipose tissue (taken close to the hind limbs) were removed, and immediately frozen in liquid N_2 and stored at -80°. Total RNA was extracted from the tissues, fractionated by agarose gel electrophoresis, and blotted onto a charged nylon membrane (Boehringer Mannheim), as described previously (Trayhurn *et al.* 1994). The mRNA encoding *ob* was detected by a chemiluminescence procedure (Trayhurn *et al.* 1994), utilizing a 33-mer antisense oligonucleotide end-labelled with digoxigenin (Boehringer Mannheim) as a hybridization probe. The oligonucleotide (5'-GGTCTGAGGCAGGGAGCAGCTCTTGGAGAAGGC-3') was based on the sequence of mouse *ob* mRNA (Zhang *et al.* 1994), and synthesized commercially (R & D Systems Europe). Hybridization conditions and the chemiluminescence detection procedure were as described previously (Trayhurn *et al.* 1994), except that CDP-*Star* (Tropix) was used as the chemiluminescence substrate (Trayhurn *et al.* 1995). Chemiluminescence was visualized by exposure of the nylon membranes to film, and quantified by densitometry or with a molecular imager (Bio-Rad) using phosphor storage screens.

The 33-mer antisense oligonucleotide designed as a probe for *ob* mRNA detected a single band of approximately 4.1 kbases on Northern blots of total RNA from white adipose tissue of both normal and diabetic mice. In each group of animals the signal was stronger in epididymal adipose tissue than in the subcutaneous fat. The level of *ob* mRNA was higher in the diabetic mice than in the normal animals for both the adipose tissue sites examined. In epididymal fat the level of the mRNA in the mutant animals was 2.4 times that in the normal mice ($P<0.05$; n 5). A much larger difference was observed between the two groups in the subcutaneous adipose tissue (19.5 times; $P<0.01$).

These results indicate that the *ob* gene is expressed more strongly in white adipose tissues of *db/db* mice than in their normal (*db/+*) siblings. Although the *db* mutation (in contrast to the *ob* mutation), does not directly involve the *ob* gene, it is evident that the lesion is associated with alterations in the expression of *ob*. If the view that the *db* mutation involves a receptor for the *ob* protein (Coleman, 1978; Zhang *et al.* 1994) is correct, then increased levels of *ob* mRNA may reflect enhanced stimulation of the expression of the *ob* gene through a feedback loop to adipose tissue.

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Altered macrophage GTPase activity may not exclusively modify TNF-induced macrophage IL1 or IL6 production. By P.S. TAPPIA, R.L. DUNN and R.F. GRIMBLE, *Department of Human Nutrition, University of Southampton, Southampton, SO16 7PX*

Cytokines are a diverse range of polypeptides that play a key role in maintaining optimal immune function (Male *et al.* 1989). Their actions ensure that the immune system is activated and nourished during infections and in response to other inflammatory stimuli. Inflammatory stimuli bring about production of the inflammatory cytokines namely tumour necrosis factor (TNF), interleukins 1 and 6 (IL1 and IL6) which are involved in the processes of inflammation, however if they are produced in the wrong context as in the case of inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease and psoriasis they contribute towards the underlying pathology of the disease. The control of over production can be achieved by macronutrients, in particular dietary fats. Fish oil supplements have been reported to bring about a 30% suppression in monocyte production of inflammatory cytokines in human rheumatoid arthritic patients (Kremer & Robinson 1991). The present study describes some mechanistic information as to how dietary fats may influence macrophage cytokine production.

Weanling rats were fed on synthetic diets containing fat (100g/kg) for a period of either 4 or 8 weeks. Macrophages were elicited with the use of an intraperitoneal injection of thioglycollate broth. Cytokine concentrations were measured by bioassay 24 h after TNF (2.5 ng/ml). GTPase activity (GTP hydrolysis) was measured in the presence or absence of TNF (2.5 ng/ml).

	4 weeks		8 weeks	
	+ TNF	- TNF	+ TNF	- TNF
Dietary fat/oil	Mean SD	Mean SD	Mean SD	Mean SD
GTPase activity (pmol/min per mg protein)				
Fish	19.5 5.9	23.8 7.0	31.0 3.8	29.2 1.7
Maize	38.1 3.2	36.6 4.0	29.1 3.3	20.5* 2.2
Olive	25.2 4.0	25.9 3.7	23.5 2.1	20.7* 2.6
Coconut	35.0 5.2	33.6 3.9	22.7 2.5	21.2* 3.1
Butter	34.6 1.1	42.2 8.4	25.9 1.8	26.3 2.8
	IL1	IL6	IL1	IL6
	Mean SD	Mean SD	Mean SD	Mean SD
Cytokine Production (ng/ml)				
Fish	1.40 0.44	2.23 0.17	1.75 0.20	2.54 0.44
Maize	1.63 0.27	2.04 0.15	4.25* 0.75	3.24* 0.92
Olive	1.56 0.10	2.19 0.19	4.05* 0.50	2.96 0.52
Coconut	1.52 0.50	1.98 0.17	1.70 0.15	1.84 0.44
Butter	1.46 0.42	2.02 0.13	2.25 0.10	2.64 0.72

* Result significantly different from corresponding fish value, $P<0.05$ (one-way ANOVA).

Dietary fats may exert their influence on altering macrophage cytokine production through, for example, changes in membrane fluidity, phospholipid composition, generation of eicosanoid and non-eicosanoid second messengers via G-protein activity. In the present study we investigated the possibility that dietary fats may, in part, exert their effect through altered G-protein activity (GTP hydrolysis) and consequent generation of second messengers, for example diacylglycerol, cyclic adenosine monophosphate, prostaglandins and leukotrienes. The results obtained suggest that even though dietary fats modify macrophage GTPase activity, the modification does not exclusively explain altered cytokine production, but may contribute toward the overall, combined effect of dietary fats.

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Zinc deficiency inhibits the differentiation of F9 embryonal carcinoma cells. By W.D. REES, SUSAN M. HAY and J.K, CHESTERS, *Divisions of Physiology and Biochemical Sciences, The Rowett Research Institute, Bucksburn, Aberdeen. AB2 9SB.*

In the rat there is a crucial period 8-10 d after conception when maternal Zn deficiency has teratogenic effects and this coincides with the main period of tissue differentiation. Attempts to investigate the role of Zn in differentiation of the early embryo are limited by the techniques available. Embryonal carcinoma cells, the undifferentiated stem cells of teratocarcinomas, have many of the properties of pluripotent embryonic cells. Treatment with retinoic acid (RA) causes the cells to differentiate into primitive endoderm, accompanied by an increased expression of alkaline phosphatase (ALP; *EC* 3.1.3.1.). The increase in ALP activity is correlated to the appearance of other markers of the differentiated phenotype (Strickland & Mahdavi, 1978). Embryonal carcinoma cells provide a simple system to investigate the effects of nutrient deficiency on differentiation in the developing embryo.

Mouse F9 embryonal carcinoma cells were cultured in 24 well multi-plates with Dulbeccos minimal essential medium supplemented with 100 ml fetal calf serum per litre. At 24 h after plating the medium was changed to the test medium containing *all-trans* retinoic acid and where appropriate 600 μM-EDTA (to chelate Zn) and metal salts. Then, 48 h later the medium was removed, cells were washed with phosphate buffered saline and dissolved in an assay buffer containing 10 mM-Tris-Cl (pH 7.5), 0.5-mM $MgCl_2$ and 1 ml Triton X-100/litre. A portion of the extract was assayed for DNA (West *et al* 1985) and a portion for ALP activity (Gianni *et al.* 1991).

In the first series of experiments the addition of 10^{-6} M-RA in the absence of EDTA produced a typical 4.6-fold increase in ALP from 1.142 (SE 0.071) mU/μg DNA to 5.268 (SE 0.304) mU/μg DNA. If EDTA was added to RA stimulated cells 2 h before the cells were harvested there was no effect on ALP activity. The addition of EDTA to the medium 48 h before the assay blocked the activation of ALP by RA (1.161 (SE 0.071) mU/μg DNA). As Zn was added back to the medium the RA-dependent increase in ALP was restored in a dose-dependent manner. EDTA alone inhibited the accretion of DNA, however whilst the addition of 25 μM-Zn SO_4 was sufficient to restore the rate of growth, over 200 μM-Zn SO_4 was required to restore the RA effect on ALP. The inhibition of the RA response by EDTA was specific for Zn, the addition of 200 μM-$CaCl_2$, Mn SO_4 and Fe SO_4 failed to produce any increase in ALP. In a second series of experiments the RA dose response was measured in control medium or in the presence of EDTA and 50 μM-Zn SO_4. Cells were treated with a range of RA concentrations and the ALP activity measured 48 hours later. Zn deficiency due to addition of EDTA produced a significant reduction in the response to RA (Fig. 1).

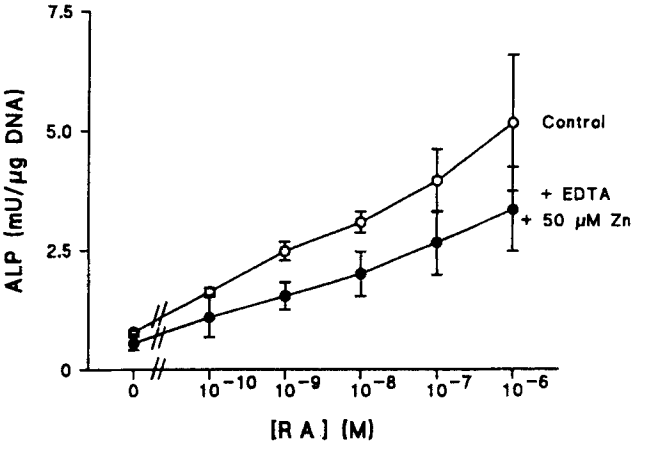

Fig. 1. Effect of retinoic acid on alkaline phosphatase activity in F9 cells. Open symbols untreated cells, closed symbols cells treated with EDTA and a suboptimal Zn supplement of (50 μM Zn). Data average of three experiments. Values are means for three experiments with standard deviations indicated by vertical bars.

In conclusion Zn deficiency changed the dose response of F9 embryonal carcinoma cells to RA and such an effect may underlie the teratogenic effects of Zn deficiency in the embryo.

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Control of selenoprotein synthesis during selenium repletion of selenium-deficient rats. By GIOVANNA BERMANO, FERGUS NICOL, JOHN A. DYER[1], ROGER A. SUNDE[1], GEOFFREY J. BECKETT[2], JOHN R. ARTHUR and JOHN E. HESKETH, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, [1]University of Missouri-Colombia, Colombia, Mo 65211, USA and [2]Cellular Endocrinology Unit, University Department of Clinical Biochemistry, The Royal Infirmary, Edinburgh EH3 9YW*

The functions of Se are mediated by up to thirty selenoproteins, many of which contain the amino acid selenocysteine. These proteins have been associated with roles for Se in cell antioxidant systems, thyroid hormone metabolism, the maintenance of fertility and possibly some anti-cancer effects (reviewed Arthur & Beckett, 1994). With such a variety of biological roles it is essential to define the mechanisms which control the incorporation of Se into functional selenoproteins, particularly when supplies of the element are insufficient to meet normal requirements. Here we describe the acute responses of the hepatic and thyroidal selenoprotein iodothyronine 5'deiodinase (5'IDI) mRNA and activities to Se-repletion in severely Se-deficient rats.

Male, Rowett Hooded Lister rats were fed on a Se-deficient diet (0.003 mg Se/kg diet) for 6 weeks from weaning. At 6, 16, 32, and 72 h before the end of the experiment, groups of six Se-deficient rats were injected i.p. with 20 μg Se/kg body weight (as Na_2SeO_3 in 9 g NaCl/l). Hepatic and thyroidal 5'IDI mRNA levels and activities were determined as described by Bermano *et al.* (1994). Results are expressed as percentage change from the values in untreated, Se-deficient rats.

	Time after Se injection (h)							
	6		16		32		72	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver 5'IDI								
activity	37	17	77**	13	135**	29	119*	35
mRNA	31	15	45	19	20**	9	-37*	4
Thyroid 5'IDI								
activity	0	8	11	14	0	13	-11	11
mRNA	22	9	20	9	68**	14	57**	11

Significant change from value in Se-deficient animal; * $P<0.05$, ** $P<0.01$.

Hepatic 5'IDI activity and mRNA were transiently increased 16, 32 and 72 h after intraperitoneal injection with Se. However, thyroid 5'IDI activity was unaffected, presumably reflecting its increase in Se deficiency (Bermano *et al.* 1994). Despite enzyme activity being unchanged 5'IDI mRNA in the thyroid gland increased two to threefold after 32 and 72 h, indicating a prolonged transcriptional response or stabilization of the mRNA, even when Se supplies were probably exhausted. An increase of thyroidal mRNA levels may therefore be a mechanism for the preservation of thyroidal selenoproteins when Se supplies are limited.

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Thyroid selenoenzyme activity and mRNA abundance in second generation selenium and iodine deficient rats. By J. H. MITCHELL, F. NICOL, G. J. BECKETT[1] and J. R. ARTHUR, *Division of Biochemical Sciences, Rowett Research Institute, Aberdeen AB2 9SB and* [1]*University Department of Clinical Biochemistry, Royal Infirmary, Edinburgh EH3 9YW*

The thyroid gland is able to retain Se, at the expense of the liver, when dietary supplies of the micronutrient are limiting. Three of the selenoproteins which occur in the thyroid gland are type I iodothyronine deiodinase (ID-I), cytosolic glutathione peroxidase (cGSHPx) and phospholipid hydroperoxide glutathione peroxidase (phGSHPx). In Se- deficient adult male rats thyroidal ID-I activity is maintained more efficiently than cGSHPx (Beech *et al.* 1995). In I- deficiency the mechanisms which stimulate thyroid hormone synthesis may produce an oxidant stress on the thyroid gland and thus increase the requirement for Se in selenoperoxidases. Therefore, to assess the interaction between Se and I in maintaining thyroid function, selenoenzyme activity and mRNA abundance were determined in the thyroid glands from second generation Se- and I- deficient rats.

Female weanling rats were fed on one of the four following diets: (1) Se- and I- adequate, control (+Se+I), (2) Se-deficient (-Se), (3) I-deficient (-I), (4) Se- and I-deficient (-Se-I). After 7 weeks the animals were mated with normal males and their offspring were killed at 19 d of age. Pup thyroid selenoenzyme activities and mRNA abundance were determined by standard techniques. Results are expressed as percentage of Se- and I-adequate control (mean values with standard errors.)

	+Se+I		-Se		-I		-Se-I	
Pup Thyroid	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ID-I activity	100	7.1	61	8.4	319	11.4	244	17.6
ID-I mRNA	100	7.1	102	9.1	216	18.5	174	19.0
cGSHPx activity	100	4.2	45	14.8	369	86.1	90	8.5
cGSHPx mRNA	100	28.2	89	7.5	500	72.1	230	22.4
phGSHPx activity	100	24.4	29	13.3	48	7.2	29	4.5
phGSHPx mRNA	100	34.2	116	32.4	280	40.1	197	13.8

Selenoenzyme mRNA levels were maintained in Se-deficient pup thyroid glands however, a differential effect was observed in selenoenzyme activity. ID-I activity decreased to 61%, cGSHPx to 45% and phGSHPx to 29% of Se-adequate controls (Table). In I- deficient glands mRNA levels were increased 2.16, 5.0 and 2.8 times for ID-I, cGSHPX and phGSHPx respectively. ID-I and cGSHPx enzyme activities were also induced but the activity of phGSHPx was decreased by 52% despite the high mRNA abundance. Selenoprotein mRNA levels were also increased in concurrent Se- and I-deficiency but again differential effects on enzyme activity were observed with ID-I activity increased, cGSHPx maintained and phGSHPx decreased to 29% of Se-adequate controls.

Iodine deficiency may stimulate the metabolic activity of the thyroid gland and increase the requirement for Se to maintain selenoenzyme activity. When dietary supplies of Se are limiting mRNA levels are induced to compensate for overall lack of the micronutrient. Furthermore, there is a preferential supply of the available Se to ID-I and cGSHPx to attempt to maintain thyroid function.

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Antioxidant supplementation decreases both endogenous and induced oxidative DNA damage in human lymphocytes. By SUSAN J. DUTHIE, AIGUO MA, MARION A. ROSS, VICTORIA L. DOBSON, KATRINA M. BROWN and ANDREW R. COLLINS. *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Habitually low intakes of fruit and vegetables are associated with a higher incidence of certain cancers. Dietary antioxidants in these foods may decrease free-radical attack on DNA and thus protect against mutation and cancer. To study the effects of antioxidant supply, a double-blind 2 x 2 factorial supplementation study (100 men, aged 50-59 years) was carried out. Age-matched groups of smokers and non-smokers received either a daily antioxidant supplement (vitamin C 100 mg, D L-α-tocopherol acetate 280 mg and ß-carotene 25 mg) or placebo for up to 20 weeks. Plasma antioxidant concentrations and endogenous DNA damage in lymphocytes (strand breaks and oxidized pyrimidines) were determined using single-cell gel electrophoresis (the "comet assay"; Collins *et al.* 1993). In addition, lymphocytes from a subset of non-smokers were challenged with different doses of H_2O_2 to determine whether antioxidant supplementation could protect against subsequent oxidative damage *in vitro*.

Table: The effect of supplementation with antioxidants on oxidised pyrimidines in lymphocyte DNA (DNA damage in arbitrary units)

	Smokers				Non-smokers			
	Placebo		Supplement		Placebo		Supplement	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Week								
5	85.9	8.3	98.2	12.6	54.7*	14.1	64.3*	8.1
10	61.1	10.2	57.3	9.5	52.6	8.1	51.1	8.5
20	53.9	10.1	26.4+	7.2	65.9	9.8	43.5+	7.5

* Significantly different from smokers on the same treatment ($p < 0.002$) using analysis of variance

\+ Significantly different from volunteers on placebo ($p < 0.002$) using analysis of variance.

Smokers had significantly elevated levels of oxidized bases compared with non-smokers at week 5 (table). Smokers on placebo showed decreased oxidised base damage throughout the study period. This may be a seasonal effect reflecting increased consumption of fresh fruit and vegetables. This remains to be established. After 20 weeks, antioxidant supplementation had significantly decreased oxidized base damage in both groups compared with the respective placebo group. Neither smoking nor antioxidant supplementation affected frequency of strand breaks (results not shown). Lymphocytes from supplemented non-smokers also showed increased resistance to *in vitro* oxidative damage. After treatment with 300 μM H_2O_2, the mean level of damage (arbitrary units) was 188 (SE 8.7) in the supplemented group compared with 250 (SE 7.8) in the placebo group ($p < 0.001$).

In conclusion, long-term antioxidant supplementation significantly decreased both endogenous and exogenous oxidative DNA damage in lymphocytes supporting the hypothesis that dietary antioxidants may protect against cancer.

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A method of transfecting C_2C_{12} myoblasts at all stages of differentiation. By FIONA E. FAIRHURST, LINDA PETRIE and JOHN K. CHESTERS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

An ability to transfect myoblasts would allow assessment of the phenotypic responses to alterations in their genetic potential and the mouse C_2C_{12} cell line could provide a suitable model system since C_2C_{12} cells can readily be induced to differentiate into contractile myotubes. However, once the myoblasts had fused, the usual calcium phosphate-based transfection protocol resulted in extensive loss of myotubes. Several transfection factors were therefore investigated to achieve the best compromise between cell survival and high transfection efficiency.

C_2C_{12} cells were maintained in Dulbecco's MEM with 120 ml fetal calf serum/l at 37° in an atmosphere of CO_2 in air (100 ml/l). Cells were routinely subcultured well before they reached confluence. However to provide myotubes, the cells were grown to confluence and then differentiation was induced by transferring them to DMEM containing 20 ml horse serum/l. Approximately 80% of cells were fused 4 d later. Transfection mixtures were prepared by combining equal volumes of filter-sterilized solutions A (0.01 vol. of both 70 mM-NaH_2PO_4 and 70 mM-Na_2HPO_4 plus 1 vol. 42 mM-HEPES, 274 mM-NaCl) and B (250 mM-$CaCl_2$). The DNA used for transfection (usually pCAT control plasmid, Promega Corporation) was purified by CsCl gradient centrifugation and added to solution B before mixing with solution A. The nature of the precipitate formed is critical for the success of the transfection. This was optimized by adding solution A to solution B drop by drop with gentle mixing which yielded a calcium phosphate precipitate with an even granular appearance when viewed at 100x magnification. Coarse precipitates tended to cause cell death while fine, barely visible precipitates resulted in poor transfection. The factors affecting transfection efficiency were investigated and their optimal conditions are indicated in the Table.

Factor investigated	Optimal procedure
Order of addition of soluitions A and B	Add A to B
Length of time A+B mixture is left to form precipitate	Use immediately
Amount of medium added to mixture before use for transfection	1 vol. mixture to 4 vol. medium
Incubator CO_2 concentration during transfection	50 ml CO_2/l
Duration of exposure of cells to transfection mixture	Overnight
Osmotic shock	2 min with 150 ml/l glycerol solution
Use of salmon sperm DNA as carrier	Decreases efficiency
Amount of DNA used for transfection	≤ 15 μg/60 mm plate of cells

Sufficient of the transfection mix to supply each of the cultures was prepared in bulk and mixed with 4 volumes of the culture medium. The cells were then incubated in the mixture (2.5 ml/60 mm dish) overnight at 37° in an atmosphere containing 50 ml CO_2/l. The following morning the mixture was removed and the cells were osmotically shocked by exposure for 2 min to a salt solution (150 mM-NaCl, 40 mM Tris-HCl pH 7.4) supplemented with glycerol (150 ml/l). After washing with the salt solution alone, the cells were then cultured in either growth or differentiation medium and in an atmosphere containing 100 ml CO_2/l until harvesting usually 72 h later. Transfection efficiency was assessed by determination of chloramphenicol acetyltransferase (*EC* 2.3.1.28) activity using the method of Nordeen *et al* (1987).

The final protocol allowed successful transfection of myotubes as well as both growing and differentiating myoblasts.

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The stability of mRNA encoding some pancreatic hydrolases is modulated by dietary protein intake in the rat. By S. CARREIRA, C. FUERI, J.-C. CHAIX and A. PUIGSERVER, *Laboratoire de Biochimie et Biologie de la Nutrition, CNRS-URA 1820, Service 342, Faculté des Sciences et Techniques St-Jérôme, 13997 Marseille cedex 20, France*

The secretion of digestive enzymes from the exocrine pancreas has been known for a long time to be modulated by nutritional substrates in the diet. The content in both pancreatic tissue and secretion of the major digestive enzymes, proteases, amylase and lipase, changes in proportion to the dietary content of their respective substrates (Wicker *et al.* 1984, 1988). The parallelism observed in changes of mRNA levels and protein synthesis, when feeding a high-protein diet, suggest that this enzymic adaptation might result directly from any nutrient or indirectly via hormone action involving the regulation of gene expression at the transcriptional level and/or a change in the stability of mRNA rather than a translational control. When feeding a protein-free diet, amylase and the subgroup of protease zymogens were largely synthesized in inverse proportion to nutritional substrates in the diet (Schick *et al.* 1984; Dakka *et al.* 1990). The regulation by a protein-free diet of the pancreatic response seems to take place at different levels, transcriptional, translational and/or at the level of mRNA stability.

The stability of pancreatic mRNA was studied in Wistar rats under three isoenergetic nutritional conditions. The rats were fed on either a high-protein diet (640g casein/kg), a protein-free diet (810g carbohydrate/kg) or a standard diet (220g casein/kg). We injected actinomycin D, an inhibitor of transcription, and directly quantified the pancreatic hydrolases mRNA levels by dot-blot hybridisation with cDNA or oligonucleotide specific probes. The calculated mRNA half-lives under each nutritional condition were normalized to the values obtained for the control rats fed on the standard diet.

mRNA	Diet					
	Protein-free		Standard		High-protein	
	Mean	SE	Mean	SE	Mean	SE
Trypsinogen I	1.68*	0.26	1	0.09	1.27*	0.13
Chymotrypsinogen B	2.65*	0.61	1	0.11	1.10	0.09
Elastase I	0.43*	0.03	1	0.14	0.44*	0.03
Procarboxypeptidase B	1.84*	0.10	1	0.08	1.01	0.07
Amylase	0.35*	0.02	1	0.08	1.18	0.37
Lipase	0.85	0.09	1	0.07	1.14	0.24

* Significantly different from the control rats fed on standard diet, $p<0.05$ (ANOVA analysis and Dunnett test)

The Table shows that nutritional regulation of the gene expression in rat pancreas is probably only transcriptional for most hydrolases when feeding rats with a high-protein diet, except for the anionic trypsinogen I and elastase I from which stability is affected, but when rats were fed on a protein-free diet, nutritional regulation of gene expression largely involves an additional control of mRNA stability. This study provides the evidence that the stability of mRNAs encoding some pancreatic hydrolases is modulated by the dietary protein content.

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Application of a new method for the measurement of circulating peptides. By MARIA HIPÓLITO-REIS, DANA WILSON, JOHN C. MacRAE and F.R. COLETTE BACKWELL, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The utilization of peptides as a source of amino acids (AA) for tissue protein synthesis in humans (Furst *et al.* 1990) and in ruminants (Backwell *et al.* 1994; Backwell, 1994) is now well established. Direct confirmation of trans-organ flux of circulating peptides is, however, difficult due to variations associated with the procedures used for the preparation of peptides from physiological fluids, which are based on the difference in AA concentrations of acid deproteinized plasma samples, before and after acid hydrolysis. As acid deproteinization techniques are not completely efficient this may result in artificially high peptide concentrations. Thus, there is currently much controversy associated with the suggestion that a substantial proportion (60-80%) of total AA uptake across the ruminant small intestine is in the form of small peptides (Koeln *et al.* 1993).

We have modified this approach to include a gel-filtration step after acid deproteinization which gives a fraction containing peptides of molecular weight <1500. Application of this method to assessment of peptide concentration in arterial blood of sheep has indicated that for some AA a substantial proportion of total AA is present in the form of peptides (MW <1500).

	Percentage of amino acid as peptide in arterial blood	
Amino acid	Mean (*n*)	SE
Serine	23.9** (5)	4.6
Glycine	53.4*** (5)	3.0
Histidine	49.4** (4)	6.1
Threonine	13.1* (5)	3.9
Isoleucine	14.5* (5)	5.1
Leucine	25.6*** (5)	1.9
Phenylalanine	25.5* (5)	9.5

Statistical significance by Student's paired *t* test, +$P<0.1$, *$P<0.05$, **$P<0.01$, ***$P<0.001$.

These values are much lower than those reported by others and probably reflect a more efficient removal of protein from the samples before hydrolysis. Measurement of peptide flux across the small intestine of sheep by this method indicates that there is no appreciable uptake of peptides of molecular weight less than 1500.

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Transfer of ^{15}N from urea to the circulating lysine pool in the human infant. By N. Yeboah[1], E. Ah-Sing[1], A Badalloo[2], T. Forrester[2], A. Jackson[3] and D. J. Millward[1], *[1]Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH, [2]Tropical Metabolism Research Centre, University of the West Indies, Jamaica and [3]Institute of Human Nutrition, University of Southampton, Southampton SO16 7PX*

Urea salvage is an extensive process in humans at all ages and in infants can account for half the urea produced. The biological importance of urea recycling depends on the metabolic fate of the salvaged N, namely the extent to which the salvaged N returns to the systemic amino N pool in the form of indispensable amino acids synthesized *de novo* by the colonic microflora or as NH_3. We are investigating this with studies of the transfer of ^{15}N, from orally administered ^{15}N dilabelled urea, to the circulating amino acid pool in the human infant during a 36 h period in children receiving treatment for severe protein-energy malnutrition. We report here preliminary results of the enrichment of lysine isolated from the urinary amino acid pool of these children.

Children were studied on admission, (after stabilization), during recovery and after recovery on diets which supplied either adequate or generous protein levels (0.53-0.69 and 2.97 g protein/kg stabilization phase, 3.05-3.1 and 4.59 g/kg during recovery and 0.52-0.62 and 3.04 g/kg after recovery). Energy supplied was 414 kJ/kg or 699kJ during recovery. ^{15}N labelled urea was administered over 36 h in a primed multiple oral dose procedure. In order to avoid blood sampling, amino acids were isolated from the urinary pool which derives from the renal arterial blood, and which was collected 6-hourly over 42 h. Lysine was isolated from urine by preparative ion-exchange with fraction collection of all column effluent and with peak identification by a manual fluorimetric procedure on each fraction. The fractions containing lysine were desalted, lyophilized, taken up into buffer and analysed for ^{15}N enrichment in a combustion isotope ratio mass spectrometer. Confirmation of the identity and purification of the lysine peak was obtained by GCMS analysis of a small sample of the final lysine solution before ^{15}N analysis.

Of the samples analysed to date (2/3 of the total) we have observed significant enrichment in the majority but not all children. Thus in one child ^{15}N-enrichment values measured in quadruplicate, were 0.0029 (sd 0.0011), 0.00455 (sd 0.0006), 0.0058 (sd 0.0009), 0.00684 (sd 0.0004), 0.01151 (sd 0.0003) and 0.01751 (sd 0.0007) atom% excess (ape) at successive 6 h periods collected after the initial 12 h collection.

Assuming that the labelling represents *de novo* synthesis of lysine then it is possible to calculate very approximate rates of lysine supply, assuming that microbial lysine has the enrichment of the urea pool at plateau and assuming that the labelled lysine which enters the body free-lysine pool is diluted by the whole-body lysine flux and has achieved a plateau enrichment: i.e. with lysine ^{15}N enrichment in blood (urine) = 0.014 ape, and urea ^{15}N enrichment = 1.6 ape, then dilution of ^{15}N = (approx.)100-fold, with lysine entry rate = lysine flux/100. Assuming whole body lysine flux (at 8g protein/kg per d) = 0.64g lysine/kg per d, then lysine entry rate = 6.4 mg lysine/kg per d or about 10% of current requirements. This is a low estimate since it assumes that: (a) urea-N with the observed labelling is the sole precursor for microbial synthesis: (b) microbial lysine has reached a plateau and (c) that the plasma pool has reached a plateau. All of these assumptions could be in considerable error with a likelihood of underestimating lysine *de novo* production.

Whilst confirmation that the labelling does reflect *de novo* synthesis rather than transamination must await completion of further studies examining this possibility, these results do support the biological importance of urea salvage as a nutritionally important source of indispensable amino acids.

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Metabolism of lactose-ureide labelled with ^{15}N and ^{13}C in humans. By R. BUNDY, A. HOUNSLOW, C. PERSAUD, J. MURPHY, S. A. WOOTTON and A.A. JACKSON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

The salvage of urea-N by the activity of the colonic microflora is an integral part of the adaptation to low protein intakes in normal people (Jackson, 1995). On low but adequate protein intakes, there is an increase in the rate at which urea-N is salvaged with the N being retained within the system. In order to study the quantitative fate of urea-N it is necessary to know the amount of urea delivered to the colonic microflora. It has been suggested that lactose-ureide might be used for this purpose, because it is resistant to digestion in the upper intestine but is readily fermented by the microflora, thereby making the urea available for hydrolysis (Heine *et al* 1995). The fate of the label has been followed in normal adults following oral ingestion of lactose-[$^{15}N^{15}N$]ureide and lactose-[^{13}C]ureide.

Lactose-[$^{15}N^{15}N$]ureide and lactose-[^{13}C]ureide were synthesized from lactose and labelled urea by the method of Hoffman (1932), with a yield of about 50%. Following recrystallization the purity was assessed by GCMS and by reaction with urease (*EC* 3.5.1.5) enzyme and assessed to be better than 99% pure.

Eight normal adults were provided with a diet which was marginally adequate in protein, 36 (SD 2) g protein/d, for 5 d. At 06.00 hours on the fourth day a single dose of lactose-[^{13}C]ureide (500 mg) was taken orally and the excretion of isotope on breath as $^{13}CO_2$ was measured over the next 48 h. Over the same period of time, from 06.00 hours, a prime (3.21 mg/kg) and intermittent doses (0.64 mg/kg per 3 h) of lactose-[$^{15}N^{15}N$]ureide were taken orally every 3 h for 18 h and urine was collected every 3 h for the measurement of enrichment in urea. Total stool was collected over days 4 and 5.

Little of the isotope (< 5%) was recovered in breath during the first 6 h following the dose of lactose-[^{13}C]ureide. The cumulative excretion of isotope as $^{13}CO_2$ as a percentage of the dose was 49 (SD 8) after 17 h and 84 (SD 21) after 48 h. The ^{15}N, as [$^{14}N^{15}N$]urea, started to appear in urine about 6-9 h after the priming dose and there was a variable rise towards plateau enrichment over the time of the study. The percentage of the dose excreted as [$^{15}N^{15}N$]urea was 5 (SD 8), and no more than 30% on average was excreted in [$^{14}N^{15}N$]urea. 20 (SD 5) % of the ^{15}N was excreted in the stool over the period of collection, so therefore about 45% of the initial dose of ^{15}N was retained within the body over the period of study.

The delay in the recovery of label is probably accounted for by the transit time from the mouth to the large bowel. Thus, these data indicate that most of the lactose-ureide taken orally escapes digestion and absorption in the small intestine, but is fermented in the large bowel with the urea moiety being hydrolysed, although there is inter-individual variability. Over half of the ^{15}N released from urea hydrolysis was retained within the body and these data conform with what has been found when [$^{15}N^{15}N$]urea was placed directly in the colon either at colonoscopy or through a colostomy (Moran & Jackson, 1990 *a*,*b*). This study reiterates the important role of the colon in whole-body N metabolism and conservation. The fate of the retained urea-N is being determined.

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Regulation of ammonia and amino acid metabolism in isolated sheep hepatocytes. By M.A. LOMAX[1], S.A. MALTBY[1], D.S.BUSS[1] and G.E. LOBLEY[2], *[1]School of Animal and Microbial Sciences, University of Reading, PO Box 228, Reading, RG6 2AJ and [2] Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

It has been proposed that hepatic amino acid deamination can be stimulated under conditions of increased NH_3 detoxification to urea by ovine liver (Lobley *et al.* 1995). We have previously reported that $^{15}NH_3$ is converted predominantly to [^{15}N,^{15}N] urea by ovine hepatocytes even in the presence of a physiological mixture of amino acids (Luo *et al.* 1995). The interpretation of this study was complicated, however, by the preferential removal by hepatocytes of glutamine and arginine from the media. We now report the effect of amino acids (AA) and dibutyryl CAMP, added to stimulate amino acid utilization, on the conversion of $^{15}NH_3$ to urea.

Hepatocytes were prepared from five fasted sheep and incubations and analytical procedures carried out as described by Luo *et al.* (1995). All incubations contained 0.2 mM-propionate and 0.75 mM-$^{15}NH_4Cl$. The concentrations (mM) of added nutrients in the media were dibutyryl cAMP (0.5), AA1 (alanine (0.16), threonine (0.15), serine (0.1), glycine (0.4)) and AA2 (3 times those of AA1).

	nmol/ mg wet wt cell per h				
	$^{15}NH_3$	UREA	^{14}N,^{15}N	^{15}N,^{15}N	^{14}N,^{14}N
NH_3	-51.30	27.54	3.08	21.81	2.65
NH_3 + AA1	-46.96	28.39	5.13	21.92	1.92
NH_3 + AA1 + cAMP	-61.54	39.10	8.54	28.22	3.02
NH_3 + AA2 + cAMP	-45.95	32.95	8.14	22.31	2.50
SED	3.56	2.02	0.50	2.10	0.61

Hepatocytes converted $^{15}NH_3$ removed from the medium to predominantly [^{15}N,^{15}N] urea with low rates of both [^{14}N,^{15}N] and unlabelled urea species being formed. Addition of AA1 significantly ($P < 0.01$) increased [^{14}N,^{15}N] urea synthesis and this was further stimulated by the addition of cAMP. Uptake of NH_3 from the medium and flux to [^{15}N,^{15}N] urea was increased ($P < 0.01$) by addition of cAMP to incubations containing AA1 but not AA2. However, even when 30% of urea formation was apparently coming from non-NH_3 sources (see AA2 + cAMP), the formation of [^{15}N,^{15}N] urea still accounted for 68% of the urea molecules synthesized.

Therefore, although NH_3 removal and detoxification to urea by ovine hepatocytes is influenced by the presence of amino acids and cAMP, the results contrast with our previous *in vivo* findings linking increases in NH_3 removal with equimolar contributions of amino acid-N towards ureagenesis (Lobley *et al.* 1995).

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Sepsis-induced long-lasting stimulation of liver protein synthesis. By DENIS BREUILLÉ[1], CATHERINE ALLAZARD[2], FRANCIS ROSÉ[1], MAURICE ARNAL[2] and CHRISTIANE OBLED[2], [1]*Clintec Technologies,Velizy, France,* [2]*Unité d'Étude du Métabolisme Azoté, INRA, Centre de Recherche en Nutrition Humaine de Clermont-Ferrand, France*

We have shown that sepsis induced an acute stimulation of liver protein synthesis which modified its contribution to whole-body protein synthesis (Breuillé et al. 1994). This anabolic effect has often been described just after a stress but the length of this reaction has received very little attention. In the present study, we used a rat model of sepsis which induced a long-lasting catabolic state. The aim was to establish if stimulation of liver protein synthesis was transient or prolonged when we compared infected with pair-fed animals.

Forty-two rats were divided into seven groups : one control non infected group killed on day 0 (C), three groups of animals Infected by means of an intravenous injection of live bacteria on day 0 and killed 2, 6 and 10 d later (I), 3 control groups, pair-fed with infected groups, after an injection of NaCl (9g/l) on day 0 (PF). Liver protein synthesis rate was measured using a flooding dose of L-[1^{13}C]valine (1500μmol/kg body weight, 30 atom % excess).

	Body-wt loss (g)		FSR (% per d)		ASR (g/d)		Orosomucoid (mg/l)		Fibrinogen (g/l)	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	0		59.9	5.1	1.38	0.27	29.8	11.6	3.47	0.10
I (2d)	40†	6	101.3*†	7.7	2.36*†	0.27	1053*†	161	7.84*†	0.40
PF (2d)	31	4	53.8	3.8	0.90*	0.06	18.5	4.1	3.28	0.79
I (6d)	59†	12	101.6*†	16.3	2.01*†	0.44	448*	203	6.86*	0.89
PF (6d)	38	6	63	7.6	1.17	0.23				
I (10d)	43†	21	97.5*†	9.5	2.01*†	0.41	372*	137	6.24*†	0.74
PF(10d)	1	6	65.8	8.0	1.42	0.29			3.84	0.25

*Significantly different from C, $p<0.05$.
†Significantly different from respective PF, $p<0.05$.
FSR, fractional synthesis rate ; ASR, absolute synthesis rate.

The Table shows that infected animals lost significantly more weight than pair-fed rats and began to recover between days 6 and 10. At 2, 6 and 10 d after infection, food intakes were respectively 10, 60 and 90% of the value observed before the day of infection. Liver FSR was not altered by food intake restriction and ASR was only decreased on day 2. On the contrary, liver protein synthesis was dramatically increased in infected groups (FSR and ASR) and this anabolic reaction of the liver was preserved until day 10. This long-lasting effect of infection was confirmed since infected animals were still inflammatory on day 10. Indeed concentrations of orosomucoid and fibrinogen peaked on day 2 but still high on day 10.

These results demonstrate that an acute infection induced a long-lasting synthesis of acute-phase proteins. Our previous results indicated that 2 days after infection liver protein synthesis represented a third of the whole-body protein synthesis (Breuillé et al. 1994). All these results suggest that the liver plays a key role in amino acid requirements of the body in inflammatory states.

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Effect of dietary proteins on the meal stimulation of albumin synthesis in humans. Relations with the precursor pool for protein synthesis. By MARC CAYOL[1], BERNARD BEAUFRERE[2], PIERRE GACHON[2] and CHRISTIANE OBLED[1], *Centre de Recherches de Nutrition Humaine, d'Auvergne, [1]Laboratoire d'Etude du Metabolisme Azoté, INRA Clermont-Ferrand-Theix, France and [2]Laboratoire de Nutrition Humaine, Clermont-Ferrand, France*

It is known that meal consumption may improve the synthesis of albumin (De Feo *et al.* 1992). However in the fed state, the calculation of the fractional synthesis rate (FSR) of liver-exported proteins is complicated because amino acids are supplied to the liver from portal and arterial blood and intracellular proteolysis. Moreover, the relative importance of these amino acid sources could be influenced by the nutritional state. In addition, the choice of the precursor pool used to estimate the FSR may affect the results. In the present study the effect of dietary proteins on the synthesis rate of albumin was investigated in two groups of five healthy volunteers using a dual tracer methodology. Subjects were fed every 20 min with isoenergetic diets containing either no dietary protein or 1 g protein / kg per d. L-[1-^{13}C]leucine (0.10 μmol / kg per min) and L-[5,5,5-^{2}H$_3$]leucine (0.15 μmol / kg per min) were administered intravenously (IV) and intragastrically (IG) respectively, for 10 h. The incorporation rate in albumin of both tracers was measured over the last 4 h of the infusion protocol. The FSR of albumin was calculated from the plateau values of the label enrichments in plasma leucine, plasma α-ketoisocaproate (KIC) or VLDL apolipoprotein B100 (apoB100).

	L-[1-^{13}C]leucine (IV)				L-[5,5,5-^{2}H$_3$]leucine (IG)			
	no protein		+ protein		no protein		+ protein	
Precursor	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Leucine	4.1	0.2	5.1*	0.2	8.8†	0.7	17.6*†	0.8
KIC	5.9	0.2	7.3*	0.3	12.0†	0.6	20.3*†	1.4
ApoB100	6.4	0.4	10.0*	0.6	6.9	0.3	13.1*†	0.8

*Significantly different from protein free diet (P<0.05, ANOVA).

†Significantly different from the route of tracer infusion with the same diet (P<0.05, paired tests).

The Table shows that albumin FSR calculated from the plasma leucine or KIC as precursor could be two- or three fold higher with the IG tracer compared with the IV tracer. In constrast, when the plateau enrichment of apoB100 was used as the precursor pool for protein synthesis, the FSR of albumin was similar for the two tracers with the protein-free diet (P > 0.05). Thus, these results support the suggestion from Reeds *et al.* (1992), that the enrichment in VLDL apoB100 could be used to probe the hepatic precursor pool. The FSR of albumin was increased by the dietary protein compared with the protein-free diet. Nevertheless, the level of stimulation was dependent on the precursor used. With the IV tracer, the albumin FSR was 24 and 56% higher when the synthesis rate was calculated with the plasma leucine and apoB100 enrichments respectively. However, with the IG tracer the FSR of albumin was increased by about 100% with dietary proteins whichever precursor was used. Finally, when using the apoB100 enrichment as precursor, the albumin FSR was slightly higher with the IG- than with the IV-labelled amino acid which could reflect a better utilization of the dietary amino acids by the liver.

The above findings indicate a specific improvement of albumin synthesis by dietary protein. Moreover, the calculated values for the FSR of liver proteins are influenced by the route of the tracer administration. However, the present results show that the measurement of the enrichment in apoB100 allows an accurate estimation of the liver precursor pool for protein synthesis.

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Estimation of the enrichment of urea N precursors. By G.D. MILANO[1], M.A. LOMAX[2] and G.E. LOBLEY[1], *[1]Rowett Research Institute,Bucksburn, Aberdeen AB2 9SB and [2]School of Animal and Microbial Sciences, University of Reading, PO Box 228, Reading RG6 2AJ*

In ruminants, hepatic conversion of NH_3 to urea may require inputs from other N sources, notably amino acids, reducing potential protein anabolism. The N atoms of urea originate from mitochondrial carbamoyl phosphate (CP) and cytosolic aspartate (ASP). N can enter both precursors from the mitochondrial NH_3 pool, either directly in the case of CP or after formation of glutamate from oxoglutarate through mitochondrial glutamate dehydrogenase (GLDH; EC 1.4.1.2) activity and subsequent transamination to ASP. Mitochondrial NH_3 and ASP pools can also be supplied with N arising from other sources (mainly amino acids) through deamination and transamination reactions, respectively. Assuming that transamination to ASP is not a rate limiting step, the direction and the rate of the GLDH reaction as compared with the N flux through the ornithine cycle, will determine the contribution of mitochondrial NH_3 to the ASP pool. Although this contribution can be calculated from the enrichments of CP (a) and ASP (a') in liver preparations *in vivo* and *in vitro* following administration of $^{15}NH_3$, direct measurement of these metabolites is difficult in practice.

An alternative approach has been adopted to estimate the upper and lower limits of the true enrichments based on the molar proportions of singly (*m1*) and doubly (*m2*) ^{15}N-labelled urea species synthesised in these preparations . According to the laws of probability the enrichments of both precursors are related to *m1* and *m2* by the equation : $1 / a + 1 / a' = (m1 / m2) + 2$. Algebraic development of this equation permits the estimation of precursor enrichments when they are assumed to be equally ($a = a'$) or unequally labelled and the value of either a or a' is known or can be estimated. This approach was applied to [^{15}N]urea species from isolated sheep hepatocytes incubated for 2.5 h in a medium containing 0.4 mM-propionate and 0.3 mM-$^{15}NH_4Cl$ in the absence (Fig. 1) or presence (Fig. 2) of a physiological mixture of amino acids (PAA) (Lomax *et al.* 1995) and from plasma of sheep infused with 150 μmol $^{15}NH_4Cl$ / min in the mesenteric vein for 3 h at the end of a 4 d administration of NH_4HCO_3 (Fig. 3).

Fig. 1

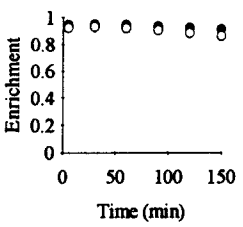


Fig. 2

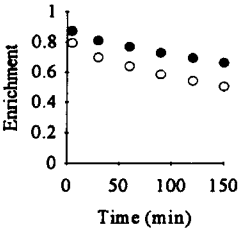
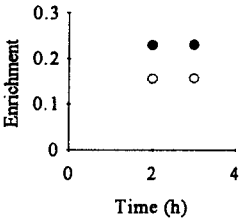


Fig. 3



(●) Enrichment when $a = a'$; (○) a' value when a was set at 0.967 (Fig. 1 and 2) or 0.45 (Fig. 3), NH_3 enrichments in medium and plasma.

In the absence of PAA the estimated limits for the enrichments declined only slightly over the incubation period suggesting that unlabelled N arising from intracellular protein degradation was small. Minimum values for a' were 0.87 indicating that mitochondrial NH_3 was the major N donor to the ASP pool. In the presence of PAA, enrichments decreased with time, probably due to extraction of amino acids from the medium, with unlabelled N sources accounting for 0.3 of the N flux through the urea precursor pool. Calculated enrichments were stable after 2.0 h of $^{15}NH_4Cl$ infusion *in vivo*. Unlabelled sources contributed 0.5 of the N flux through the precursor pool while not less than one third of the aspartate N arose from NH_3. The results indicate that liver GLDH activity in sheep is directed towards net synthesis of glutamate and that a substantial proportion of aspartate N is derived from NH_3 even in fasting conditions.

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The influence of sulphur amino acids on urea-nitrogen salvage. By T.S. MEAKINS and A.A. JACKSON, *Department of Human Nutrition, University of Southampton, Southampton SO16 7PX*

Previously, we demonstrated that the rate of urea-nitrogen salvage was determined by the rate of appearance of urea in the body. However, at protein intakes below requirement levels, large amounts of urea were required to enhance salvage by the colonic bacteria (Meakins & Jackson, 1994). S amino acids were most limiting in the diet and methionine supplementation can improve N balance on low protein intakes (Zezulka & Calloway, 1976; Kies & Fox, 1978). The aim of the present study was to determine whether S amino acids might be acting as a limiting factor and whether their addition to a diet inadequate in protein exerted any effect upon the urea salvage system.

Six adult females were given four diets for a period of 5d each, containing either (a) 26 g protein/d (4.16 g N/d), (b) 26 g protein/d with 600 mg methionine (4.22 g N/d), (c) 26 g protein/d with 6.9 g urea (7.36 g N/d), (d) 26 g protein/d with 600 mg methionine and 6.9 g urea (7.42 g N/d). Urea kinetics were measured using the prime/intermittent oral dose method over the final 24 h of each dietary period (Jackson *et al*, 1984).

Urea...	N Balance (mgN/kgperd)		Production (mgN/kgperd)		Salvage (mgN/kgperd)		Excretion (mgN/kgerd)	
Intake	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
26 g protein (P)	-37[a]	3	88[a]	8	20[a]	4	68[a]	6
26 g P + methionine	-30[a]	9	84[a]	9	18[a]	7	69[a]	10
26 g P + urea	-19[b]	4	91[a]	9	32[a]	12	113[b]	13
26 g P + urea + methionine	-37[a]	4	82[a]	13	23[a]	11	115[b]	10

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

N balance was negative on the diet providing 26 g protein with or without methionine. Addition of the urea supplement improved N balance significantly due to a corresponding increase in urea-N hydrolysis in the colon. This is in contrast with the previous study in that males consuming 30 g protein/d were unable to utilize 6.9 g of urea to improve N balance. When urea and methionine were given together, N balance was the same as with 26 g protein alone, with no difference in the rate of urea-N hydrolysis. Endogenous urea production remained constant on all four dietary regimens. With the addition of urea to the diet there was a significant increase in the excretion of urea.

These results demonstrate that on this marginal intake of protein, women are able to utilise urea-N by recycling in the colon to improve N balance. However, the addition of methionine to this diet does not improve N balance, possibly due to the inability of the colonic bacteria to handle the additional load of amino acids. Hence, it is unlikely that sulphur amino acids play a limiting role in the urea-N salvage system.

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Altered insulin response to a glucose load in rats following exposure to a low-protein diet *in utero*. By C.L.PICKARD, H.D.McCARTHY, R.F.BROWNE and A.A.JACKSON, *Department of Human Nutrition, University of Southampton, Southampton SO16 7PX*

Epidemiological studies have demonstrated that poor intrauterine growth is linked to non-communicable diseases in adulthood, such as type II diabetes, hypertension and coronary heart disease (Barker, 1993). Using a rat model, we investigated the insulin response to an intravenous glucose load in offspring previously exposed to a low-protein diet *in utero*.

Female Wistar rats were fed on semi-purified diets containing either 180 or 90 g casein/kg diet for 2 weeks before mating and throughout pregnancy. Within 8 h of birth, dams were transferred onto standard laboratory chow (CRMX, SDS). Upon weaning, the offspring were housed individually and transferred onto a macronutrient self-selection diet based on maize starch/sucrose, casein and lard/maize oil (McCarthy et al. 1994). At age 20 weeks, six animals of both sexes in each dietary group were fasted for 24 h before an intravenous glucose tolerance test (GTT, 2 g/kg body weight) administered at time zero. Blood samples were removed at time points -10, -5, 0, 5, 10, 15, 20, 30, 40 and 60 min. Plasma was separated and frozen and later assayed for insulin using a commercial radioimmunoassay kit (Diagnostics Systems Laboratories, Texas), and also glucose concentration.

In males, GTT profiles were similar between dietary groups, with peak glucose concentrations of 51.9 (SE 1.9) mmol/l and 49.7 (SE 1.8) mmol/l for the 90 g/kg and 180 g/kg groups respectively. The whole glucose load had been cleared by 60 min in both groups. Initial peak insulin response was lower in the 90 g/kg group (7.8 (SE 1.8) ng/ml *v.* 10.6 (SE 1.6) ng/ml, $P = 0.21$, NS). However, in the 90 g/kg group, a second peak in plasma insulin concentration occurred after 20 min, such that after 60 min, plasma insulin concentration was significantly higher in the 90 g/kg group (15.2 (SE 3.0) ng/ml *v.* 5.2 (SE 1.4) ng/ml, $P = < 0.05$). The area under the curve for the 90 g/kg group was 43% higher than for the 180 g/kg group ($P = 0.15$, NS).

In females, glucose clearance was again similar between groups (90 g/kg, 42.2 (SE 0.6) mmol/l; 180 g/kg, 37.2 (SE 1.8) mmol/l), along with initial peak insulin levels lower in the 90 g/kg group (7.9 (SE 1.5) ng/ml *v.* 11.4 (SE 3.14) ng/ml, $P = 0.33$, NS). Rats in the 90 g/kg group again showed a second insulin peak (9.4 (SE 2.8) ng/ml *v.* 5.2 (SE 3.3) ng/ml, $P = 0.35$, NS). The area under the curve was increased by 17% (P=0.54, NS)

In conclusion, this study has shown that although rate of glucose clearance appears to be identical in both groups of rats, this is at the expense of larger increases in insulin secretion in the 90 g/kg group. This suggests a degree of insulin resistance in these animals, although the nature of this alteration in insulin sensitivity is at yet unknown.

This work is funded by the British Heart Foundation

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Effect of a maternal diet low in protein on glucocorticoid-sensitive brain enzyme activities. By D.S.GARDNER, S.C.LANGLEY-EVANS and A.A.JACKSON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

The hypertension observed in the offspring of rats exposed to a maternal diet low in protein is, in part, dependent on the glucocorticoid status of the mother (Langley-Evans *et al.* 1995). The enzyme 11β-hydroxysteroid dehydrogenase protects the developing fetus from excessive maternal glucocorticoids. The activity of this enzyme is decreased in placental homogenates of rats fed on a low-protein diet (Phillips *et al.* 1994). Rats administered dexamethasone during pregnancy (Benediktsson *et al.* 1993) have offspring which are hypertensive in adulthood. In order to provide an index of glucocorticoid status and action within the fetuses and offspring of rats exposed to a maternal diet low in protein, the activity of four brain enzymes, two glucocorticoid-sensitive and two-insensitive, were assayed in whole brain of day 20 fetuses and term neonates, and specific brain regions of weanlings.

Twenty-one female Wistar rats were fed on a 180 g casein/kg diet for 2 weeks before mating and during pregnancy. A further twenty-one females were fed on a 90 g casein/kg diet following the same protocol. Six rats in each group were killed at day 20 gestation, and the remainder proceeded to term. Upon giving birth, all dams were transferred to a laboratory chow diet. The offspring of six rats in each group were killed within 12 h. The remaining pups were killed at weaning (age 4 weeks) following determination of systolic blood pressure. Whole brain samples were obtained from the day 20 fetuses and neonatal rat pups. The brains of the weanlings were removed and the hypothalamus, hippocampus and cerebellum were dissected out. Assay of the specific activity of three glucocorticoid 'marker' enzymes, malate dehydrogenase (MD;1.1.1.37), glutamine synthetase (GS;6.3.1.2) and glycerol phosphate dehydrogenase (GPDH;1.1.1.8) was carried out on the samples obtained.

Brain region	Maternal diet (g casein/kg)	Specific Activity (nmol converted/min per mg protein) GS Mean	*n*	SEM	GPDH Mean	*n*	SEM
Hypothalamus	180	186.1	10	3.7	43.6	10	2.47
	90	236.6*	9	8.5	52.9*	10	2.60
Hippocampus	180	152.3	9	6.5	24.0	9	2.39
	90	186.8*	9	13.4	30.1	10	2.89
Cerebellum	180	184.0	10	8.1	14.8	9	1.30
	90	242.6*	10	12.1	21.0*	9	0.90

* Mean values were significantly different from the 180 g casein/kg dietary group, $P < 0.05$.

The two glucocorticoid-sensitive enzymes showed significantly increased activity in the brain regions of the low-protein-exposed group relative to the 180 g casein/kg-fed controls. The activities of GS and GDPH increased 31% and 42% respectively in the cerebellum of the 90 g casein/kg-exposed group. This was a specific effect as the activity of the steroid-insensitive enzyme MD was unaltered due to dietary experience. Neonatal GDPH activity was significantly increased ($P<0.05$) in the low-protein exposed group. No effect was observed in fetal brains.

These results are consistent with the hypothesis of *in utero* programming of later disease by maternal nutrition being mediated through over-exposure of the fetus to maternal glucocorticoids.

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Effect of methionine addition to a high-protein diet on growth and energy utilization in the rat.
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It has been reported that methionine supplementation improves growth rates in rats maintained on a high-casein (up to 350g/kg) diet (Donoso *et al.* 1964). Methionine addition to semi-purified diets containing very high amounts of casein (up to 700g/kg) is frequently reported in rat studies (Imai *et al.* 1986). The aim of the present study was to examine the effect of methionine addition to casein-rich diets.

Female Sprague-Dawley rats were weaned onto isoenergetic synthetic diets containing either 580g (HP) or 570g casein +10g methionine (HP+M), 50g sunflower-seed oil and 210g carbohydrate /kg diet. A third group was fed on a normal protein diet (NP: 170g casein, 620g carbohydrate and 50g sunflower-seed oil /kg diet). Growth rate was monitored regularly, N balance and body composition were measured in rats after 9 weeks on the respective diets.

Energy intake and food efficiency were similar in the three dietary groups. The HP+M diet did not increase the growth rate of rats above that exhibited by either the HP- or the NP-fed rats. Furthermore, N balance, body composition and carcass gross energy were all similar between HP and HP+M groups. However, in rats fed on either of the high-protein diets relative and absolute body fat were 21% lower than that of controls. In addition, about a 3-fold increase in N balance was observed in rats fed on the high-protein diets.

	NP ($n5$)		HP ($n7$)		HP+M ($n7$)		Dietary effect
Variable	Mean	SE	Mean	SE	Mean	SE	(ANOVA)
Live body weight(g)	240.5	7.1	226.9	8.0	223.3	5.3	NS
Energy intake KJ/d	239	8.1	244	7.7	249	7.1	NS
Dry body composition							
Fat (g/kg)	472	7.9	414	6.6	398	16.4	$P<0.01$
Protein (g/kg)	478	6.6	526	9.0	529	9.9	$P<0.01$
Ash (g/kg)	95.5	5.3	100.1	2.2	111.7	6.1	NS
Gross energy KJ/g carcass	29.4	0.88	27.3	0.58	26.3	0.34	$P<0.01$
Nitrogen balance							
Nitrogen intake (mg/d)	378	8.0	1171	43.0	1291	74.0	$P<0.001$
Faecal nitrogen (mg/d)	32.7	1.79	38.1	1.57	39.5	2.05	$P<0.05$
Urinary nitrogen (mg/d)	181	9.9	714	12.0	782	47.1	$P<0.001$
Nitrogen balance (mg/d)	164	8.8	418	38.4	470	61.9	$P<0.01$

The present results indicate that methionine addition to an already casein-rich diet has no functional value. It may also be inferred that prolonged intake of a high-protein diet can modify body composition enhancing protein accretion and decreasing fat deposition.

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Effect of food restriction on protein metabolism in obesity treated by gastric bypass surgery. By J. M. HIBBERT[1], D. C. GORE[1], J. N. CLORE[2], H. J. SUGERMAN[1] and L. WOLFE[1], *Departments of [1]Surgery and [2]Internal Medicine, Medical College of Virginia, Richmond, USA*

There is little information on the effects of food restriction on whole-body protein turnover (WBPT) in humans and some discrepancies have been observed. In the early studies Steffee *et al.* (1976) used oral [^{15}N]-glycine and showed that both synthesis and degradation of protein were increased when dietary protein intake was restricted. Subsequent investigations using [^{13}C]-leucine or [^{15}N]-glycine have failed to confirm this result, demonstrating instead reduction of both protein synthesis and degradation with low-protein and protein-free diets (Garlick *et al.* 1980). This discrepancy was thought to be due to long periods of [^{15}N]-glycine infusion in the early study, compared with subsequent shorter protocols (Garlick *et al.* 1991), the long infusion presumably fostering recycling of the label.

The present study was undertaken to explore further the effect of food restriction on WBPT, protein synthesis and proteolysis. Measurements were made in seven obese subjects taking adequate diets and 2 weeks after their treatment by gastric bypass surgery which causes mandatory food restriction. At 2 weeks post-operatively, the effect of surgery is expected to be minimal. During each study the corresponding diet was given as small 3-hourly meals. A single oral dose of [^{15}N]-glycine (100 mg) was given and turnover rates were calculated from the cumulative excretion of ^{15}N in urinary NH_3 over 12 h (Waterlow *et al.* 1978), thereby estimating protein (Pr) metabolism in peripheral tissues.

	Body wt (kg)	Intake (gPr/kg per 12 h)	WBPT (gPr/kg per 12 h)	Synthesis (gPr/kg per 12 h)	Degradation (gPr/kg per 12 h)
Adequate diet					
Mean	131	0.93	2.14	2.13	1.21
SD	23	0.20	0.22	0.23	0.20
Restricted diet					
Mean	122	0.28	2.24	2.23	1.97
SD	22	0.11	0.61	0.65	0.66
P value	0.0003	0.00008	NS	NS	0.04

Significance was obtained by the student's paired T-test

After 2 weeks of food restriction, protein intake was reduced by 70%. Significant weight loss (7%) was accompanied by slightly increased protein synthesis (5%) and significantly increased proteolysis (63%).

These preliminary results indicate that after 2 weeks of severe food restriction in gastric bypass patients proteolysis is significantly increased. The results appear to concur with those of Steffee *et al.* (1976) that food restriction may be associated with increased protein turnover. This metabolic alteration could be preceding loss of lean body mass during weight loss after gastric bypass.

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The effect of weaning practice on fermentation capacity in breast-fed babies By A.M. PARRETT, E. LOKERSE and C.A. EDWARDS, *Department of Human Nutrition, Glasgow University, Yorkhill Hospitals, Glasgow, G3 8SJ*

It is well established that the faecal flora of breast-fed (BF) babies differs from that of formula-fed (FF) babies (Edwards *et al.* 1994). BF babies have bifidobacteria and lactobacilli as predominant organisms in contrast to FF babies who have a higher proportion of enterobacteria and *Bacteriodes* spp. (Balmer and Wharton 1989). This is related to the decreased incidence of infectious diarrhoea in BF babies (Howie, 1990). The profiles of faecal short chain fatty acids (SCFA), the products of carbohydrate fermentation, also differ. BF babies produce mainly acetic acid and lactic acid, and we have previously demonstrated (Parrett *et al.* 1994) that BF infants are less able to ferment complex carbohydrates than FF babies.

It is not known whether the pattern of fermentation products and the low ability to ferment complex carbohydrates persist through the weaning process. When weaning begins, BF babies are exposed to a variety of new foods including dietary fibres and starch. The amount of starch reaching the colon is likely to be substantial because of the immature digestive tract in young babies (Verity and Edwards 1994). The populations of bacteria will have to adapt before they can ferment these new substrates and it is not clear how long this process takes. If the babies are presented with a substrate they are unable to ferment, it will pass through the colon unmetabolized and may cause a large increase in stool output. It is during the weaning process that BF babies are most susceptible to diarrhoea. It is therefore important to understand the development of fermentation capacity during weaning.

In the present cross-sectional study, an *in vitro* faecal incubation system was used to assess the ability of BF babies to ferment simple and complex carbohydrates. Fresh faecal samples were obtained from twelve exclusively BF babies (preweaning), seven early weaning (liquidized food) BF babies and eight late weaning (chopped food) BF babies and processed within 1 h of passage. Cultures containing 32g/l faeces and 1g/l carbohydrate (glucose, lactose, raftilose, soyabean polysaccharide and guar gum) in a tryptone basic salts medium were incubated anaerobically at 37°. A control culture with no carbohydrate was also incubated. After 24 h, SCFA and lactic acid in culture supernatant fractions were measured by GLC. Results were compared by Mann-Whitney U tests after subtraction of values from the control culture.

	Total SCFA concentration (μmoles/ml)					
	Breast-fed preweaning		Breast-fed early weaning		Breast-fed late weaning	
	Median	Range	Median	Range	Median	Range
Blank	18.0	5.6 - 28.0	13.4	0 - 22.5	18.8	12.1 - 29.3
Glucose	56.4	0 - 77.6	68.5	57.9 - 98.8	61.3	28.6 - 120.4
Lactose	48.7	0 - 63.0	60.9	30.7 - 83.6	63.3	48.3 - 96.0
Raftilose	31.0[a,bb]	3.6 - 48.9	57.1	2.5 - 70.6	68.6	22.0 - 113.4
Soyabean polysaccharide	12.3[bb]	6.9 - 40.2	10.8**	6.3 - 23.9	35.5	23.2 - 78.4
Guar gum	6.4b	0.1 - 57.3	18.4*	0 - 40.5	45.4	15.6 - 62.0

a $P<0.05$ preweaning compared with early weaning; b $P<0.05$, bb $P<0.01$ preweaning compared with late weaning; * $P<0.05$, ** $P<0.01$ early weaning compared with late weaning. All by Mann-Whitney U tests.

Babies at each stage of weaning were able to ferment simple sugars equally well. Ability to ferment raftilose, a fructo-oligosaccharide, increased in early weaning but ability to ferment complex carbohydrates did not develop until late weaning. Moreover, during late weaning the babies were still less able to ferment complex carbohydrates than the sugars. These results show that the development of fermentation capacity for complex carbohydrates is a slow process and indicate that dietary fibre and resistant starch in weaning foods may escape fermentation and are likely to have a significant effect on stool output.

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Colonic fermentation capacity is reduced in children with cystic fibrosis By A.M. PARRETT[1], D. CLARK[1], K. LOGAN[1], N. MORTON[1], T.J. EVANS[2] and C.A. EDWARDS[1], [1]*Department of Human Nutrition, Glasgow University, and* [2]*Yorkhill Hospitals, Glasgow G3 8SJ*

Carbohydrate which enters the large intestine is fermented by the colonic bacterial microflora producing short-chain fatty acids (SCFA). SCFA are easily absorbed and help salvage energy (Livesey, 1990). This mechanism may be more important in children with cystic fibrosis (CF) who have small-intestinal malabsorption of carbohydrates and fat, even when taking pancreatic enzyme supplements. Children with CF, however, frequently use antibiotics which may decrease the colonic salvage capacity by inhibiting bacterial fermentation. Bacteria also hydroxylate malabsorbed fats to hydroxy fatty acids (HFA) which have several adverse effects. They are inhibitory to the colonic bacteria, damage the colonic mucosa, stimulate secretion and propulsive motor activity, and may be involved in free-radical damage (Ammon & Phillips, 1974; Bull *et al*, 1988). In the present study, faecal SCFA and HFA and fermentation capacity for simple and complex carbohydrates of children with CF were compared with those of normal, healthy children of the same age.

Fresh faecal samples were collected from eight children with CF and eight healthy controls of similar age (1-9 years, median 5 years 1 months (CF) and 4 years 7 months (controls)) and processed within 1 h of passage. Cultures containing 32g/l faeces and 1g/l carbohydrate (glucose, lactose, raftilose, soyabean polysaccharide) in a tryptone basic salts medium were incubated anaerobically at 37°. A control culture with no carbohydrate was also incubated. After 24 h, SCFA and lactic acid in culture supernatant fractions were measured by GLC. In addition, faecal HFA was determined by GLC after extraction and conversion to methyl esters (modified method of Pearson, 1972). Results were compared by Mann-Whitney U tests after subtraction of values from the control culture.

Faecal cultures from normal children produced significantly more total SCFA from glucose, raftilose and soyabean polysaccharide than those of CF children ($P<0.05$). Of the total SCFA a significantly lower median molar proportion was butyrate in the CF children when glucose was fermented, 24.5 (range 0-212.8) compared with 181.4 (range 2.6-331.6). Butyrate is believed to be an important fuel for the colonic mucosa (Roediger, 1982) and to stimulate intestinal mucosal cell proliferation (Sakata, 1987). There was no significant difference in lactic acid production. CF children had significantly higher faecal hydroxystearic acid (CF 0.9,(range 0.1-3.3) mmol/l; controls 0,(range 0-0.2)) which may influence colonic function.

In conclusion, children with CF have reduced ability to ferment carbohydrate compared with normal children and this may severely reduce their capacity to salvage malabsorbed energy from the colon.

	Total SCFA concentration (μmol/ml)					
	Control Group			Cystic Fibrosis Patients		
	Median	Range		Median	Range	
Blank	20.24	12.85	- 48.87	13.56	6.49	- 31.31
Glucose	54.71*	38.23	- 73.27	19.49	0	- 64.84
Lactose	56.72	42.95	- 68.94	31.2	0	- 60.21
Raftilose	58.19*	27.52	- 82.20	26.47	0	- 63.73
Soyabean polysaccharide	31.79***	25.70	- 50.67	18.41	7.94	- 23.37

* $P<0.05$, *** $P<0.001$, compared with control group by Mann-Whitney U test.

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Is the ileostomist a good model for studies of starch digestion in man? By M.D. ROBERTSON[1], J.C. MATHERS[1], V. MISHRA[2] and G. LIVESEY[3], *Departments of [1]Biological and Nutritional Sciences and [2]Medicine, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU and [3]Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA*

Quantitative estimates of the extent of small-bowel digestion and of the supply of materials to the large bowel have been obtained using healthy human ileostomists (Andersson, 1992) but the reliability of this model remains uncertain. The objective of the present study was to compare the kinetics of digestion of a starch-rich food in both ileostomists and intact volunteers.

Six normal men (mean age 40 (range 22-56) years and six male ileostomists (mean age 53 (range 40-60) years) were admitted to the metabolic facility after an overnight fast. Cannulas were placed in a radial vein (for blood sampling) and in an ante-cubital vein (for infusion of [6,6-^{2}H]glucose). Two hours later, each subject consumed a test meal of 50 g [U-^{13}C]peas (*Pisum sativum*). Blood glucose concentration, ^{13}C-enrichment in exhaled CO_2 and total CO_2 production were measured at frequent intervals.

Fasting and peak blood glucose concentrations were similar in both groups but time to peak glucose concentration was significantly ($P<0.05$) longer for ileostomists (163 and 179 min for controls and ileostomists respectively). There was a second late (420 min post-meal) rise in blood glucose concentration in the ileostomists which was not seen in intacts. $^{13}CO_2$-enrichment was slower to increase but peaked at a similar level in ileostomists. The decline in $^{13}CO_2$ enrichment 420 mins post-meal was faster in the ileostomists.

The assumption that the kinetics of digestion within the stomach and small bowel of healthy ileostomists is the same as that in intact subjects was not supported by this study. The results suggest that digestion of a starchy food may be slower in ileostomists and that the time course of substrate supply is influenced by the presence of a functioning colon. Delayed gastric emptying in ileostomists might explain the extended time to peak glucose concentration. However, it should be noted that the ileostomists were, on average, older than the intact subjects and a possible confounding effect of age cannot be excluded although an earlier study from our group failed to detect any significant influence of ageing on brush border hydrolases or activity of the Na^{+}-dependent glucose transporter in duodenal mucosa (Wallis *et al.* 1993).

M.D.R. holds a BBSRC CASE studentship in collaboration with the Institute of Food Research, Norwich Laboratory.

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Dietary zinc prevents intestinal dysfunction in malnourished milk-sensitized guinea-pigs. By N.DARMON, M.A. PELISSIER, P.CHAPPUIS, C.CANDALH, M.A.BLATON, R.ALBRECHT, J.F.DESJEUX and M.HEYMAN, *INSERM U-290, 107 rue du Fg St-Denis, 75010 Paris, France.*

Oxidative stress and sensitization to food proteins are thought to play an important role in the intestinal dysfunction associated with malnutrition. We recently reported that sensitization to cow's milk proteins in guinea-pigs was enhanced during malnutrition. As Zn possesses antioxidant and immunoregulating properties, we studied the ability of dietary Zn to prevent intestinal dysfunction in malnourished guinea-pigs sensitized to cow's milk.

Control and malnourished guinea-pigs received a diet containing 300g or 40g protein/kg, and a normal Zn content (45 ppm). Another group of malnourished animals received the 40g protein/kg diet supplemented with 1800 ppm Zn. In the three diets, milk proteins were included to trigger intestinal anaphylaxis. We assessed intestinal function in vitro in jejunal segments mounted in Ussing chambers. Ionic conductance was assessed as an index of chloride secretion, and the increase in short-circuit current (Isc) induced by mast-cell mediators was used as an index of chloride secretion. Intestinal permeability was measured by [^{3}H]-mannitol and horseradish peroxidase (EC 1.11.1.7) fluxes. In intestinal homogenates, we measured the activities of superoxide dismutase (EC 1.15.1.1), Se-dependent glutathione-peroxidase (EC 1.11.1.9) and catalase (EC 1.11.1.6) to assess antioxidant defences, and the level of thiobarbituric acid-reacting substances (TBARS) as an index of free radical damage. Systemic milk sensitization was measured in serum samples by anti-ß-lactoglobulin IgG and IgE, using ELISA and by passive cutaneous anaphylaxis respectively. Intestinal anaphylaxis was tested in Ussing chambers by the Isc response to ß-lactoglobulin.

In malnourished animals, supplemented with Zn or not, the enzymic antioxidant activities and the TBARS level were not modified compared with controls, suggesting that malnutrition did not induce oxidative stress in the guinea-pig intestine. In malnourished animals, ionic conductance, Isc responses to mast-cell mediators, as well as mannitol and horseradish peroxidase transports were significantly increased compared to controls, indicating a hyperactivation of chloride secretion and an increased intestinal permeability. Malnourished animals presented higher IgG and IgE levels and intestinal anaphylactic response than controls, suggesting that malnutrition enhanced milk sensitization. By contrast, malnourished Zn-supplemented animals presented a lower ionic conductance than malnourished ones and Isc responses to mast cell-mediators as well as mannitol and horseradish peroxidase transports returned to control values. Zn-supplementation prevented the increase in antibody responses due to malnutrition, and inhibited intestinal anaphylaxis, even when compared with controls.

This study demonstrates the ability of Zn to prevent intestinal dysfunction during experimental malnutrition. The inhibition of intestinal anaphylaxis by Zn may contribute to this improvement.

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How the fatty acid composition of previous high-fat diets affects lipid absorption in rats. By P. DEGRACE, C. CASELLI, E. MIANOWSKI, I. NIOT, A. BERNARD and H. CARLIER, *Laboratoire de Physiologie de la Nutrition, EA DRED 580, Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation, Université de Bourgogne, 1 Esplanade Erasme, 21000 Dijon France*

Taking into account that in the postprandial state, intestinal lipoprotein fatty acids are derived from endogenous and exogenous sources (Shiau *et al.* 1985), 14 week-old Wistar rats were prefed for 4 weeks with diets containing either medium-chain triacylglycerols (MCT: 49% 8:0, octanoic acid, 43% 10:0, decanoic acid), sunflower-seed oil (SSO: 66% 18:2 *n*-6, linoleic acid), or menhaden oil (MO: 22% 20:5 *n*-3, eicosapentaenoic acid (EPA) + 22:6 *n*-3, docosahexaenoic acid (DHA)) 150 g/kg. Were investigated fatty acid composition of bile in fasting rats and intestinal lymph both in fasting rats and during absorption of a lipid emulsion. The lipid emulsion which was composed of monopalmitin (30 μmol), [^{3}H] oleic acid (30 μmol), linoleic acid (25 μmol) and [^{14}C] arachidonic acid (5 μmol) emulsified with sodium taurocholate, was infused intraduodenally. In fasting rats, either bile or intestinal lymph was harvested for 6 h. After infusion of the lipid emulsion, both the mass of recovered fatty acids in lymph and the time course for the appearance of the two labelled fatty acids showed that the maximum lipid absorption took place during the first 3 h after the lipid infusion. This absorption was accompanied by the presence of enlarged chylomicrons. Therefore, fatty acid composition analysis of intestinal lipoproteins in the postprandial state was carried out on lymph collected during this time period.

Whatever the high-fat diet, in fasting rats the fatty acid pattern of intestinal lymph reflected the composition of bile phospholipid fatty acids (Shrivastava *et al.* 1967). Moreover, except for palmitic acid, the weight percentage of which remained in the range from 23 - 29%, a strong effect of the previous diet was observed on bile and thus on mesenteric lymph fatty acid composition. This significant impact of the previous diets persisted on the fatty acid pattern of lymph after the lumenal lipid infusion.

Fatty acid	Palmitic 16:0		Oleic 18:1		Linoleic 18:2 *n*-6		Arachidonic 20:4 *n*-6		EPA 20:5 *n*-3		DHA 22:6 *n*-3	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Emulsion	31.1		34.2		28.3		6.3					
MCT	28.7	3	26.4	2.5	16.4	1.0	7.8	1.0	0.6	0.6	0.2	0.1
SSO	14.0	1.8	12.2	1.3	30.2	4.6	5.9	1.3	0.2	0.2	0.2	0.1
MO	23.8	3.0	14.6	2.2	8.0	1.6	2.8	0.8	7.6	1.9	6.2	1.4

Fatty acid composition (g/100g total fatty acids) of the lipid emulsion and of characteristic fatty acids of the mesenteric lymph collected during the 3 h following the lumenal lipid infusion. Values are means with their standard errors for five rats per group.

In MCT prefed rats, the metabolism of octanoic and decanoic acids yielded palmitic, stearic and oleic acids which were recovered in the bile and lymph of the fasting rats. Their persistence in the lymph after the lipid infusion maintained the closest similarity of the fatty acid pattern between the intestinal lymph and the lipid emulsion. In the SSO prefed rats, whatever the nutritional state of the rat, a significant persistence of linoleic acid and the presence of arachidonic acid were observed in bile and in lymphs of fasting and infused rats. In the MO prefed rats, despite some similarities with the MCT prefed rats, this was the group for which the endogenous contribution seemed to be particularly efficient giving dissimilar fatty acid patterns between the lipid emulsion administered and the recovered intestinal lymph. For example, although absent from the emulsion, a significant proportion of *n*-3 polyunsaturated fatty acids persisted in the lymph (Herzberg *et al.* 1992) accompanied by a decrease in arachidonic acid. All these results highlighted the contribution of endogenous fatty acids in intestinal lipoprotein formation in the postprandial state.

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Intestinal metabolism of α-linolenic acid during its absorption in the rat and mouse. By A. BERNARD[1], C. CASELLI[1], V. OKRASZEWSKI[1], P. BESNARD[1], J.P. BLOND[2] and H. CARLIER[1], [1]*Laboratoire de Physiologie de la Nutrition, EA DRED N° 580, Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation, 1, Esplanade Erasme, Université de Bourgogne, 21000 Dijon, France and* [2]*Unité de Nutrition Cellulaire et Métabolique, UFR Sciences de la Vie, France*

Intestinal absorption constitutes a first parameter of the bioavailability of polyunsaturated fatty acids. The goal of the present work was to investigate several aspects of linolenic acid (18:3 *n*-3) metabolism during its intestinal absorption and particularly to study if*in vivo*, this fatty acid is converted into docosahexaenoic acid (DHA) (Chen & Nilsson, 1994).

Mesenteric lymph was collected from adult Wistar rats for 6 h after a duodenal infusion of an equimolar mixture of [1-^{14}C] linolenic acid, oleic acid and monopalmitoylglycerol, emulsified by sodium taurocholate. During the 6 h, the recovered radioactivity in the lymph reached 13340 (SD 535) nmol equivalents linolenic acid *v.* 24100 (SD 731) nmol infused. Linolenic acid appeared to be more rapidly and efficiently absorbed than linoleic acid (Bernard *et al.* 1991) (Fig 1). The radioactivity of the different lipid classes analysed by TLC showed a high proportion of triacylglycerols: 90.3% (SD 0.9) compared with phospholipids: 2.7% (SD 0.3) during the first hour of absorption. This distribution of the linolenic acid in esterified lipids corresponds to the synthesis of chylomicrons visualized by electron microscopy.

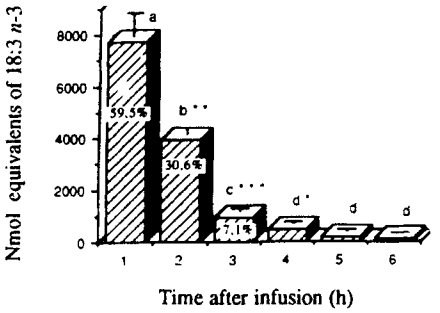

Fig.1. Appearance of 18:3 *n*-3 in lymph

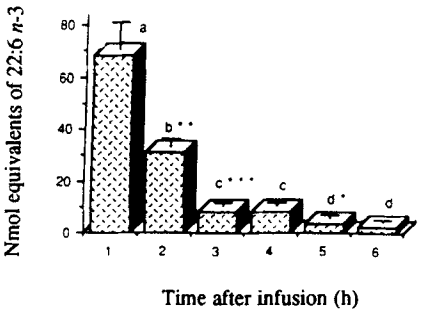

Fig.2. Appearance of DHA in the lymph

When assigned with different superscript letters, values were significantly different, *** P<0.001, ** P<0.01, * P<0.05.

The analysis of labelled fatty acids by HPLC (Ulmann *et al.* 1991) shows the presence in the lymph of DHA (Fig 2). The DHA recovered in 6 h in the lymph corresponds to 0.9 (SD 0.1)% of the 18:3 simultaneously absorbed. This observation suggests an intestinal conversion of linolenic acid without excluding a liver origin through biliary phospholipids and/or blood.

On a second group of rats, the bile was collected for 6 hours after a duodenal infusion of the same lipid mixture. The total radioactivity recovered in the bile: 44.3 (SD 0.6) nmol equivalents 18:3 *n*-3 during 6 h was not sufficient to show that the DHA recovered in the lymph derived from bile only, resulting from a conversion at the liver level.

Finally, a third set of experiments was carried out with mouse jejunal explants incubated in a lipid medium containing 0.8 mM linolenic acid and, 0.4 mM monoleoylglycerol in Dulbecco, NCTC emulsified by sodium taurocholate. At 30 min after incubation, 17.3 (SD 0.6) nmol equivalents of DHA were recovered to give 1.9% (SD 0.2) of the total Eq nmol of 18:3 *n*-3 in the explants. This result supports the hypothesis that the DHA may not originate from an uniquely hepatic conversion.

In conclusion, this work shows that 18:3 *n*-3 is rapidly absorbed in lipoprotein form, largely integrated in triacylglycerols. This absorption is accompanied by successive and alternative desaturations and elongations. Although low, this conversion appears essentially to originate from the intestine.

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Dose-response effects of dietary carrageenan (from *Eucheuma cottonii*) on blood lipids and gut tissues of rats. By C.S. ROPER, W. BAL and J.C. MATHERS, *Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU.*

Marine algae are considered to be an underutilized resource within Europe. Whilst they are the source of several phycocolloids used in the food industry, seaweeds are not eaten in nutritionally-important quantities. As part of a systematic investigation of the nutritional value and physiological effects of food grade seaweeds, the present study investigated responses to inclusion of a non-viscous carrageenan phycocolloid from the red seaweed *Eucheuma cottonii* (EC) in rats.

Semi-purified diets containing 0, 25, 50, 75 and 100 g EC/kg and 100, 75, 50, 25 and 0 g cellulose/kg were fed to male Wistar rats (five rats per treatment) for a period of 3 weeks. The remainder of the diet was of constant composition offered at 15 g/d. Responses to diets were examined using orthogonal polynomials within an analysis of variance.

	Dietary *Eucheuma cottonii* (g/kg)					SEM	yP value	
	0	25	50	75	100		Linear	Quadratic
Plasma conc. (mmol/l)								
Total cholesterol	0.77	0.76	0.69	0.67	0.66	0.05	0.063	0.755
HDL cholesterol	0.29	0.26	0.25	0.23	0.23	0.03	0.062	0.618
Triglyceride	0.88	1.04	0.77	0.70	0.82	0.11	0.179	0.770
Tissue weight (g)								
Small intestine	5.28	5.84	6.34	6.08	7.15	0.26	0.000	0.782
Caecum	0.34	0.38	0.46	0.46	0.46	0.03	0.003	0.118
Colon	0.98	1.03	1.17	1.22	1.31	0.02	0.001	0.974
Caecal pH	6.99	7.16	7.38	7.41	7.30	0.07	0.001	0.010

Food consumption by all diet groups was essentially complete and there were no differences in the body-weight gain ($P = 0.425$). Increasing EC at the expense of cellulose resulted in linear reductions in plasma total and HDL-cholesterol, but not plasma triacylglycerols. The HDL: total cholesterol ratio was unaffected by diet ($P = 0.898$). Gut tissue weights increased linearly with increasing carrageenan, although there was no change in crypt-cell proliferation rate (results not shown). Caecal pH increased linearly with additional carrageenan. This raised pH suggests either a change in the buffering capacity within the caecum or the production of alkaline end-products (e.g. NH_3) or both.

These results indicate a dose-response effect of this dietary carrageenan which has mild hypocholesterolaemic, but not hypotriacylglycerolaemic, effects. The hypocholesterolaemic effect occurred in the absence of stimulated fermentation in the large bowel (no fall in caecal pH). The cause of the gut tissue hypertrophy remains uncertain and requires further investigation.

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Comparative gastrointestinal responses to guar gum and a seaweed polysaccharide (sodium alginate) in rats. By C. J. SEAL and J. C. MATHERS, *Human Nutrition Research Centre, University of Newcastle upon Tyne, Wellcome Research Laboratories, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP*

Dietary soluble non-starch polysaccharides (NSP) stimulate cell proliferation and muscle mass in both the small intestine and large bowel (Goodlad *et al.* 1992), and have hypocholesterolaemic effects in experimental animals and man (Overton *et al.* 1994). The present experiment was designed to compare the effects of inclusion of guar gum (GG) and a seaweed polysaccharide (sodium alginate, SA), each at two concentrations, on gut tissue mass, large-bowel fermentation and plasma cholesterol concentration.

Groups of five individually housed male Wistar rats, initial average weight 100 g, were fed on 15 g/d of one of five semi-purified diets containing 0, 50 or 100 g GG or SA/kg diet respectively. The added NSP replaced some of the maize starch in the diet which otherwise remained constant and contained (g/kg) maize starch 250 (control diet), casein 200, sucrose 288, groundnut oil 150, vitamin premix 20, mineral premix 40, and Cr_2O_3 (indigestible marker) 2. After 14 d adaptation and a 7 d balance period, the animals were killed and samples of intestine and heart blood collected for analysis. Due to inappetence, two rats from the 100 g SA/kg diet were removed from the experiment. Statistical analysis was by GLM using the Minitab package.

	DIET						Probability of contrast*			
	Control	50GG	100GG	50SA	100SA	EMS	1	2	3	4
Small-intestine length (m)	1.07	1.29	1.24	1.21	1.23	46.9	0.002	0.003	0.958	0.075
Caecum tissue weight (g)	0.54	0.85	1.02	0.78	1.00	0.015	0.000	0.457	0.854	0.407
Colon tissue weight (g)	0.97	1.13	1.23	1.14	1.28	0.044	0.026	0.745	0.781	0.963
pH of caecal contents	6.62	6.32	6.14	6.75	6.74	0.023	0.006	0.573	0.000	0.000
Caecal total VFA (mmol/l)	79.5	101.5	105.6	62.3	70.4	12.6	0.069	0.462	0.001	0.000
Plasma cholesterol (mmol/l)	0.91	0.83	0.72	0.79	0.69	0.034	0.064	0.967	0.814	0.725

EMS, error mean square.
* 1, Linear (L), and 2, quadratic (Q) effect of added NSP; 3, LxNSP source interaction; 4, QxNSP source interaction.

Caecal and colonic tissue weights increased linearly with additional NSP, regardless of source, and small-intestinal length was increased by about 15%. Increased caecal fermentation was evident in rats fed on GG but not those fed on SA in which the pH of caecal contents was not different from the control group, and the concentration of VFA in caecal contents was not increased. Plasma cholesterol concentrations decreased linearly ($P = 0.064$) with additional GG and SA. These results confirm both the trophic response of intestinal tissues and hypocholesterolaemia associated with increased intake of soluble NSP. In the case of rats fed on SA, these responses are independent of fermentation in the large intestine and potential mechanisms are being investigated further.

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Modulated role of proteolytic enzymes in the digestion of food proteins. By L. SAVOIE[1], K. ARVANITI[1], L. GIGNAC[1] and N. MERCIER[1], (introduced by M.F. FULLER[2]), [1]*Département de nutrition humaine, Université Laval, Québec, Canada,* [2]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Digestion of food proteins in single-stomached animals is affected by the sequential or simultaneous action of various proteolytic enzymes. The level and composition of digestive juices, in the stomach as well as in the small intestine, are modulated by the amount and quality of food (Corring, 1980).With regards to proteins, recent results showed that the overall digestibility was not affected by the composition of pancreatic enzyme mixtures. However, the form and the rate of release of amino acids was modified but not according to the proportion of enzymes in the mixtures (Valette *et al.* 1993).

The present study showed the impact of pepsin (*EC* 3.4.23.1) and trypsin (*EC* 3.4.21.4) on the digestion of casein, fish and soyabean proteins. Protein sources were hydrolysed for different periods of time with various enzyme:substrate (E/S) ratios. At optimal pH (1.9), there was rapid production of TCA-soluble material (MW < 10 000 D) after 30 min digestion (E/S=1/50). With a lower enzyme concentration, equivalent levels of digestion could be reached after a longer period. None of these treatments affected specifically the proportion of peptide bonds hydrolysed (as measured by O-phtaldialdehyde method) in the TCA-soluble material.

	TCA-soluble material (%)				Peptide bonds hydrolysed (mg Leu equivalent)			
E/S	1/50		1/250		1/50		1/250	
Digestion period (min)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fish proteins								
30	55.8	14.0	31.8	3.2	37.2	4.2	19.1	3.3
120	71.2	5.7	51.9	6.6	54.5	4.3	33.9	5.2
Casein								
30	23.7	2	8.6	1.1	14.2	0.6	6.7	0.4
120	44.9	3.2	24.4	5.6	22.8	3.3	12.8	1.0
Soyabean proteins								
30	51.8	6.9	20.4	36.	22.2	0.9	9.0	0.3
120	68.7	10.1	45.3	3.5	36.3	2.5	19.8	2.5

These mixtures were, in a second step, hydrolysed by pancreatin with simultaneous dialysis of digestion products (MW < 1 000 D) (Savoie & Gauthier, 1986). After 6 hours, the levels of digestion products were respectively 55, 45 and 47 % for fish, casein and soyabean and were not affected by the previous intensity (duration or E/S) of peptic hydrolysis. In the case of fish and casein, no differences were noted when pepsin prehydrolysis was omitted, while this produced a 35 % reduction with soyabean.

On the other hand, with equivalent pepsin digestion of the three substrates, inhibition of tryptic activity of pancreatin by soyabean trypsin inhibitor type II S resulted in a 50 % reduction of fish and casein digestibility, but only of 11 % for soyabean protein. These findings suggest that, from a strictly enzymic point of view, the specific impact of enzymes on the nature and rate of release of absorbable materials was related to the protein structure.

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Glutamine utilization by the healthy rabbit intestinal epithelium, *in vitro*, using [^{14}C]-and [^{15}N] glutamine. By R.N. MAZUMDER, S.K. NATH, M. RONGIER, D. DARMAUN and J.F. DESJEUX, *Unité de Recherches sur les Fonctions Intestinales, le Métabolisme et la Nutrition, INSERM U. 290, Hôpital Saint-Lazare, 75010 Paris, France*

L-Glutamine is considered to be a potential candidate for use in oral rehydration solutions, the mainstay of treating dehydration due to diarrhoea. This is based on the fact that glutamine stimulates Na absorption in the small intestine of animals and patients with cholera. An additional potential advantage of glutamine utilization is its central role in energy metabolism. In the search for a relationship between energy utilization from glutamine and stimulation of Na absorption by glutamine, we found unexpected results (Nath *et al.* 1992) : in our *in vitro* model of experimental bacterial diarrhoea in the rabbit, we found that glutamine was actively absorbed and stimulated electrogenic Na absorption, but it was not metabolized by the transporting epithelium. The aim of the present study was to assess the presence of glutamine metabolism in an isolated piece of rabbit ileum, *in vitro*.

L-Glutamine (gln) transport across isolated ileum of ten healthy weanling rabbits was studied in the fasting state. To pieces of tissue in the Ussing chamber both 10 mM [^{15}N] gln and 185 Bq [U-^{14}C] gln were added to either the mucosal or serosal side (hot side) ; unlabelled 10 mM gln was added to the other side (cold side). After a 30 min period of stabilization, electrical variables were recorded using voltage-clamping as an index of steady electrolyte transport and samples were collected from the cold side for 180 min. Samples were used for measurement of ^{14}C dpm in a scintillation counter for gln-equivalent flux calculation and of [^{15}N] gln in a gas-chromatograph-mass-spectrometer for gln flux calculation.

Addition of 10 mM gln to Ringer's solution stimulated short-circuit current by (64.5 (SE 16.9) μA/cm^2 ; $P < 0.05$). The gln-equivalent ^{14}C flux from mucosa to serosa was greater (1.06 (SE 0.22) μmol/h per cm^2) than the [^{15}N] gln flux (0.34 (SE 0.10) μmol/h per cm^2 ; $P < 0.05$) ; no difference was observed in the serosa to mucosa fluxes (^{14}C, 0.45 (SE 0.08) v. [^{15}N] gln, 0.32 (SE 0.12). The resulting net flux was significantly greater ($P < 0.05$) when measured by ^{14}C (0.64 (SE 0.15) than [^{15}N] (gln 0.02 (SE 0.09) μmol/h per cm^2).

These results indicate that during transport of electrolytes and glutamine across isolated rabbit ileum in a fasting state, the C constituents of glutamine are transferred to other molecules.

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Endogenous nitrogen and amino acid flow in the terminal ileum of preruminant calves fed on skimmed milk, soluble wheat and soyabean isolate proteins. By G.H. TOLMAN and G.M. BEELEN, *TNO Nutrition and Food Research Institute, Department of Animal Nutrition and Physiology ILOB, Wageningen, The Netherlands*

The nutritional value of a dietary protein is mainly determined by its true ileal amino acid digestibility and by the amount and re-utilization of endogenous protein secreted in the gut lumen in response to the ingested protein.

In the present work, studies of endogenous N and amino acid flow in the terminal ileum and of true digestibilities of N and amino acids were made in preruminant calves of 70 kg live weight, which received diets containing either skimmed milk powder (SMP; *n* 4), a 50:50 mixture of SMP and soluble wheat protein (SWP+SMP; *n* 2) or soyabean isolate (SI; *n* 3) as the only source of protein. N uptake on average was 45.7 g/d. A ^{15}N-isotope dilution technique (Schulze, 1994) was used to measure the endogenous N flow at the distal ileum. Amino acid composition was determined on digesta.

In calves fed on SMP, 95% of the ileal N flow was of endogenous origin. This indicated that the true ileal N digestibility of SMP was close to 100%. Values for true ileal N digestibility of SWP+SMP and SI were found to be 97.5% and 96.4% respectively.

To calculate the endogenous flow of amino acids of the SWP+SMP- and the SI-fed animals, it was assumed that (1) the true digestibility of amino acids of SMP is 100%, (2) the amino acid composition of the ileal digesta for the SMP-fed animals represents that of the endogenous secretions and (3) the amino acid composition of the endogenous protein flow at the distal ileum is relatively constant and independent of the diets tested in this experiment. Thus, endogenous amino acid flows for the SWP+SMP and SI treatments were calculated as follows:

$$\frac{\text{endogenous N flow, test}}{\text{endogenous N flow, SMP}} \times \text{endogenous amino acid flow, SMP.}$$

In the Table amino acids are included with true ileal digestibilities differing 2½% or more from the true ileal N digestibility (not for SMP).

	Endogenous flow terminal ileum (g/d)			Undigested flow terminal ileum (g/d)			True ileal digestibility (%)		
	SMP	SWP/SMP	SI	SMP	SWP/SMP	SI	SMP	SWP/SMP	SI
N	4.72	5.08	4.88	0.00	1.12	1.68	100[a]	97.5[ab]	96.4[b]
Leu	1.43	1.54	1.48	0.00	0.31	1.67	100	98.7	92.7
Lys	1.45	1.56	1.50	0.00	-0.14	0.06	100	100.7	99.6
Cys	0.68	0.73	0.70	0.00	0.25	0.20	100	94.5	94.8
Thr	1.88	2.02	1.94	0.00	-0.03	0.52	100	100.3	95.7
Ala	1.05	1.13	1.08	0.00	0.11	0.93	100	98.5	92.3
Asp	2.00	2.15	2.07	0.00	0.21	2.09	100	98.5	93.6
Glu	3.65	3.93	3.77	0.00	5.03	2.04	100	94.2	96.7
Gly	0.98	1.05	1.01	0.00	0.32	1.10	100	94.8	90.8
Pro	1.41	1.52	1.46	0.00	2.39	0.52	100	92.6	96.7

[a,b]Means without a common superscript differed significantly: $P<0.05$.

Relatively higher amounts of Cys, Glu, Gly and Pro were present in the undigested part of the SWP, whereas undigested SI protein contained correspondingly more Leu, Ala, Asp and Gly. It appears that peptide chains composed of these amino acids are difficult for the calf to digest.

Schulze, H. (1994). Endogenous ileal nitrogen losses in pigs: dietary factors. PhD Thesis, Wageningen

Digestion of maize starch and native pea starch in the small intestine of pigs. By H. EVERTS, R.A. DEKKER, B. SMITS and J.W. CONE. *Institute for Animal Science and Health, ID-DLO , Department of Nutrition of Pigs and Poultry, PO Box 160, 8200 AD Lelystad, The Netherlands*

In the Dutch net energy system, complete enzymic digestion of starch at the end of the small intestine is assumed. However, this digestion can be incomplete and the rates of digestion can differ between sources of starch. Therefore we have measured, in a cross-over design with four cannulated pigs (Mroz *et al.* 1993), the ileal digestibility of two diets at a feeding level of 870 kJ digestible energy/$kg^{0.75}$. Diet M contained 650 g maize starch/kg and diet P 650 g native pea starch/kg. The remaining 350 g/kg supplied all amino acids, fat, fibre, minerals and vitamins. The diets and the chyme samples were analysed for water-insoluble starch (WIS), water-soluble starch (WSS), maltose and glucose. Starch digestibility was calculated on the basis of the sum of glucose units originating from WIS, WSS, maltose and glucose. Ileal digestibility of starch on diet M was 0.983 and on diet P was 0.975. Ileal digestibility of protein and of some amino acids (cystine, serine, threonine, phenylalanine and tyrosine) was significantly lower on diet P than on diet M.

In a slaughter experiment the same diets were fed at the same feeding level to twelve animals (six on diet M and six on diet P). After 14 d on the diet, all animals were killed 3.5 h after feeding. Immediately, chyme samples were collected from the stomach, from the small intestine (SI A: the first 1/3, SI B: the second 1/3 and SI C: the third 1/3) and from the caecum. Analysed levels of WIS, WSS, maltose and glucose in the diets and the chyme samples were (g/kg DM):

	Diet		Stomach		SI A		SI B		SI C	
Treatment	M	P	M	P	M	P	M	P	M	P
Water-insoluble starch	550	636	239	598	14	200	28	38	33	24
Water-soluble starch	72	0	240	6	52	34	26	10	4	2
Maltose	3	3	55	3	39	55	22	18	1	1
Glucose	12	4	4	1	212	180	114	228	9	42

Digestibility of the starch up to the different parts of the intestinal tract was calculated using Cr_2O_3 as a marker. Starch digestibility up to the stomach was low on diet M and negative on diet P. In the other parts of the small intestine and the caecum the starch digestibility was:

	SI A	SI B	SI C	Caecum
Diet M	0.63	0.86	0.99	0.99
Diet P	-0.04	0.80	0.98	0.99

Pea starch lowered the ileal digestibility of protein (0.78 *v* 0.75) and some amino acids. There was no difference in the ileal digestibilities of the two types of starch, but the rates at which they were digested did differ (maize > native pea). The lower protein and amino acid digestion on diet P could be explained by a higher endogenous secretion due to this lower digestion rate. Availability of glucose and amino acids in portal blood is reported by Van der Meulen *et al.* (1995).

Mroz, Z., Bakker, G.C.M., Jongbloed, R., Jongbloed, A. & van der Honing, Y. (1993). *Journal of Animal Science* **71**, 160.

Van der Meulen, J., Bakker, J.G.M., Smits, B. & De Visser, H. (1995). *Proceedings of the Nutrition Society* (In the Press).

Portal appearance of glucose and amino acids in the pig after feeding maize starch and native pea starch. By J. VAN DER MEULEN, J.G.M. BAKKER, B. SMITS and H. DE VISSER, *Institute for Animal Science and Health, ID-DLO, Department of Nutrition of Pigs and Poultry, PO Box 160, 8200 AD Lelystad, The Netherlands*

Although ileal starch digestibility of maize starch and native pea starch did not differ, maize starch was digested faster than pea starch, and pea starch decreased the ileal digestibility of protein and some amino acids (Everts *et al.* 1995). In the present study the portal appearance of glucose and amino acids (AA) was measured after feeding those two types of starch.

Diets consisted of 650 g maize starch (diet M) or native pea starch (diet P)/kg and the remaining 350 g/kg supplied all amino acids, fat, fibre, minerals and vitamins. The diets were fed at a feeding level of 870 kJ digestible energy/$kg^{0.75}$ twice daily (06.00 and 18.00 hours) to four female pigs (initial weight 39.0 (SD 2.4) kg) in a cross-over design. The pigs were fitted with catheters in the portal vein and mesenteric vein and artery. Portal vein blood flow was determined by the *p*-aminohippuric acid dilution technique (Yen & Killefer, 1987). Blood samples were taken 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 h after morning feeding to determine absorption of glucose and AA.

Portal vein blood flow did not differ between the diets and averaged 36.5 (SD 4.6) ml/min per kg. Compared with diet P, absorption of glucose from diet M was significantly higher in the first 6 h after feeding, not different 8 h after feeding and significantly lower thereafter. The amount of glucose absorbed over 12 h from diet M was higher than from diet P ($P=0.054$) and accounted for 97% and 72% respectively of the glucose supplied to the small intestine. Absorption of AA from diet M 2 to 4 h after feeding was higher and thereafter lower than from diet P, but these differences were not significant. The total amount of AA (TAA) absorbed over 12 h from diet M was lower than from diet P, but this difference was only significant for essential AA (EAA) and not for non-essential AA (NEAA).

	Portal flux (mmol)				Portal appearance* (%)			
	Diet M	Diet P	SED	*P*	Diet M	Diet P	SED	*P*
Glucose	1759	1265	183	0.05	97	72	10	0.05
EAA	183	225	13	0.04	80	96	4	0.02
NEAA	71	89	22	0.48	34	42	11	0.52
TAA	265	326	33	0.14	61	73	7	0.16

* Based upon amounts ingested corrected for ileal digestibility.

It can be concluded that absorption of glucose from maize starch was faster than from pea starch, which is in agreement with the rate of ileal starch digestion (Everts *et al.* 1995). Although ileal starch digestibility did not differ between maize and pea starches (Everts *et al.* 1995), the total amount of glucose absorbed over 12 h was lower for pea starch. This implies that utilization of glucose by the gastrointestinal wall or fermentation of starch in the intestine differs after feeding maize and pea starches. The portal appearance of AA was higher after feeding pea starch. However, the ileal AA digestibility was lower for pea starch, which may have been caused by the slower starch digestion resulting in a higher endogenous secretion (Everts *et al.* 1995). The slower digestion of pea starch may, on the other hand, have provided a more gradual supply of glucose (energy) to the gastrointestinal wall which may have spared AA and/or may have prevented competition between AA and glucose for transport carriers.

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Inter-animal variability in the energy expenditure of splanchnic tissues in preruminant calves.
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Preruminant veal calves, reared solely on milk diets are often known to present sub-deficiencies in Fe which result in reduced haemoglobin levels and which may impair growth. Indeed, packed cell volumes (PCV) are known to be highly variable in a preruminant calf population as compared with those in older ruminants. Any modification of the transport of O_2 in blood will have consequences on the O_2 supply to tissues. The question was thus raised as to whether such a phenomenon could interfere with the oxidative metabolism of splanchnic tissues, mainly the portal-drained viscera (PDV) and liver which contribute 17.2 and 12.8% respectively to total energy expenditure of standing animals (Ortigues *et al.* 1995).

A total of eleven 2-3-week-old preruminant male Holstein x Friesian calves were surgically equipped with blood catheters at the PDV and liver levels (Durand *et al.* 1988). All calves were fed on a commercial milk replacer in two equal daily feedings for a daily gain of 1 kg/d (Ortigues *et al.* 1995). After 2 weeks of adaptation, blood samples were taken 6.5, 7.0, 7.5 and 8.0 h after the morning meal, for PCV and total blood O_2 determination. Splanchnic blood flows were estimated according to Durand *et al.* (1988) at 47 and 5.6 ml/min per kg live weight at the portal venous and hepatic arterial levels respectively. Animals were slaughtered immediately after the last sampling for partial dissection.

Average live weight was 66.4 (SE 2.37) kg of which 4.35 (SE 0.201) % was adipose-tissue free PDV and 2.51 (SE 0.083) % liver, and average live weight gain was 993 (SE 47.8) g/d. Only one hepatic catheter was non functional for the experiment.

Blood PCV levels averaged 0.23 (SE 0.021) with a range varying between 0.15 and 0.32. Arterial, portal and hepatic concentrations of O_2 averaged 4.12 (SE 0.436), 2.86 (SE 0.388) and 2.26 (SE 0.408) mmol/l respectively. The inter-animal variability in arterial concentrations (A) was much higher (CV 35.1%) than that noted in adult ruminants (e.g. CV 7.5% in ewes, Ortigues & Durand, 1995) and was tightly correlated to PCV levels according to the following relationship $A = 20.29\ PCV - 0.56$ (r^2 0.93, $P<0.01$). Total O_2 consumption of PDV and liver averaged 3.84 (SE 0.299) and 2.67 (SE 0.221) mmol/min and remained uncorrelated to PCV. However, extraction rates of O_2 (ER) which amounted to 32.90 (SE 3.153, range 22.54-52.27) % and 31.06 (SE 4.592, range 12.22-53.69) % at PDV and liver level, were significantly and negatively correlated to PCV in the PDV : $ER_{PDV} = -120.01\ PCV + 60.61$ (r^2 0.62, $P<0.01$) and liver $ER_L = -185.16\ PCV + 74.10$ (r^2 0.77, $P<0.01$). Finally, the metabolic activity of PDV and hepatic tissues averaged 81.3 (SE 7.24) and 96.6 (SE 7.88) μmol O_2/h per g fresh tissue, confirming previously published results (Ortigues *et al.* 1995). It is noteworthy that the metabolic activity of PDV (MA_{PDV}) increased significantly with PCV : $MA_{PDV} = 224.69\ PCV + 29.42$ (r^2 0.41, $P<0.05$).

In conclusion, Fe sub-deficiencies in preruminant calves detected in terms of highly variable PCV levels are determinant for O_2 supply to tissues. The latter might then be able to satisfy their O_2 requirements by significantly modulating their O_2 extraction rates. Nevertheless, the influence of PCV on the metabolic activity of digestive tissues suggests that tissue O_2 consumption is partly determined by O_2 supply. Consequently, in energy metabolism experiments that are conducted at tissue level with preruminant calves, it would seem highly advisable to allot animals to treatments according to their PCV levels.

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Volatile fatty acid absorption and water flux in the rumen of sheep in relation to the volatile fatty acid supply. By S. LOPEZ and F.D.DEB. HOVELL, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

Volatile fatty acids (VFA) produced by fermentation in the rumen are the major energy source for the ruminant. Three lambs nourished by intragastric infusion of nutrients (VFA and McDougall's buffer into the rumen, casein into the abomasum) were used in a Latin square design to study the effect of VFA supply to the rumen on VFA absorption and water flux. The basal rate of VFA infusion was 270 mmol/h, using a 2.1 M VFA solution with (mmol/mol total VFA): acetate 650, propionate 250 and butyrate 100. VFA supply was modified by changing the concentration of the infusate, to give experimental rates of 135, 394 and 511 mmol/h. The volumes and ratio of VFA to buffer were kept constant. All animals received the basal infusion rate of VFA during the first 2 h, and then VFA at the experimental rate for the next 7.5 h. Rumen samples were taken at 0 h, 2 h, and every 1.5 h thereafter. Rumen volumes and liquid outflows were estimated using PEG and Cr-EDTA. (Drinking water and casein were withdrawn on experimental days.)

Treatment (VFA infusion, mmol/h)...	135	394	511	SED
Total VFA (mmol/l)	71[a]	155[b]	194[c]	6.6
Rumen pH	6.8[a]	5.7[b]	5.4[b]	0.13
Rumen osmolality (mOsmol/kg)	218[a]	316[b]	363[c]	6.9
Rumen volume (litres)	5.2	5.4	5.2	0.16
Liquid outflow (ml/h)	391	408	408	12.1
Water absorption rate (ml/h)	-14	-23	-19	10.5
VFA absorption rate (mmol/h)	105[a]	332[b]	437[c]	10.9

Rumen VFA concentration and rumen osmotic pressure (OP) increased and pH declined linearly ($P<0.01$) with VFA infusion rate. The proportion of VFA supply absorbed from the rumen was higher as the VFA infusion rate was increased (0.775, 0.844 and 0.854 respectively), and calculated as the slope of the linear relationship between VFA infused (x) and VFA absorbed (Y) was Y = 0.88x (SE 0.014) - 13.6 (SE 5.09). There was no significant ($P>0.05$) effect of VFA infusion rate on rumen volume, liquid outflow rate and net flux of water across the rumen wall (the difference between liquid infused and outflow), although for VFA concentrations up to 77.5 mM a trend for increasing liquid outflows associated with increasing VFA concentrations was observed, whereas for VFA concentrations above 77.5 mM, liquid outflow remained stable at an average rate of 408 ml/h. Surprisingly we were unable to detect differences in water absorption or outflow associated with changes in OP, unlike previous work (von Engelhardt, 1970; Lopez *et al*, 1994).

The support of the Scottish Office, Agriculture and Fisheries Department and of the Spanish Comisión Interministerial de Ciéncia y Tecnologia is gratefully acknowledged.

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Comparison of apparent absorption of essential amino acids from the small intestine and their net flux across the mesenteric and portal drained viscera of lambs. By JOHN C. MacRAE, LES A. BRUCE, DAVID S. BROWN and DAVID A.H. FARNINGHAM. *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

Growing lambs (30-35 kg live weight) prepared with trans-organ catheters (into the arterial (AA), mesenteric (M) and portal (P) vasculature) and intestinal cannulations (into the duodenum and/or jejunum and ileum) were fed on pelleted lucerne ration (710 g DM, 18 g N/d) sufficient to maintain N (N retention 0.2 (SE 0.19) g N/d). The disappearances of essential amino acids (EAA) from the small intestine (SI) were compared with the net flux of these across the mesenteric (MDV) and portal (PDV) viscera. Recovery of apparently absorbed EAA in the MDV (1.04 (SE 0.025)) was greater ($P<0.05$) than in the PDV (0.70 (SE 0.044)), even though all the MDV drainage enters the PDV.

	mmol/d					
	Apparent absorption from SI		MDV flux		PDV flux	
	Mean	SEM	Mean	SEM	Mean	SEM
Leucine	45.3	1.5	43.9	12.0	31.3*	2.9
Valine	36.6	1.1	38.4	3.5	22.1*	2.5
Lysine	32.5	0.05	29.4	2.4	17.0*	2.6
Isoleucine	29.2	0.8	32.8	2.3	19.9*	1.7
Threonine	30.8	0.5	31.3	2.0	24.0*	1.6
Phenylalanine	23.5	0.9	25.2	2.0	19.3*	2.2
Methionine	8.7	0.5	9.3	1.7	5.2*	0.8
Histidine	7.5	0.7	8.3	2.2	6.7	1.7
Proportional recovery of apparently absorbed EAA			1.04 (SE 0.025)		0.70 (SE 0.044)	

*Significantly less than MDV flux, $P<0.05$.

The consistently high recovery of apparently absorbed EAA across the MDV seems to question the earlier suggestion, derived from the PDV flux data of Tagari & Bergman (1978) that EAA are selectively and preferentially used by gastrointestinal tract tissues during absorptive metabolism. Instead, the disparity between the MDV and PDV fluxes ($P<0.05$) probably indicates net utilization of arterial EAA for protein synthesis in regions of the gastrointestinal tract which are not drained by the MDV. This could occur if a proportion of the degradation products from protein turnover in these regions entered the lumen of the tract rather than the venous drainage. If this is the case, then these results might, at least in part, substantiate the earlier suggestions (MacRae & Reeds, 1980) that a substantial proportion of duodenal digesta protein could be of endogenous rather than dietary (microbial) origin.

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Rat serum HDL_2-HDL_3 compositions are not affected by the consumption of different gluten intakes whereas lecithin-cholesterol-acyl-transferase activity is altered. By S. SENOUCI[1], M.Y. LAMRI[1], D. AIT-YAHIA[1], M. MEGHELLI-BOUCHENAK[1] and J. BELLEVILLE[2], [1]*Laboratoire de Physiologie Animale et de la Nutrition, Institut des Sciences de la Nature, Université d'Oran, Algérie,* [2] *Unité de Nutrition Cellulaire et métabolique, Université de Bourgogne, Dijon, France.*

Diets containing casein generally induce hypercholesterolaemia whereas vegetable proteins have a hypocholesterolaemic effect (Terpstra *et al.* 1991; Kurowska & Caroll, 1992). The aim of the present study was to determine whether lecithin-cholesterol-acyl-transferase (LCAT; EC 2.3.1.43) activity is sensitive to dietary vegetable protein levels.

Changes in LCAT activity were studied in growing rats, during the consumption of different gluten diets (100, 150, 200 and 300 g/kg) for 28 d. LCAT activity was determined by conversion of [^{3}H] cholesterol into [^{3}H] esterified cholesterol (Glomset & Wright, 1964 modified by Knipping, 1986). Serum HDL_2-HDL_3 were separated by discontinuous gradient ultracentrifugation (Meghelli-Bouchenak *et al.* 1987), and their lipid and apolipoprotein compositions were determined. Apolipoprotein electrophoresis was assayed according to the method of Irwin *et al.* (1984). The amount of HDL_2-HDL_3 expressed in g/l was the sum of the concentrations of triacylglycerol, phospholipid, unesterified cholesterol, cholesteryl ester and apolipoprotein contents.

With 100 and 150 g/kg gluten diets LCAT activity (nmol/ml/h) was reduced to about 60% of the values obtained with the 200 and 300 g/kg gluten diets ($P < 0.005$). However, HDL_2-HDL_3 amounts and their protein and lipid compositions were similar in the four groups after 28 d consumption of these different diets, except for HDL_2-HDL_3-apolipoprotein A-I, a cofactor for LCAT activity which increased with the 300 g/kg gluten diet compared with the 100 g/kg and 150 g/kg gluten diets.

Gluten (g/kg)	100		150		200		300	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LCAT (nmol/ml/h)	5.39^b	1.19	5.36^b	1.51	8.66^a	0.50	8.90^a	1.45
HDL_2-HDL_3 (g/l)	1.33^a	0.55	1.50^a	0.21	1.53^a	0.52	2.14^a	0.36
Apo A-I (g/l)	40.50^b	11.90	43.10^b	10.04	51.00^{ab}	7.50	60.00^a	2.28

^{a,b,c} Means values within a row with unlike superscript letters were significantly different, $P < 0.05$.

It could be suggested that low LCAT activity and apo A-I, in the 100 and 150 g/kg gluten diets were not sufficient to impair significantly HDL2-HDL3 metabolism. Moreover, the reduced LCAT activity could be explained by LCAT hepatic synthesis reduction induced by low vegetable protein diets.

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Taurine metabolism in cats fed on commercial canned or dry foods. By O. P. BALLEVRE [1], F. MONTIGON [1], J. VUICHOUD [1], K. JOHNSTON [2], S. MARTI[1] and L. B. FAY [1], *[1]Nestec Ltd. (Friskies Research) PO Box 44, CH 1000 Lausanne 26, Switzerland and [2] Department of Small Animal Medicine and Surgery Royal Veterinary College, University of London, North Mymms, Hatfield AL9 7TA*

Taurine is an essential amino acid for cats and the requirement differs depending on the processing of the food, i.e. 96 for canned and 60 μg/kJ for extruded dry food respectively (Douglas *et al.* 1991). Up to now the mechanism of this difference has not been established. The use of [^{15}N] taurine tracer in cats showed that taurine degradation was lower when extruded dry food rather than canned food was fed (Ballevre *et al.* 1993). However, in this study, taurine intake was 62% lower with dry than with canned food. The objective of the present study was to evaluate taurine metabolism in cats fed on canned and dry foods with similar taurine concentrations.

Five European cats were fed successively on canned and dry commercial foods for 6 weeks each. In the two foods, taurine concentrations were 115 and 131 μg per kJ respectively. At the end of each period, an oral bolus dose of [^{15}N]taurine (63 mg) was given and urine was collected for the following 5 d. [^{15}N]taurine was analysed by gas chromatography mass spectrometry (Staempfli *et al.* 1993). Total urinary ^{15}N and [^{15}N]urea were analysed by isotope ratio mass spectrometry. Fecal and urinary taurine were analysed by amino acid analyser. Taurine production rate (TAU PR) was calculated from single exponential fitting of taurine tracer : tracee ratio. Taurine synthesis and degradation were calculated as the difference between TAU PR and taurine intake and taurine excretion in urine and feces respectively.

	Taurine Intake	Taurine Excretion	Taurine Balance	^{15}N taurine	^{15}N-urea	^{15}N other	Taurine PR	Taurine Synthesis	Taurine Degradation
	μmol/kg per d			% total urinary ^{15}N			μmol/kg per d		
Canned diet	280	132	148	73.5	12.7	14.3	447	167	315
SE	18	15	12	4.6	1.5	3.2	38	24	25
Dry diet	344 *	265 *	79 *	91.4 *	5.7 *	3.3 *	584	240	318
SE	23	19	13	2.2	0.9	2.3	64	53	47

* Significantly different by paired *t* test from canned food, $P<0.05$.

Whole blood taurine concentration was significantly higher in cats fed on the dry (570 (SE 40) μmol/L) compared with the canned food (400 (SE 22) μmol/L) reflecting a better metabolic conservation of taurine despite the fact that taurine balance was 47% lower in cats fed on dry compared to canned food. For both foods, half of the ^{15}N dose was recovered in urine in 5 d. A significantly higher proportion was recovered as [^{15}N]taurine with dry than with canned food. As a consequence [^{15}N]urea represented 5.7 and 12.7% of total urinary ^{15}N for dry and canned food respectively. This suggests a higher degradation rate of taurine in cats fed on canned food. By contrast none of the taurine kinetics (PR, synthesis, degradation) was significantly modified by the type of food. Taurine synthesis had a tendency to be higher with dry food which implies a better overall balance. This can be illustrated by a significantly higher amplification factor (1- synthesis/net balance) with dry than with canned food (4.1 (SE 0.9) and 2.1 (SE 0.2) respectively).

Therefore better conservation of taurine in cats fed on dry food could be the result of an increase of substrate cycling by modification of both synthesis and degradation. Furthermore comparison of these results with those of the previous experiment (Ballevre *et al.* 1993) suggests a stimulation of taurine degradation with increasing taurine intake. Further work is required to better understand the complex metabolism of this special amino acid.

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Number and affinity of subcutaneous insulin receptors during growth in Large White and Meishan pigs. By M. CAMARA and J. MOUROT, *Station de Recherches Porcines, INRA, 35590 St Gilles, France*

It is now well recognized that pig subcutaneous adipose tissue is the main site of energy storage of the body and that adipocytes are sites of high metabolic activity. Studies examining endocrine regulation of adipocyte processes have reported that insulin plays a prominent role in the regulation of glucose metabolism and in fatty acid mobilization. However, the variations of fatty acid synthesis in pig backfat with age do not allow any conclusion about either the enzymes involved in these processes, the substrate availability or the insulin sensitivity. The present work was conducted to study possible differences in number and affinity of insulin receptors of backfat adipocytes during animal growth.

Genetically lean Large White (LW) and obese Meishan (MS) male pigs were slaughtered at 21, 80 and 180 d life, (eight animals per age group). Backfat adipose tissue was removed between the 13^{th} and 14^{th} lumbar vertebra. The binding of ^{125}I porcine insulin to isolated adipocytes was studied after digestion of adipose tissue with collagenase.

Two populations of receptors (I and II) with different affinities (K_{D1} and K_{D2}) have been identified. In LW pigs, the numbers of receptors I and II (R_1 and R_2) differed with age ($P<0.05$). The affinities of these two receptors (K_{D1} and K_{D2}) did not vary significantly during growth. In MS pigs, the binding intensity differed with age ($P<0.05$). The affinities of both classes of receptors did not change during animal growth. The number of type I receptors varied significantly ($P<0.05$) with animal age whereas the number of receptors II did not change during animal development.

	Large White					Meishan				
Age (d)	Size*	K_{D1} 10^{-10}M	K_{D2} 10^{-8}M	R_1 10^{-12}M	R_2 10^{-9}M	Size*	K_{D1} 10^{-10}M	K_{D2} 10^{-8}M	R_1 10^{-12}M	R_2 10^{-9}M
21	44.6	2.9	1.2	4.1	3.0	45.8	2.1	1.3	4.9	2.6
80	46.3	2.5	0.9	2.4	4.5	50.9	0.01	1.3	1.6	0.1
180	58.8	4.2	1.2	3.5	0.3	60.8	1.0	1.5	4.2	1.9

* diameter of adipocyte (μm)

The affinity of type I receptors (K_{D1}) was significantly higher in MS pigs ($P<0.001$) than in LW pigs (1.05×10^{-12} M and 2.47×10^{-10} M in 80 d old MS and LW pigs respectively) and could explain the higher lipid synthesis in young MS pigs due to the higher substrate availability. The affinity of type II receptors was similar in both breeds of pigs. The number of type I receptors (R_1) was higher in LW pigs ($P<0.05$) whereas the number of type II receptors (R_2) was similar in both breeds.

Thus, both number and affinity of insulin receptors change during animal growth and are correlated with the variations of adipose cell size. They could explain at least in part the variations with age in the synthesis capacity and in the lipid content of adipocytes between the two breeds of pigs.

Urea kinetics in healthy pregnant women eating habitual and 60 g protein diets. By I. McCLELLAND, C. PERSAUD and A.A. JACKSON. *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

There are increased demands for protein during pregnancy and the FAO/WHO/UNU (1985) expert consultation recommended an additional intake of 6 g protein/d, based upon limited balance data. Dynamic measurements of protein turnover or urea-N salvage can provide additional information on the ability of the diet to satisfy the demands for protein and N.

Nine healthy pregnant women were recruited from antenatal clinics at 16 weeks gestation. They were studied at 16, 24 and 32 weeks gestation. Urea kinetics were measured over 48 h using the single-dose oral presentation of [$^{15}N^{15}N$]-urea (Jackson *et al.* 1993) first while the women were consuming their habitual protein intake and again 4 d later after acclimatization to a diet which provided 60 g protein /d.

		Intake		Urea production		Urea excretion		Urea salvage		Salvage/production
				(mgN/kg per d)						(%)
Week	Intake	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean
16	habitual	184	49	207	50	123	38	84	38	40
	60 g protein	153	14	163*	44	92*	28	71	6	44
24	habitual	181	54	209	36	96	39	112	30	55
	60 g protein	144	17	164	65	71	26	93	73	51
32	habitual	162	36	150	47	81	26	69	34	45
	60 g protein	136	14	152	51	69	23	83	43	53

* Significantly different from habitual, ANOVA: $P < 0.05$.

The values for urea kinetics on the habitual intake were similar to those obtained previously with the use of the intermittent dose method (Unpublished results). Salvage increased absolutely and as a percentage of production from the first to the second trimester, indicative of an increase in demand. Habitual intake, production and salvage fell to the third trimester. On the 60 g protein diet production and excretion were significantly reduced in the first trimester, but by the third trimester were similar to values on the habitual intake. On 60 g protein salvage as a percentage of production increased progressively from the first to the third trimester, but there was wide variability amongst individuals and the differences were not statistically significant. The changes in urea kinetics were much more subtle than have been observed in women in Jamaica (Forrester *et al*, 1994), but suggest that although both diets were adequate to sustain pregnancy adaptation took place on 60 g protein.

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Nitrogen or protein intake and urinary 5-oxoproline excretion in adults. By C. PERSAUD, T. MEAKINS, R. BUNDY and A.A. JACKSON, *Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX*

In normal adults low levels of 5-oxoproline are excreted in urine, but there may be wide variability amongst individuals. Because excretion increases when the glycine pool is artificially depleted (Jackson *et al.* 1987), we have used the rate of excretion as an index of glycine status, especially during growth. The endogenous formation of glycine is reduced on low N intake (Yu *et al.* 1985) and we were interested to know how protein or N intake influenced 5-oxoproline excretion. Urinary excretion of 5-oxoproline over 24 h was measured in individuals who were taking control diets for at least 5 d as part of other studies. The experimental diets were based on 30, 35, 70 g of protein, and 30 g of protein supplemented with either 6.9 or 13.7 g of urea. Urinary 5-oxoproline was isolated by short column chromatography, hydrolysed and measured as glutamic acid.

The increase in 5-oxoproline excretion was seen on the fifth day on the low-N diet, but not earlier (Table, Wilcoxon rank sum test). On day 5 there was a graded decrease in 5-oxoproline excretion as protein intake increased from 30 to 35 to 70 g protein (r -0.54; P=0.012, n 21). Similarly on day 5, individuals taking 30 g protein and supplemented urea, there was a graded decrease in 5-oxoproline excretion as the total N intake increased from 4.8 to 8 to 11.2 g N/d (r -0.58; P=0.012, n 18). The fall in excretion with increased protein intake was not different to the fall in excretion on the increased N intake (overall, r-0.59; P<0.001)

Intake (Protein+urea) (g N/d)	5-Oxoproline (μmol/d) (mean (95% CI))			*P* d1 v d5
	Day 1	Day 4	Day 5	
4.8	234(152-233)	276(212-340)	510(259-761)	0.001
8.0	219(24-462)	228(140-316)	392(306-480)	0.05
11.2	327(113-541)	283(170-397)	277(236-318)	NS

The results show that in healthy people the level of 5-oxoproline is related to the dietary intake of N, either as protein or as protein with urea-N, once 4 d has been allowed for equilibration on the diet. As the *de novo* formation of glycine is reduced on a low-protein diet (Yu *et al.* 1985), we would interpret our findings as indicating that the increase in 5-oxoproline after 4 d is a reflection of the time required to deplete the endogenous pool of glycine. Further, it may be that the urea-N is used either directly or indirectly to sustain the endogenous formation of glycine.

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Factors influencing postprandial protein utilization in adults and the elderly. By A. FEREDAY[1], N. GIBSON[2], M. COX[2], D. HALLIDAY[3], P.J. PACY[2] and D.J. MILLWARD[1]. [1]*Centre for Nutrition and Food Safety, University of Surrey, Guildford GU2 5XH,* [2]*Nutrition Research Unit, St. Pancras Hospital, London NW1 0PE, and* [3] *Human Metabolism Research Group, St. Mary's Hospital Medical School London W2 1PG*

Nitrogen homeostasis in man requires that postabsorptive losses are repleted in the postprandial state. The efficiency of postprandial protein utilization in individuals is an important physiological component of their ability to maintain N balance. This efficiency will be determined by the partition of the amino acid intake between oxidation (O) and net protein deposition which is dependent on changes in rates of protein synthesis (S) and proteolysis (D). The main factors which mediate changes in these processes are the postprandial increases in insulin and amino acids but it is not known how changes in the sensitivity of these three processes to feeding influences the efficiency of postprandial protein utilization or the extent of any change with age. We have described a stable-isotope procedure for measuring the efficiency of postprandial protein utilization (PPU; Gibson *et al.* 1994) based on assessment of leucine balance (intake-oxidation), during a constant infusion of [^{13}C]leucine in subjects in the postabsorptive state and fed successively low- and high-protein meals. PPU is calculated from the slope of the leucine balance-intake relationship between the two intake levels and evaluation of leucine kinetics enables S and D to be measured. We report here an investigation of the way variation in the sensitivity of S, D and O to feeding influence postprandial protein utilization in normal adults and in a group of ten elderly men and women, some of the results having previously been presented (Fereday *et al.* 1994).

Ten men and five women aged 19 and 58 years, and five men and five women aged 68-91 years, were studied, all in good health. We measured [^{13}C]leucine balance, during a single 9 h prime dose constant infusion of [1-^{13}C]leucine with three 3 h phases, (i) the postabsorbtive state, (ii) low-protein feeding (30 min feeding of 1/24 of daily energy needs and 2% protein energy) and (iii) high-protein feeding (30 min feeding of approximately 14% protein energy), the protein fed at the habitual intake level based on a dietary questionnaire and 24 h urinary N collections. The frequent small meals ensured a metabolic steady state. Leucine balance was calculated as leucine intake minus leucine oxidation, calculated from $^{13}CO_2$ excretion and plasma α-ketoisocaproate enrichment, measured during the third hour of each 3 h phase.

PPU varied between 0.58 and 0.99 (0.80 mean: cv 13%), with the elderly group not different from the younger adults overall (0.795 mean: cv 16%) since both lowest (0.58) and highest values (0.99) were found in this group. Overall protein utilization was mediated by significant inhibition of D, stimulation of S and was accompanied by a stimulation of O. As expected the magnitude of PPU was inversely correlated with the increase in O. The main determinant of PPU was the inhibition of D since stimulation of S was associated with a parallel stimulation of O and negatively correlated with PPU. These relationships were not different in the elderly. A likely mechanism is that subjects who exhibit maximal inhibition of D on feeding exhibit minimal postprandial increases in tissue free amino acid concentrations thereby limiting increases in amino acid oxidation and, coincidentally, protein synthesis.

Variation in insulin levels with feeding did not account for any variability in postprandial responses. A calculated amino acid sensitivity of D (% inhibition/incremental amino acid intake) did predict PPU (r 0.782) and in the elderly those with low PPU values (0.68 (SD 0.06); n 5 compared with 0.91(SD 0.57); n 5) had values for amino acid sensitivity of D which were only 20% of the high PPU group. Thus these results show that variation in the efficiency of postprandial protein utilization between individuals reflects variation in the amino acid sensitivity of the inhibition of proteolysis to feeding with low levels accounting for the reduced efficiency of postprandial protein utilization which is observed in some elderly individuals.

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VLDL binding by isolated hepatocytes in rats fed on animal or vegetable protein diets (100, 200 or 300 g casein or soyabean proteins/kg). By J. PROST, M. NARCE, S. PEYRON and J. BELLEVILLE, *Nutrition Cellulaire et Métabolique, Université de Bourgogne, Dijon, France*

As we have previously shown that soyabean protein are hypotriacylglycerolaemic compared with casein, we have investigated whether protein origin (animal or vegetable) and levels modify VLDL binding by isolated hepatocytes.

Twenty-four male Wistar rats weighing 80 (SD 5) g were divided into six groups. Rats were fed for 4 weeks on diets containing (g/kg) 100, 200 or 300 casein (C10, C20 and C30) or highly purified (98% purity) soyabean proteins, (SP10, SP20 and SP30) with 50 soyabean oil, 50 cellulose, 20 vitamins, 40 minerals, and starch to make up to 1 kg. [^{3}H] VLDL and total lipoproteins were prepared using the technique of Meghelli-Bouchenak et al. (1991). Isolated hepatocytes were obtained according to Seglen's technique (1973). Proteins were measured on the pellet using Shacterle & Pollack's technique (1973). For each assay, isolated hepatocytes corresponding to about 100 µg protein were incubated with [^{3}H] VLDL in a total volume of 150 µl Krebs-Henseleit (containing 20 mg / ml bovine serum albumin) for 60 min at 37°, under continuous stirring. After incubation, fractions were poured over presoaked glass microfibre filters (Whatman GF/C) under vacuum. The filters were rapidly washed with 25 ml incubation buffer, dried and counted. Saturation analysis was performed by hepatocyte incubation with labelled [^{3}H] VLDL (25 µg protein/ml, specific radioactivity 500 Bq/µg protein) with or without unlabelled total lipoproteins (200 µg/ml). Specific binding was determined by subtracting non-specific binding ([^{3}H] VLDL + total lipoproteins) from total binding ([^{3}H] VLDL).

Protein source and level	C 10		C 20		C 30		SP 10		SP 20		SP 30	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
VLDL receptors (µg VLDL/mg hepatocytes)	20.1^{d}	7.6	59.4^{b}	4.9	21.5^{d}	7.2	39.0^{c}	4.3	70.5^{a}	11.8	36.5^{c}	11.5
Plasma triacylglycerols (mmol/l)	0.25^{b}	0.04	0.77^{a}	0.15	0.71^{a}	0.05	0.27^{b}	0.05	0.25^{b}	0.11	0.35^{b}	0.36

^{a,b,c} Means values within a row with unlike superscript letters were significantly different. $P < 0.05$.

The table shows that rats fed on casein diets developed fewer hepatocyte VLDL receptors than those fed on soyabean proteins. Compared with diets containing 200 g protein/kg the 100 or 300 g casein or soyabean proteins/kg diets induced lower VLDL binding by hepatocytes. Plasma triacylglycerols were significantly diminished with the diets containing 200 or 300 g soyabean proteins/kg compared with diets containing the same casein levels.

These results indicate that soyabean protein diets compared with casein diets enhanced more VLDL binding by hepatocytes, which may partly explain the hypolipaemic effect of these vegetable proteins.

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Postprandial serum promoted cholesterol efflux depends on fatty acid composition of the oral load. By SANA SAKR[1], CLAUDE SENAULT[1], DOMINIQUE VACHER[1], NATHALIE FOURNIER[2], MALIKA HAOURIGUI[3], NICOLAS GAUSSERES[1], and ANIK GIRARD-GLOBA[1], *[1]Groupe Lipoprotéines and [3]INSERM U224, Faculté de Médecine X. Bichat, BP 416, 75870 Paris Cedex 18, France and [2]Laboratoire de Biochimie, Hôpital Broussais, 75014 Paris, France*

The regular ingestion of monounsaturated fatty acids (MUFA), mainly oleic, is known to be associated with a relative protection against cardiovascular heart disease risk. We have tested the hypothesis that differences in chylomicron metabolism after a single fat load can sufficiently modify high density lipoproteins (HDL) to improve capacity for reverse cholesterol eflux, an essential step in the prevention of atherogenesis.

Serum-promoted cholesterol efflux was measured *in vitro* on [^{3}H]-cholesterol labelled Fu5AH cells after ingestion, by six normolipidaemic women, of each of four fat loads differing only in fatty acid composition. In parallel, HDL_2 and HDL_3 subfractions were isolated by ultracentrifugation and characterized by their chemical composition. Cholesterol efflux was higher 4, 6 and 8h after oleic-sunflower (73% MUFA), 8h after mixed oil (39% MUFA and 50% polyunsaturated fatty acids (PUFA)) or beef tallow (50% saturated fatty acids and 45% MUFA) ($P<0.02$ or $P<0.05$, compared with fasting values). No change occurred after sunflower (67% PUFA). Percent HDL_3 phospholipids was higher 6 and 8h after oleic-sunflower and 8h after mixed oil ($P<0.01$ or $P<0.05$, compared with fasting values). Variation in cholesterol efflux correlated positively with variation in percent HDL_3 phospholipids after oleic-sunflower ($r=0.929$; $P=0.007$ at 6h). Using purified HDL_3, efflux was higher 6h after oleic-sunflower ($P<0.05$) but not after sunflower, and variation in cholesterol efflux correlated with variation in HDL_3 phospholipid concentration in medium ($r=0.913$; $P=0.011$). Thus the capacity of serum to promote cholesterol efflux in the postprandial state is influenced by the nature of ingested fats. The protective effect of MUFA against atherogenesis might be partly mediated by an enhanced ability of postprandial serum to accept cell cholesterol as a consequence of higher phospholipid availability on HDL_3 particles.

Gastric lipase activity is modulated by dietary fat intake in human subjects. By M. ARMAND [1,2,4], M. HAMOSH [2,4], J. S. DIPALMA [3], J. GALLAGHER [5], S. B. BENJAMIN [5], J. R. PHILPOTT [2,4], P. HAMOSH [3,6] and D. LAIRON [1]. *[1]INSERM Unité 130, National Institute of Health, Unité de Recherche sur le Transport des Lipides, 18 Avenue Mozart, Marseille, France, [2] Division of Developmental Biology and Nutrition, [3] Division of Gastroenterology, [4] Department of Pediatrics, [5] Division of Gastroenterology, Department of Medicine, [6] Department of Physiology and Biophysics, Georgetown University, Medical Center, Washington DC, USA*

In rats (Hamosh, 1978; Armand *et al.* 1990), rabbits (Borel *et al.* 1991) and pigs (Armand *et al.* 1992) preduodenal lipase, lingual or gastric depending on the animal species, adapts to dietary fat . The aim of the present study was thus to determine whether the amount of dietary fat might modulate the activity of gastric lipase (EC 3.1.1.3) in healthy human subjects.

Six healthy subjects (age 29 (SE 3 years) two males, four females) were asked to undertake two 2-week periods of either high-fat (about 50% energy as fat) or low-fat (about 25% energy as fat) diet. As checked by dietary records, mean daily fat intakes were 116 g (53% of energy) and 40 g (23% of energy) respectively. After every diet period and an overnight fast, the subjects were equipped with a naso-gastric tube and gastric juice was collected. The collection period lasted 2 h, the first hour under basal conditions and the second hour after pentagastrin (6 μg/kg) stimulation. Gastric juice volume, acid output and pH were determined. Gastric lipase and pepsin activities were measured by conventional assay methods at 15 min intervals and total enzyme output was calculated.

Higher mean outputs of gastric lipase and pepsin were observed after the high-fat diet under baseline conditions (744.8 *v.* 445.7 U/h and 107 677 *v.* 78 505 U/h respectively). After pentagastrin stimulation, significantly higher outputs ($P<0.05$) were observed following the high-fat diet period for gastric lipase (1322.5 *v.* 875.3 U/h) and pepsin (191 751 *v.* 128 961 U/h). Diets had no effect on gastric juice volume, acid output and pH.

There was a positive correlation between gastric lipase output after pentagastrin stimulation and the daily fat intake (r 0.84, $P = 0.0007$) whereas no correlation was found with the daily intake of protein, carbohydrate or energy.

In conclusion, the activities of gastric lipase and pepsin were higher in healthy subjects 2 weeks after changing from a low-fat to a high-fat diet. Thus, this study reports for the first time that a high-fat diet leads to an increased activity of gastric enzymes in humans and the results obtained indicate that the change in gastric lipase secretion is specific to the fat content of the diet. The ability of gastric lipase to adapt to the fat content of the diet would result in more extensive hydrolysis of fat in both stomach and upper small intestine, thus allowing for an assimilation of fat even in the presence of limited amounts of pancreatic lipase.

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Effects of *n*-3 polyunsaturated fatty acids on lipid storage-related enzymes in adipose tissue of sucrose-fed insulin resistant rats. By A. QUIGNARD-BOULANGE[1], S. FLUTEAU-NADLER[2], S.W. RIZKALLA[2], B. ARDOUIN[1], M. KABIR[2], J. LUO[2] and G. SLAMA[2], *[1]INSERM U177, Institut Biomédical des Cordeliers, Paris, [2]INSERM U 341, Centre Hospitalier Hôtel-Dieu, Paris, France*

Previously we showed that inclusion of *n*-3 polyunsaturated fatty acids in a lard-enriched diet ameliorates the diet-induced insulin resistance in rat adipose tissue. As the high-lard diet dramatically decreased lipid storage-related enzyme activities such as fatty acid synthetase (FAS; EC 2.3.1.85) and lipoprotein lipase (LPL; EC 3.1.1.34), addition of 15% of *n*-3 polyunsaturated fatty acids to this high-fat diet prevented the decrease in these enzyme activities (Benhizia *et al.* 1994). These findings prompted us to examine the effects of dietary *n*-3 polyunsaturated fatty acids on the metabolic capacity of adipose tissue within another rat model of insulin resistance induced by sucrose feeding.

Five-week-old Sprague-Dawley rats were fed for 3 weeks on a diet containing 50% energy as sucrose, 20% as protein and 30% as fat. Fat in the diet was provided either by standard oil (9% palm oil, 9% lard, 6% corn oil, 6% peanut oil) (group SC), standard oil (10%) + fish oil (20%; Maxepa, Pierre Fabre Medicament, France) (group SP) or standard oil (10%) + olive oil (20%) (group SO).

	SC		SP		SO	
	Mean	SE	Mean	SE	Mean	SE
Body weight (g)	315	3.9	305	7	302	10
Triacylglycerolaemia (g/L)	2.9	0.2	0.77*	0.08	4.57*†	0.56
Retroperitoneal adipose tissue						
- 2 pads weight (g)	3.1	0.3	2.5*	0.3	2.8†	0.1
- LPL (U/g)	18.4	3.3	57.7*	9.2	22.7†	6
- FAS (U/mg prot.)	22.6	4.1	40.6*	7.3	9.5*†	1.6

*Significantly different from SC group, $P < 0.01$.

†Significantly different SO versus SP group, $P < 0.05$.

The table shows that fish-oil compared with standard-oil feeding decreased hypertriacylglycerolaemia induced by sucrose feeding. Fish-oil-fed rats showed similar body weight but exhibited a significant 20% decrease in retroperitoneal fat-pad weight when compared with control and olive-oil-fed rats. Replacement of control oil by 20% olive oil as compared with control oil diet markedly decreased FAS specific activity by 50% and did not significantly modify LPL activity in adipose tissue. By contrast adipose tissue LPL activity in fish-oil-fed rats was 3-fold higher than with the control diet while FAS activity was increased by 40%.

The partial replacement of control oil by fish oil in a high-sucrose diet prevents the deleterious effect of sucrose feeding on the development of hypertriacylglycerolaemia and hyperinsulinaemia. These findings also show a specific effect of *n*-3 polyunsaturated fatty acids which results in a marked increase in LPL activity associated with a sustained FAS activity in adipose tissue. To know whether polyunsaturated *n*-3 fatty acids may exert their specific effects through direct regulation of gene expression is under investigation.

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Provision of Asian foods in hospital. By P.C. McGLONE, J. W. T. DICKERSON and G.J DAVIES.
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It is now 18 years since Hill *et al* (1977) reported malnutrition in surgical patients in a British hospital. However, the problem still largely exists today. McWhirter & Pennington (1994) recently assessed 500 patients, revealing that 200 of these were undernourished on admission to hospital and 112 of these when re-assessed on discharge had lost a mean of 5.5% of their body weight. One reason for patients becoming malnourished is not consuming the food provided. Little research has focused on the types of foods available in British hospitals and particularly on the availability of foods suitable for minority groups which could lead to malnutrition in these groups.

In the present study eighty questionnaires were completed by hospital caterers to ascertain the availability of Asian foods in British hospitals. Two hospitals from each of five areas with large Asian populations were selected for analysis.

Area of hospital	Hospital beds (n)	Total Asian population (n)	Percentage Asian sub - groups				Reported availability of Asian foods	Availability of Asian foods on the menu
			P	I	B	O		
Inner London+	350	220391	1.2	3.0	2.8	1.8	√	√
Inner London+	735						√	√
Outer London+	650	413349	1.4	6.5	0.4	1.6	√	not supplied
Outer London+	350						√	√
Lancashire+	663	51208	1.9	1.5	0.2	0.1	√	√ -Gujarati
Lancashire*	500						√	®
Grt Manchester+	450	97478	2.0	1.2	0.5	0.2	x	x
Grt Manchester+	1000						√	√
West Yorkshire+	520	124849	4.0	1.7	0.3	0.2	√	√
West Yorkshire+	238						o	√-Urdu

+ Acute hospitals, * psychiatric hospital; P, Pakistani; I, Indian; B, Bangladeshi; O, Other Asian; ®, Asian foods available on request; x, no Asian foods available; o, did not specify.

These findings indicate that there are differences in the provision of foods in hospitals within areas. The results also indicate that there are discrepancies in what the caterer thinks is being provided and what is provided. The size of the hospital does not determine the availability of Asian foods. The results also suggest that the cultural diversity within the Asian group is not fully appreciated by the providers of hospital food. The language in which the menu is presented is of paramount importance for food choice. ' Foods available on request ' suggests that communication between patients, ward staff and the catering department are important if patients are to receive appropriate food.

In conclusion, the results from this investigation highlight the need for more research into the types of foods available in hospital for ethnic minorities and the acceptability of these foods by the patients.

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Comparison of a food-frequency questionnaire with a 4 d weighed record in South Asians. By N.A.KARIM[1] and B.M.MARGETTS [1,2], [1]*Institute of Human Nutrition and* [2]*Wessex Institute of Public Health Medicine, University of Southampton, Southampton SO16 7PX*

Food-frequency questionnaires (FFQ) are often the preferred method in epidemiological studies as they are simple,easy to administer and require minimal effort from the subjects themselves (Boeing *et al.* 1989). However to date no FFQ has been calibrated for use in South Asians. Thus as part of a bigger study to investigate the relationship between nutritional status and diabetes in South Asians,we compared a FFQ which consisted of seventy-five food items usually consumed by the South Asians with a 4 d weighed record (WR). The FFQ was administered first by interview and then followed by the 4 d WR. A total of fifty-eight South Asians (twenty-three men,thirty-five women) between the ages of 19 and 76 years of Pakistani,Gujerati Indian and Punjabi ethnic groups participated in this study.

	WR mean (95%CI)	FFQ mean (95%CI)	adj FFQ mean (95%CI)	MD %MD FFQ	MD %MD adj FFQ
Energy (kJ)	7816 (7125,8502)	10301 (9301,11297)	8878 (8095,2309)	2485 +32	1062 +14
Fat (g)	82 (72,92)	123 (107,138)	89 (79,99)	41 +49	7 +9
CHO (g)	235 (215,255)	282 (258,307)	279 (256,302)	47 +20	44 +19
Protein (g)	60 (54,65)	72 (65,79)	65 (60,71)	12 +21	5 +9

MD, mean difference (FFQ-WR); %MD, (mean FFQ-mean WR)/mean WR; adj FFQ, adjusted FFQ with with adjustments made to meat portions and fat frequencies; CHO, carbohydrate.

The Table presents the comparison of mean nutrient intakes by FFQ, adjusted FFQ and WR with 95% confidence interval. Ideally the mean difference between measures should be less than or equal to 10%; and only fat and protein in the adjusted FFQ measured in this study showed this level of agreement. Rank correlation coefficients showed a low correlation between dietary methods suggesting a weak association. This implies that both the absolute levels and ranking of the subjects are different between methods. For epidemiological studies,comparing across ranges of intake, the FFQ may provide reasonable results, but this study shows that further developments of the FFQ in this population are required.

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Changing dietary intake: a plate model approach. By A. S. ANDERSON and M. E. J. LEAN, *Department of Human Nutrition, Royal Infirmary, Glasgow G31 2ER*

Dietary recommendations for diabetes (DNSG, 1988) advocate about 50% energy from carbohydrate, <35% from fat, <10% from saturates. However, dietary studies throughout Europe have shown that patients fail to achieve these goals.

To demonstrate the proportions of dietary items in everyday meals, a plate model approach has been developed to establish the sizes of food helpings on a plate with divisions approximating to a meal with 35% energy as fat, 55% as carbohydrate and 10% as saturates (Armstrong and Lean 1993). Two adhesive discs representing the food portion have been produced, one with three segments (e.g. meats, potatoes, vegetables) and one with two segments (e.g. spaghetti, meat/vegetable sauce).

To assess this approach, sixteen non-insulin dependent diabetic patients were recruited from routine clinics after completing 4d weighed diet studies. A dietitian demonstrated the plate model approach and spent up to 10 min explaining the proportions of food which should be eaten at meals, emphasizing the importance of carbohydrate-rich foods and decreasing amounts of high-fat foods. Fasting triacylglycerol and total serum cholesterol levels, height and weight were also measured at baseline.

At 6-10 weeks later subjects repeated the 4d weighed diet study and blood tests. Ten subjects (seven men and three women, mean age 57.2 (SD 8.5) years), completed diaries and blood tests on both occasions. Six took oral hypoglycaemic drugs, four were on diet alone.

	Baseline		Post plate-model		Significance
	Mean	SD	Mean	SD	
Energy (KJ)	8079	1954	6837	1205	NS
% energy fat	37.9	4.5	31.5	6.0	P <0.01
% energy SFA	13.6	3.9	10.0	4.3	P <0.05
% energy MUFA	10.9	3.0	8.8	3.2	NS
% energy PUFA	5.6	1.8	4.1	2.2	P <0.01
% energy carbohydrate	41.9	6.4	46.9	6.7	P <0.05
% energy protein	18.5	3.0	20.1	2.3	NS
% energy alcohol	1.2	2.0	1.7	3.3	NS
Dietary fibre (g)	17.4	3.3	17.5	5.9	NS
BMI (KG/M^2)	32.0	6.4	32.0	6.2	NS
Serum triacylglycerol (mmol/l)	2.3	1.1	2.7	1.2	NS
Serum cholesterol (nmol/l)	6.1	1.4	6.3	1.6	NS

SFA, saturated fatty acids, MUFA, monunsaturated fatty acids, PUFA, polyunsaturated fatty acids

Following dietary and plate model advice there was a significant reduction in the % energy derived from fat and saturated fat with a corresponding increase in the % energy derived from carbohydrate. No changes were detected in serum triacylglycerol, total cholesterol, or BMI over this period.

Simple, visual advice based on a plate model thus helps patients to achieve a diet in accordance with current dietary recommendations.

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Understanding dietary change: perceptions of ten dietary changes. By M. M. RAATS, P. SPARKS, M. A. GEEKIE and R. SHEPHERD. *Institute of Food Research, Reading RG6 2EF*

The recent Department of Heath (1994) report specifies how dietary targets might be achieved. However, these targets are unlikely to be realized unless more effective methods can be devised for influencing the dietary choices of consumers. Methods for understanding dietary change are needed. As part of a larger study assessing the effect of dietary information feedback, 170 university staff members completed a questionnaire about making ten dietary changes (see Table 1). The questionnaire was designed according to an extended form of the theory of planned behaviour (Ajzen, 1988). The following variables were measured: behavioural intention ('I intend to..'), attitude ('How harmful or beneficial would it be..?' and 'How unenjoyable or enjoyable would it be..?'), subjective norm ('Most people who are important to me think I should..'), perceived control ('How easy or difficult would it be..?'), anticipated affect ('How displeased or pleased would you be..?') and perceived need to change ('To what extent do you feel that you need to..?'). The ten dietary changes studied were perceived differently by the subjects (Table 1). Attitude (unenjoyable/enjoyable), anticipated affect and perceived need to change were important in predicting intention to make the ten dietary changes studied (Table 2). These results suggest that the roles of perceived need to change and anticipated affect within the structure of the theory of planned behaviour merit further attention.

Table 1. *Mean scores and standard errors for behavioural intention to make ten dietary changes (1 to 9), attitude (harmful/beneficial) (-4 to +4), attitude (unenjoyable/enjoyable) (-4 to +4), subjective norm (1 to 9), perceived control (1 to 9), anticipated affect (1 to 9) and perceived need to change (1 to 9)*

	Behavioural intention (*n* 102)		Attitude (harmful-beneficial) (*n* 102)		Attitude (unenjoyable-enjoyable) (*n* 109)		Subjective norm (*n* 112)		Perceived control (*n* 110)		Anticipated affect (*n* 109)		Perceived need to change (*n* 104)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	mean	SE	Mean	SE	Mean	SE
Eat fewer biscuits	5.9	0.2	1.6	0.1	-0.7	0.2	5.6	0.2	4.6	0.2	6.2	0.2	4.6	0.3
Eat fewer cakes	5.5	0.2	1.7	0.1	-0.6	0.1	5.4	0.2	4.2	0.2	6.1	0.2	4.8	0.3
Eat fewer chips	5.4	0.3	1.5	0.2	-0.2	0.1	5.3	0.2	4.3	0.2	5.9	0.2	5.1	0.2
Eat less butter/margarine	5.5	0.2	1.4	0.1	-0.4	0.1	5.1	0.1	4.8	0.2	5.8	0.2	4.9	0.2
Eat less cheese	4.8	0.2	1.2	0.1	-1.0	0.1	5.3	0.1	5.1	0.2	5.1	0.2	4.8	0.2
Eat less fat	5.9	0.2	2.1	0.2	0.0	0.1	5.6	0.2	4.9	0.2	6.6	0.2	4.2	0.2
Eat less red meat	5.3	0.3	1.1	0.1	-0.3	0.2	5.0	0.2	4.5	0.2	5.4	0.2	5.4	0.2
Eat more bread	4.7	0.2	0.8	0.1	0.4	0.1	5.1	0.1	4.3	0.2	5.2	0.1	5.4	0.2
Eat more fruit	6.3	0.2	2.6	0.1	1.7	0.1	6.1	0.2	3.3	0.2	7.1	0.1	3.5	0.2
Eat more vegetables	6.3	0.2	2.6	0.1	1.3	0.2	6.2	0.2	3.6	0.2	7.0	0.1	3.9	0.2

Table 2. *Multiple regressions of behavioural intentions to make ten dietary changes (each row refers to a separate multiple regression for that particular dietary change), on two attitude measures, subjective norm, perceived control, anticipated affect and perceived need to change (significance levels refer to final β-coefficients)*

	n	R^2	Attitude (harmful-beneficial)	Attitude (unenjoyable-enjoyable)	Subjective norm	Perceived control	Anticipated affect	Perceived need to change
Eat fewer biscuits	138	0.35	NS	**	NS	NS	*	***
Eat fewer cakes	131	0.33	NS	***	NS	NS	*	***
Eat fewer chips	124	0.50	NS	***	NS	NS	***	***
Eat less butter and margarine	141	0.42	**	**	NS	NS	*	***
Eat less cheese	141	0.30	NS	NS	NS	NS	**	***
Eat less fat	143	0.34	NS	*	NS	NS	***	***
Eat less red meat	127	0.58	NS	NS	NS	NS	***	***
Eat more bread	147	0.44	NS	NS	**	NS	**	***
Eat more fruit	153	0.47	NS	NS	NS	***	***	***
Eat more vegetables	149	0.42	NS	*	NS	NS	**	**

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

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Changes over seven years in dietary patterns in the Health and Lifestyle Survey population.

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A previous communication (Whichelow & Prevost 1995) described the four main dietary patterns identified by principal component analysis from thirty-nine food items in 8675 of the 1984/5 Health and Lifestyle Survey (HALS1) respondents. The first component, "Healthy", had high weightings for fruit, salad and other foods high in dietary fibre and low in fat, whereas the second, "Bulk", was predominantly composed of high-carbohydrate foods and carcass meat. The third, "Junk", diet was weighted towards high-fat and convenience foods and the fourth, "Sweet", was positively weighted for confectionery, cakes and biscuits but negatively for all vegetables. Analysis of the data from the follow-up Survey in 1991/2 (HALS2) showed that the associations of foods were unaltered.

Each individual achieves a score on each of the diets, resulting from their pattern of food consumption. A high score indicates that a respondent favours that diet. Any individual can score high (or low) on more than one diet. Comparison of the two surveys shows a marked change in overall scores, in the same respondents at HALS2 in 1991/2 for the "Healthy", "Bulk" and the "Sweet" diets (Table).

Diet		"Healthy"		"Bulk"		"Junk"		"Sweet"	
HALS		1	2	1	2	1	2	1	2
Men									
Non-manual	Mean	65	374	100	-264	85	41	39	279
(n 970)	SE	29	28	32	31	29	29	31	32
Manual	Mean	-528	-178	263	-100	165	90	-163	51
(n 1253)	SE	25	26	27	28	31	30	30	29
Women									
Non-manual	Mean	559	846	-137	-410	-57	-49	127	328
(n 1386)	SE	25	24	25	24	23	24	26	26
Manual	Mean	26	368	-111	-413	-127	-105	23	189
(n 1556)	SE	24	24	24	24	23	23	24	25

The marked age variations in scores seen for all diets at HALS1 were maintained at HALS2. These findings demonstrate considerable changes in the British diet but also indicate a polarization of eating habits. The "Healthy" eaters at HALS1, who tend to be non-smoking, prudent drinkers, have further improved their diets, whilst those with a preference for the poor diet, "Junk", where smokers and heavy drinkers predominate, have not changed their eating habits. The change in the "Bulk" diet scores indicates a drop in energy consumption.

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Comparison of dietary assessment methods for energy and fat in a group of young and middle-aged men. By D.WEBB, J.KNAPPER, H.JORDAN, C.C.CULVERWELL, C.KESSEL, J.A.TREDGER and C.M.WILLIAMS, *The Nutrition and Food Safety Research Centre, University of Surrey, Guildford, GU2 5XH.*

Measurement of habitual diet is an important aspect of nutritional research. In the present study, fifteen young men, mean age 22.3 (SD 2.0) years, and fifteen middle-aged men, mean age 46.7 (SD 5.2) years, undertook test meal studies to investigate the acute effects of monounsaturated fatty acids on postprandial lipoprotein metabolism. Assessment of their habitual background diet was by means of a 3d diary and a food-frequency questionnaire (FFQ). Both methods were simple and unobtrusive and their use was well understood and accepted by the volunteers.

Results showed that the estimates of total energy and fat obtained from the 3d diary records were very similar to values currently reported for the UK adult male population (Gregory *et al.* 1990). Values for total energy and fat obtained from the FFQ were, however, significantly higher and this was particularly notable for the younger age group ($P<0.008$, both energy and fat in young men; $P<0.02$ both energy and fat in middle-aged men).

There was a poor correlation between the two assessment methods for total energy and fat intake. On a qualitative basis (% energy), when the data from the two groups were combined, there was a strong correlation between the two methods for % energy from total fat (r 0.55; $P<0.002$) and SFA (r 0.57; $P<0.001$). Caution is necessary, however, when interpreting data from FFQ's, as this method of dietary assessment is more usually applied to population studies. For food diaries, a longer recording period may be required to assess habitual dietary intake more thoroughly (Bingham *et al.* 1994).

Daily Intake	Young men (n 15)				Middle aged men (n 15)				British Adults Survey†			
	3 d diary		FFQ		3 d diary		FFQ		Men 16-24		Men 35-49	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Energy (MJ)	10.87	0.64	15.31	1.38	10.25	0.55	12.18	0.48	10.29	0.20	10.46	0.13
Total Fat (g)	108	8.2	163**	17.3	106	7.5	127*	8.1	103*	2.1	103.3	1.5
(% energy)	(37)	(15)	(49)	(1.3)	(39.5)	(5.5)	(40.3)	(1.7)	(37.9)	(0.4)	(37.1)	(0.3)
SFA (g)	39	3.6	64	7.5	40	3.8	50.9	4.0	41.6	1.0	42.0	0.7
MUFA (g)	32	2.8	60	8	33	2.8	48.4	3.9	32.4	0.7	31.5	0.5
PUFA (g)	18	1.8	24	2	19	2	23.2	2.4	16.2	0.5	16.3	0.4

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
†Gregory *et al.* 1990. *$P<0.02$; **$P<0.008$

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The use of *n*-3 polyunsaturated fatty acid-enriched foods in a dietary intervention study of middle-aged men. By C.Pike, J.A. Lovegrove, M.C. Murphy and C.M. Williams, *The Nutrition and Food Safety Research Centre, University of Surrey, Guildford, GU2 5XH*

Increasing interest in the beneficial nutritional properties of long-chain *n*-3 polyunsaturated fatty acids (PUFA), the low amounts of these fatty acids currently consumed in the UK diet together with consumer resistance to consumption of oily fish, the main dietary source of long-chain *n*-3 PUFA, have led to proposals for the production of manufactured foods enriched with these fatty acids.

The present study has evaluated the use of eleven food items, enriched with long-chain *n*-3 PUFA, in a randomized controlled cross-over design. The aims of the study were to increase the intakes of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) from habitual levels of intake of approximately 0.1g/d to levels of approximately 1.8g/d, without altering the intake of other nutrients or energy, and to assess the palatability and acceptability of the enriched food items compared with the conventional (control) products.

Nine normolipidaemic male subjects (mean age 50 (SD 7.2) years, BMI 25.7 (SD 2.6) kg/m^2 were recruited to participate in a 2 x 21 d randomized single-blind cross-over trial of *n*-3 PUFA and control foods. The food items used were bread, biscuits, cakes, ice-cream, orange drink, milk shake, low fat spread, pasta, mayonnaise, vinaigrette and milk shake powder. Enrichment was achieved by incorporation of a fish-oil product (ROPUFA, Hoffman La Roche Ltd), either directly, or in a microencapsulated form, into the enriched food items during manufacture. Control foods were prepared and packaged in an identical fashion to the enriched products. An exchange system was used to help advise the subjects on the amounts of foods needed to be eaten each day. Each food portion was assigned an exchange value, where one exchange was equivalent to 0.2g EPA/DHA. Subjects were asked to consume 9 exchanges/d, equal to 1.8g EPA/DHA/d. At the end of each 21 d period the subjects were asked to score the acceptability and palatability of the individual food items using a linear analogue score ranging from 0 to 10, where 0 was "not at all palatable" and 10 was "very palatable". Habitual nutrient intakes and intakes whilst on the 21 d interventions were assessed by means of 3 d diet records, with nutrient analysis using the FOODBASE nutrient analysis programme.

The mean palatability score for the eleven enriched foods was 6.85 (SD 0.96) compared with a score of 7.44 (SD 0.85) for the control foods. For the individual food items there were very few differences apart from a significantly lower palatability score for the enriched orange drink compared with the control product (enriched 5.9 (SD 3.28) v. control 8.01 (SD 1.38); $P = 0.04$.

Dietary analysis showed energy intakes of subjects to be higher during the dietary intervention periods compared with the habitual diets (enriched diet 11.49 (SD 1.28) MJ/d, control diet 11.26 (SD 1.96) MJ/d v. 9.41 (SD1.37) MJ/d; $P< 0.05$). There was also a gain in weight by the subjects over both study periods (enriched diet 1.16 (SD 1.41) kg, control diet 0.54 (SD 1.04) kg).

It is concluded that manufactured foods can be used to achieve dietary enrichment with *n*-3 PUFA and that these are palatable and acceptable over a short time period. However it is clear that unless the enriched foods are used to substitute for similar foods in the habitual diet, alterations in intakes of other nutrients can occur. This may have implications for the interpretation of data from intervention studies when these foods are used as the mode of *n*-3 PUFA enrichment.

Weight and length of Glasgow infants compared with Tanner and Whitehouse standards and new British standards for growth. By S.A.H. SAVAGE, J.J.REILLY, and J.V.G.A DURNIN, *University of Glasgow, Department of Human Nutrition, Yorkhill Hospitals, Glasgow G3 8SJ*

The infant growth standard which has been widely used in the UK for the past 30 years is that of "Tanner and Whitehouse" (Tanner *et al.* 1966). This study, with measurements made only every 3 months, did not pay particular attention to growth in early infancy. It may also be possible that infant growth patterns have changed in the last 30 years due perhaps to changes in feeding practice. New British standards (Freeman *et al.* 1995) are now available where more frequent measurements have been made over the rapid period of growth in the first 6 months of life.

A study was undertaken in Glasgow to assess growth and the factors which might influence growth in a representative sample of 127 infants. Infants were followed longitudinally from birth to 2 years of age, although only the results for the first year are presented here. Measurements were made monthly to 6 months then at 9, 12, 18 and 24 months. The following anthropometric measurements were made: weight; length; triceps and subscapular skinfolds; head, mid-upper arm, calf and thigh circumferences. The breast-feeding rate among these infants was 41% (1 week), 39% (1 month) and 36% (2 months) and the median age of introduction of solid food was 11 weeks, range 4-35 weeks (Savage *et al.* 1994).

A comparison of Glasgow data with Tanner and Whitehouse and the new British standards for weight (kg) and length (cm) was carried out. Results are presented as mean and standard deviation of weights and lengths for Glasgow, Tanner and Whitehouse and new standards. Glasgow data are expressed as a SD score relative to Tanner and Whitehouse and new standards in order to make comparisons. In order to simplify the presentation of results comparisons are presented at 2 and 12 months.

	Glasgow		Tanner		New		Tanner	New
	Mean	SD	Mean	SD	Mean	SD	Mean SD - Score	
Weight (kg)								
2 months - boys	5.60	0.62	4.95	0.59	5.43	0.66	1.05	0.25
- girls	4.91	0.66	4.64	0.56	5.15	0.61	0.51	-0.40
12 months - boys	10.42	1.15	10.21	1.12	10.05	1.05	0.19	0.34
- girls	9.52	1.29	9.73	1.13	9.62	1.00	-0.18	-0.08
Length (cm)								
2 months - boys	58.2	2.2	57.7	2.1	58.3	2.1	0.36	-0.16
- girls	56.5	2.2	56.4	2.2	57.0	2.0	0.30	-0.12
12 months - boys	76.1	3.0	76.2	2.8	75.8	2.5	-0.03	0.12
- girls	74.1	2.8	74.2	2.7	74.0	2.4	-0.02	0.05

Infants in our sample tended to be heavier than those of Tanner and Whitehouse at 2 months of age (mean SD score +1.05 for boys, +0.51 for girls) and were more similar to those of the new standards although the girls tended to be lighter. The difference for length between our data and the two standards was much less marked but infants tended to be longer compared with Tanner and Whitehouse at 2 months and slightly shorter than the new standards. By the end of the first year there was less difference between the weights and lengths from the three studies.

The first 6 months of life is critical for child growth, so it is essential to use appropriate standards when assessing growth. In conclusion the new British standards appear to be more appropriate than those of Tanner and Whitehouse when assessing weight and length in Glasgow infants. However, we have observed some differences in growth between the new standards and Glasgow infants. It seems then that any reference values must be used with caution when used to assess growth in local populations.

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Comparison of weight and height based indices for assessment of the risk of death in severely malnourished children. By C. PRUDHON[1,2], A. BRIEND[2,3], D. LAURIER[4], I.Y. MARY[4] and M.H.N. GOLDEN[5], *[1]Action Internationale Contre la Faim (AICF); [2]INSERM U290, Paris, France; [3]ORSTOM, Paris, France; [4]INSERM U263, Centre de Bioinformatique, Université Paris 7, France; [5]University of Aberdeen, Aberdeen, AB9 2ZD.*

To compare the efficacy of different centres treating malnourished children there is a need to have a simple objective method of adjusting the crude mortality rates to allow for differences in the initial nutritional state of the patients. We compared different anthropometric indices based on weight and height to predict the risk of death among severely malnourished children.

Measurements were made son 1441 children attending nine therapeutic feeding centres in three African countries, established during emergencies. Anthropometric data from 1047 children who survived were compared with those of 147 children who died during treatment. The most appropriate power of height in the Optimal Ratio of Weight to Height (ORWH) for assessing the risk of death (weight/height$^{\beta}$) was determined by logistic regression. The model included survival as the dependent variable and the logarithms of weight and height as the independent variables, with adjustment for differences between therapeutic feeding centres.

The ORWH for all children, with and without oedema, was weight/height$^{1.74}$ (95% CI of ß estimate: 1.65-1.84). Admission ORWH, BMI (weight/height^2), weight-for-height expressed as a percent of the median (WHP) or Z-score (WHZ) of the National Centre for Health Statistics standards, were compared as predictors of death. The Receiver Operating Curves (ROC; sensitivity vs 1-specificity), showed that ORWH, BMI and WHP were equivalent and each superior to WHZ.

As BMI is much easier to calculate than WHP, WHZ or ORWH and does not depend upon standards and tables, we conclude that BMI can be conveniently used to adjust mortality rates for nutritional status when comparison between treatment centres is made. BMI may also be useful for making rapid management decisions on individual children.

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Use of the waist:height ratio to assess the risks of obesity. By M.A. ASHWELL[1], S.R.E. LEJEUNE[1] and K. McPHERSON[2]. [1]*British Nutrition Foundation, High Holborn House, 52-54 High Holborn, London WC1V 6RQ, and* [2]*Department of Public Health and Policy, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

Abdominal obesity is now considered an important clinical entity; the amount of visceral fat, and its proxy, the waist to hip circumference ratio (WHR), is often a better predictor of subsequent CHD than is total obesity as measured by the BMI (Kissebah & Krakower, 1994). The *Health Survey for England 1992* (Breeze *et al.* 1994) included values for weight, height, waist, hips, blood pressure, blood cholesterol, smoking behaviour and diabetic status for men and women aged between 16 and 85 years. The prediction equation generated by the Framingham Heart Study was applied to this data for 1411 men and 1481 women aged between 30 and 74 years to establish the probability of a CHD event within 6 years (Anderson *et al.* 1990).

Stepwise regression was used to discern the strongest discriminatory components of WHR and BMI for CHD risk. The strongest two-parameter discriminator was found to be the ratio of the waist circumference and height (WHTR) (r^2 0.15 for men ($P<0.001$); r^2 0.10 for women ($P<0.001$)).
BMI was a much weaker discriminator of CHD risk (r^2 0.026 for men ($P<0.001$); r^2 0.028 for women ($P<0.001$)). Furthermore, the relationship of WHTR with CHD risk was statistically significant at all ages for women and up to age 60 for men whereas BMI was not significantly related to risk beyond age 45 in either sex.

We are currently validating this conclusion using prospective data collected on Swedish men and women (Lapidus *et al.* 1984; Larsson *et al.* 1984). Preliminary analysis of follow-up data has shown that WHTR is a better predictor than BMI for myocardial infarction and all-cause mortality, even when controlling for age and smoking.

Use of WHTR instead of BMI has the following advantages: (i) treatment can be prioritized on those people who are most likely to benefit from weight reduction resulting in the reduction of the high risk visceral fat deposits; (ii) certain individuals, with high BMI but low WHTR, can be reassured about their low risk status and can be discouraged from weight reduction.

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The relationship between arm-span and height in different ethnic groups. By S.L. REEVES, C. VARAKAMIN and C.J.K. HENRY, *School of Biological and Molecular Sciences, Oxford Brookes University, Headington, Oxford OX3 0BP*

In recent years arm-span has been proposed as a proxy for stature, notably in subjects such as the elderly who show extensive spinal curvature (Kuczmarski, 1989). Whilst arm-span may be a practical measure to estimate height, it is necessary to establish the relationship between height and arm-span in different ethnic groups, in order to assess its validity and universal application as a clinical measure.

Males (*n* 272) of mixed nationalities and an average age of 23.6 (SE 2.2) years were studied. Subjects were divided into four broad ethnic groups: (a) Afro-Caribbean, (b) Asian, (c) Caucasian and (d) Oriental. Arm-span and height were measured using standard procedures (Lohman *et al.* 1988) amongst other anthropometric variables.

		Arm-span - height (cm)		Arm-span / height (cm)		
Ethnicity	*n*	Mean	SE	Mean	SE	*r*
Afro-Caribbean	50	7.11** ††	3.69	1.04	0.03	0.89
Asian	50	4.93**†	2.09	1.03	0.02	0.84
Caucasian	103	2.05	2.01	1.01	0.02	0.87
Oriental	69	1.92	1.97	1.01	0.02	0.85

Arm-span and height measurements were significantly different within ethnic group, ** $P<0.01$.

Arm-span - height was significantly different from all other groups, † $P<0.05$, †† $P<0.01$.

For Afro-Caribbean and Asian subjects arm-span was significantly different from height. Afro-Caribbeans showed greatest deviation from the assumed arm span / height of 1.00. Significant differences between the ethnic groups with regards to arm-span - height were also noted. All groups however exhibited good correlations between arm-span and height.

While arm-span may be a valuable proxy for height, these ethnic differences need to be recognized. Further work is in progress and attention will now be focused on variation between arm-span and height amongst female ethnic groups.

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Predicting body composition in adults. By THANG S. HAN[1], PAUL DEURENBERG[2] and MICHAEL E.J. LEAN[1], *University Department of Human Nutrition, [1]Royal Infirmary, Glasgow G31 2ER, and [2]Bromenweg 2 6703 HD, Wageningen, the Netherlands.*

Ageing is associated with increased body fat, particularly the intra-abdominal fat mass. Subcutaneous skinfolds (Durnin & Womersley, 1974) tend to underestimate percentage body fat (BF%) in the elderly (Reilly *et al.* 1994). Waist circumference has been shown to correlate highly (r 0.89, P <0.001) with intra-abdominal fat mass measured by magnetic resonance imaging (Han *et al.* 1995). Employing waist circumference may improve body fat prediction.

BF% of sixty-three men and eighty-four women aged 18-65 years, was calculated from body density (Siri, 1961) measured by underwater weighing (BF%: 5-52% of body weight). Linear regression for each sex showed that waist circumference, adjusted for age, significantly predicted BF%, explaining 78.8% (men) and 70.4% (women) of variance.

These equations and those of Durnin & Womersley (1974), were validated in a separate Dutch population of 146 men and 238 women aged 18-83 years. Total variance explained was equal for the two methods (men: r^2 70%; women: r^2 74%). The mean BF% difference from BF% by underwater weighing was close to zero with each predictive method, with 95% CI about the mean difference in BF% ranging from 8.5 to 9% of body weight. Plots of this mean prediction error against age showed no bias in predicting BF% using waist circumference equations for men (slope = -0.23, r -0.06, P = 0.51) or women (slope = 0.03, r 0.08, P = 0.24), but systematic underestimation of BF% by the subcutaneous skinfold method was observed with increasing age in men (slope = -1.0, r -0.14, P <0.01) and was most marked in women (slope = -1.34, r -0.35, P <0.001). Examination for the bias in separate age groups showed errors in all subjects by the waist method and subjects below 60 years by the skinfold method were similar, but there was marked underestimation of up to 12 BF% in men and 15 BF% in women above 60 years by the skinfold method (Table).

Magnitude of BF% prediction errors (% body weight) by waist circumference and skinfold methods in different age groups

		Men			Women		
Method	Age (y)	Mean	95% CI	*n*	Mean	95% CI	*n*
Waist circ	18-39	-0.5	-9.2, 8.2	48	0.6	-7.7, 8.9	138
	40-59	-1.4	-9.0, 6.1	58	0.9	-8.7, 10.5	60
	60-83	-0.7	-9.6, 8.2	40	0.9	-7.4, 9.3	40
Skinfold*	18-39	-1.6	-8.7, 5.5	47	-0.9	-8.6, 6.7	138
	40-59	-1.4	-9.8, 7.0	56	-0.5	-9.5, 8.5	58
	60-83	-3.4	-12.0, 5.2	40	-5.7	-14.8, 3.4	40

*Durnin & Womersley (1974).

The skinfold method gives good prediction of BF% in younger groups but after validation in an independent sample, this study indicates that regression equations employing waist circumference (adjusted for age) can be applied confidently to predict BF% of all ages:

BF% (men) = 0.567 × waist circumference (cm) + 0.101 × age (years) - 31.8

BF% (women) = 0.439 × waist circumference (cm) + 0.221 × age (years) - 9.5

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Prediction of limb muscle mass in men over 75 years of age. By N.J. FULLER[1], M.A. LASKEY[1,2], P. PAXTON[3] and M. ELIA[1], *[1]MRC Dunn Clinical Nutrition Centre, Cambridge CB2 2DH, [2]Department of Nuclear Medicine, Addenbrooke's Hospital, Cambridge CB2 2QQ and [3]Lensfield Medical Practice, Cambridge CB2 1EG*

Knowledge of muscle mass in the elderly is becoming increasingly important because strategies that improve muscle function may help to prevent or delay some of the functional decline associated with ageing. An alternative to the traditional bedside assessment of muscle mass by anthropometry (limb circumference and skinfold thickness) has been proposed that utilizes measurement of impedance and an assumed resistivity for each major limb component (Brown *et al.* 1988). The aim of this preliminary study was to establish whether use of this impedance technique in elderly men might potentially improve on anthropometric estimates of limb muscle mass, obtained by dual-energy X-ray analysis (DXA - Lunar model DPX, software version 3.6) as reference (Fuller *et al.* 1992).

Details of the fifteen free-living elderly men (aged 76-87 years) who volunteered for the study were: weight 71.6 (SD 11.7) kg; height 1.71 (SD 0.09) m; body fat 24.7 (SD 6.2) % of body weight (determined by DXA). Arm, leg and total limb muscle mass was assessed using the DXA model (Fuller *et al.* 1992) and appropriate indices of limb muscle mass (limb segment muscle cross-sectional areas and volumes) were obtained using anthropometry and the prediction equation involving impedance (Brown *et al.* 1988), modified slightly to obtain estimates of the entire limb and applied to both arm and leg.

Muscle mass of the arm was 3.64 (SD 0.80) kg, the leg was 12.66 (SD 2.25) kg, and total limb was 16.30 (SD 2.93) kg (22.9 % body weight and 30.7 % fat-free mass, using DXA). These values were substantially lower (with similar inter-individual variation) than in younger men (Fuller *et al.* 1992) also studied with DXA. Relationships between DXA and anthropometric or impedance indices are given in the Table as correlation coefficient (r) and standard error of the estimate (SEE).

	Impedance indices of :-				Anthropometric indices of :-			
	Muscle area		Muscle volume		Muscle area		Muscle volume	
	r	SEE (kg)	r	SEE (kg)	r	SEE (kg)	r	SEE (kg)
Arm	0.69	0.58	0.71	0.57	0.67	0.59	0.71	0.56
Leg	0.62	1.77	0.76	1.47	0.58	1.83	0.69	1.62
Total limb	0.76	1.90	0.85	1.56	0.61	2.33	0.71	2.07

In general, the results indicate that predictions of muscle mass (assessed by DXA) using equations incorporating impedance (Brown *et al.* 1988) were considerably better than those based on anthropometry alone for this group of elderly men. Evidently, both anthropometric and impedance predictions were improved if a measure of segment length was also incorporated, as were predictions based on the combined results of individual limbs. Further validation of these predictions using preferred alternative reference methods such as MRI or CAT scans is desirable.

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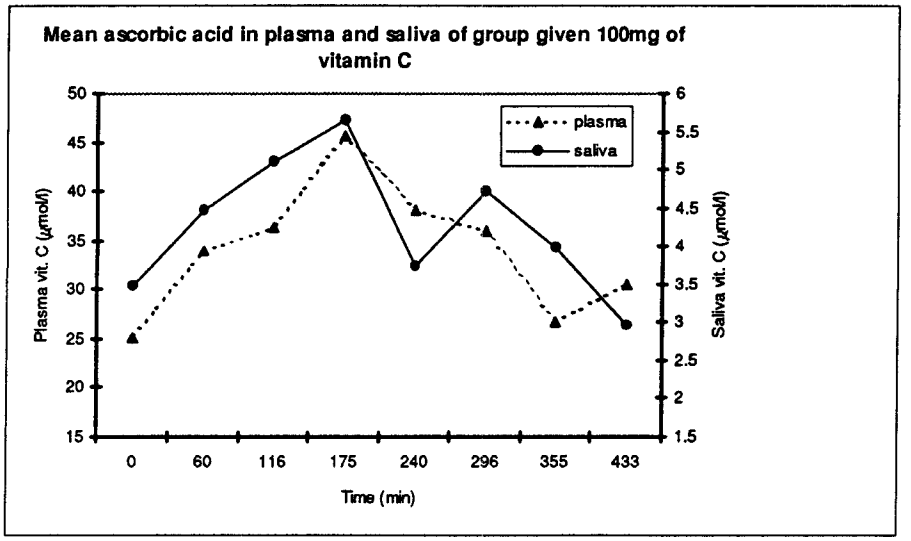
Fuller, N.J., Laskey, M.A. & Elia, M. (1992). *Clinical Physiology* **12**, 253-266.

A comparison of saliva and plasma ascorbic acid. By THOMAS K. K. HA, JOSEPHINE COONEY and MICHAEL E.J. LEAN. *Department of Human Nutrition, University of Glasgow, Glasgow G31 2ER.*

Plasma ascorbic acid is widely available as a recognized and accurate marker of recent ascorbic acid (vitamin C) intake and status. The utility of saliva as a similar index of ascorbic acid intake and status has been limited by the small concentrations of ascorbic acid in saliva (approximately 10 times less than plasma) and the use of spectrophotometric methods in measurement. This has led researchers to dismiss saliva measurements (Leggott et. al.,1986).

The present study employs a HPLC for ascorbic acid analysis and a novel saliva collection method to compare ascorbic acid in saliva and venous blood, to determine if saliva might be used as a biomarker for ascorbic acid intake.

Saliva was obtained by chewing on a piece of cotton-wool until moistened, the cotton wool was then placed inside an empty syringe and the saliva extracted by reinserting the plunger. Saliva and venous blood were obtained simultaneously from volunteers, and preserved using 1mg dithiotrietol (DTT) per ml of blood or saliva. Samples were obtained fasting and at hourly intervals for 8 h after an oral dose of 100mg ascorbic acid. The plasma was separated from blood and stored at -80°. The saliva was frozen at the same temperature. Before analysis, the specimens were thawed, deproteinized with 3% metaphosphoric acid (MPA), then centrifuged. The supernatant fraction was analysed by HPLC using a reversed-phase analytical column and electrochemical detection.



In results from five subjects (*n* 39 samples), plasma ascorbic acid ranged from 4.7 to 64 µmol/l and saliva ascorbic acid from 0.68 to 12.8 µmol/l. The figure above shows mean trends during the 8 hour period. Our methodology for plasma ascorbic acid has an interassay CV of 5.4% and for saliva a CV of 6% for duplicate samples. A strong correlation of r 0.80; $p<0.001$ was found between concurrent plasma and saliva ascorbic acid concentrations.

Ascorbic acid can be measured accurately in human saliva and can be explored as a non-invasive method to determine recent ascorbic acid intake and status.

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Measurement of stable isotope [^{13}C]ascorbate using gas chromatography with mass spectrometry (GCMS). By A.P.IZZARD, L.J.C.BLUCK and C.J.BATES, *MRC Dunn Nutrition Laboratory, Downhams Lane, Milton Road, Cambridge. CB4 1XJ*

Vitamin C is probably the most popular vitamin supplement, however there is no international consensus on requirements. Nutritional surveys employ assays which correlate more closely with recent intake than with long term ascorbate status. For more revealing kinetic studies in man, stable-isotope labelled ascorbate is needed. However only one such study has been attempted, and this was 30 years ago (Atkins *et al*. 1964). Here we report how quadrupole GCMS might be applied, in a routine laboratory method, to assay the ^{13}C-isotope enrichment of ascorbate in biological samples.

Ascorbate must be converted to a volatile derivative, stable at 150-300°, for GC separation and MS analysis. Trimethylsilyl (TMS), tertbutyldimethylsilyl (TBDMS) and butylboronic acid derivatives of ascorbate were compared for their performance on the GCMS, ease of preparation and stability during storage. Their use for isotope ratio measurements is affected by the sizes of the derivatized ascorbate molecules and the presence of atoms, such as silicon, with a high abundance of naturally occurring heavy isotopes. These factors dilute the contribution of the ascorbate label and reduce sensitivity. The TMS derivative best fulfilled the essential criteria for detection and precise measurement of ascorbate enrichment.

Characterization of the complex mass spectra of ascorbate-TMS was needed to identify the fragments carrying the labelled ^{13}C, derived from the C-1 position of ascorbate.Accuracy and repeatability of isotope ratio measurements were assessed and further improved using single ion monitoring (SIM). The standard errors below for C isotope ratios were very similar when ascorbate-TMS was analysed on either a GCMS or a gas chromatograph combustion isotope ratio mass spectrometer (GC-C-IRMS), the latter being more sensitive for isotope ratio work.For GCMS the standard error for an isotopomer ratio measurement (i.e. M^+/M molecular isotope ratio) was found to be about 0.002, whilst GC-C-IRMS gave a standard error of 0.00003. Since the ascorbic acid used as the dose is labelled in one position only these two figures are directly comparable.

An oral dose of 50 mg (99% APE) [1-^{13}C]ascorbate is expected to give an immediate increase of twice the naturally occurring isotopomer ratio of the plasma ascorbate pool and an increase of 1.04 times the isotopomer ratio of the total body pool, at equilibrium. This level of ^{13}C isotope enrichment of ascorbate is easily measured by GCMS, as judged by the standard errors above, without the requirement of the extra sensitivity provided by GC-C-IRMS.

Quantitative ascorbate measurement in biological samples was achieved by addition of an internal standard. Inositol was chosen because it behaved similarly to ascorbate in terms of TMS derivatization and MS performance, and appeared after a suitable elution time from the GC. If added at a concentration of 10 or 20 ng/μl, when ascorbate concentration varied between 10-100 ng /μl, the correlation between the ascorbate peak and inositol peak was better than 99%, with a coefficient of variance below 2% at each concentration .

A method of preparing biological samples has also been developed, satisfying both the requirement for acidic stabilisation of ascorbate in aqueous solutions and of maintaining compatibility with the chemistry of TMS derivatisation.

This analytical and preparative experience is now being used to design a human study of ascorbate kinetics by dilution of a single oral dose of [1-^{13}C]ascorbate in the plasma ascorbate pool,with measurements also of ascorbate and its metabolites (such as oxalate) in the urine, during 2 weeks post dose.

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The effect of ascorbate loading on ascorbyl radical formation in patients with sepsis and healthy subjects. By H. F. GOODE[1], M. J. DAVIES[2] and N. R. WEBSTER[1], [1]*University of Aberdeen Department of Anaesthesia & Intensive Care, Aberdeen Royal Infirmary, AB9 2ZB and* [2]*University of York, Department of Chemistry, York YO1 5DD*

Intensive care patients with sepsis have been shown to have abnormally low concentrations of protective antioxidants including ascorbic acid, tocopherol and the carotenoids and high levels of the products of free radical attack associated with clinical disease severity (Richard *et al* 1990; Downing *et al* 1993; Goode *et al* 1995). Animal studies of antioxidant therapy in order to combat such increased oxidant stress in sepsis have been encouraging and similar therapy including ascorbic acid administration, has been advocated in humans (Sawyer *et al* 1989). However, although ascorbic acid is a potent antioxidant it is also a powerful reducing agent, and it can act as a pro-oxidant by maintaining Fe as Fe^{2+} and thus available for participation in radical-generating reactions. The amount of unbound 'catalytic' metal ions present in the circulation may play a key role in determining the levels of free radicals produced and the hence the degree of oxidant stress. Elevated levels of catalytic metal ions have been demonstrated in a number of diseases including sepsis (Galley & Webster 1995). Both of these actions result in the generation of ascorbyl radicals, which can be used to monitor the extent of oxidative stress.

We have investigated the effect of infusion of ascorbic acid on ascorbyl radical concentrations in patients with sepsis syndrome, defined according to standard clinical and biochemical criteria. We measured baseline plasma total vitamin C and serum bleomycin-detectable 'free' Fe concentrations, and ascorbyl radical production using electron paramagnetic resonance (EPR) spectroscopy before and after intravenous infusion of ascorbic acid in seven patients with sepsis and nine healthy control subjects. Pre-infusion blood samples were centrifuged immediately and the plasma/serum frozen at -20° for Fe and vitamin C assays. EDTA-anticoagulated plasma was added to an equal volume of the spin trap agent N-t-butyl-α-phenylnitrone (PBN), incubated for 1 h at 20°, centrifuged, and the supernatant fraction stored in liquid N_2 until EPR analysis. Ascorbic acid (1 g in 10 ml saline) was infused intravenously to both patients and controls over a period of 10 min. After a further 5 min, post-infusion EDTA blood samples were obtained, and treated as above for EPR studies.

Vitamin C concentrations were markedly lower in patients compared with healthy subjects (0.55 (SD 0.28) mg/ml compared with 1.71 (SD 0.46) mg/ml, $P<0.0001$) and 'free' Fe was higher (32.3 (SD 15.3) mmol/l compared to 4.7 (SD 2.5) mmol/l in the control group ($P<0.002$). Pre-infusion ascorbyl radical concentrations (arbitary units) were not significantly different between patients and controls (1.9 (SD 1.6) units and 1.1 (SD 0.3) units respectively, NS). Post-infusion ascorbyl radical concentrations increased in both controls (14.6 (SD 4.3) units, $P<0.0001$ *v.* pre-infusion samples) and patients (3.2 (SD 0.73) units, $P<0.001$ *v.* pre-infusion samples).

The post-infusion radical concentration in healthy subjects was significantly higher than in patients with sepsis ($P<0.0001$), suggesting sub-optimal basal vitamin C levels and increased scavenging of a constant oxidant pool by ascorbate in the controls. In the ascorbate-depleted patients infused ascorbate was rapidly consumed, either via the promotion of redox cycling of Fe or as a result of radical scavenging i.e. by acting as an antioxidant. This study demonstrates markedly different handling of infused ascorbate in patients with sepsis and healthy subjects and clearly further studies are needed to elucidate the relative anti- and pro-antioxidant mechanisms of ascorbate metabolism in patients with raised 'free' Fe levels.

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Are high fetal vitamin C concentrations due to placental synthesis? By A.LOBAN[1], A.T.GIBSON[2] and H.J.POWERS[1], *[1]University Department of Paediatrics, University of Sheffield, S10 2TH and [2]The Jessop Hospital for Women, Sheffield S3 7RE*

It has been observed that neonates have high plasma levels of vitamin C compared with adults. We have found that high plasma vitamin C concentrations in premature babies at birth are associated with poor outcome (Silvers *et al.* 1994). Maternofetal transport of vitamin C occurs against a concentration gradient, maintaining a higher vitamin C concentration in the fetus than in the mother. An additional factor may be fetal synthesis of vitamin C, although this has not been established (De Fabro, 1967).

A further possibility is that placental synthesis of vitamin C occurs, and we have investigated this in human tissue. Vitamin C synthesis was measured in homogenates from maternal and fetal sides of twenty-two human placentas, obtained from births at 23-41 weeks gestation. Homogenates were incubated at 37° with and without gulonolactone, which is the substrate for gulonolactone oxidase (EC 1.1.3.8), the absence of which prohibits the synthesis of vitamin C in human tissue. Samples were taken regularly over 25 min, for the fluorimetric measurement of total vitamin C and dehydroascorbic acid using the Cobas Bio Autoanalyser. The method was validated using homogenates of rat liver. The results were analysed using the paired T test and analysis of variance.

There was no significant change in the amount of total vitamin C in the placental homogenates over 25 min incubation at 37° either with or without substrate. The concentration of total vitamin C in the maternal side of the placenta was 1.00 mmol/l of which 7% was dehydroascorbic acid. Values in the fetal side of the placenta were not significantly different. There was a significant fall (9%) in the concentration of dehydroascorbic acid, over 25 min incubation, in the fetal side of the placenta ($p=0.024$) which appeared to be inhibited by gulonolactone. This may reflect a reduction of dehydroascorbic acid to ascorbic acid, supporting a previous suggestion that the placenta has a role in reducing dehydroascorbic acid to ascorbic acid and that dehydroascorbic acid is the main form of vitamin C transported by the placenta (Choi & Rose, 1989).

High fetal vitamin C concentrations do not appear to be due to placental synthesis of vitamin C; the placenta may have the ability to reduce dehydroascorbic acid to ascorbic acid.

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De Fabro, S. (1967). *Société Argentine de Biologie* **162**, 284-285.

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

Superoxide dismutase activity in sixty nine premature babies from birth over the first week of life. By K.M. SILVERS,[1] A.T. GIBSON[2] and H.J. POWERS[1], [1]*University Department of Paediatrics, Sheffield Children's Hospital, S10 2TH,* [2]*Jessop Hospital for Women, Sheffield S3 7RE*

Clinical and experimental data have provided evidence that cell damage mediated by reactive-O-species contributes to the pathogenesis of events in a variety of diseases such as bronchopulmonary dysplasia. Aerobic metabolic processes within mammalian cells have necessitated the development of mechanisms to protect cellular components against O-induced damage. For example, the highly reactive superoxide radical is reduced to H_2O_2 by superoxide dismutase (SOD; EC 1.15.1.1).

There is some evidence to associate prematurity with reduced antioxidant activity in babies. A reduced concentration and activity of SOD has been found in lung tissue from premature infants when compared with term babies (Autor *et al.* 1976; Kugo *et al.* 1989). In addition, expression of antioxidant enzymes in fetal lung has been reported to increase during the late gestational period. In contrast, there is no evidence for significant developmental changes in SOD activity of erythrocytes (Ripaldo *et al.* 1989). SOD is inducible in neonates of some species at birth but there is some evidence to suggest that this may not be true for premature neonates (Autor *et al.* 1976).

Subjects were recruited with a mean gestational age of 28.72 (SD 2.12) weeks and birth weight of 1230 (SD 340) g. Blood was collected within 2 hours of birth and on days 1, 2 and 7 thereafter. Erythrocytes were separated by centrifugation and washed with saline (9g NaCl/l) and a haemolysate was prepared in 3 volumes of distilled water. SOD activity was measured spectrophotometrically by monitoring the autoxidation of pyrogallol. The method according to Marklund & Marklund (1974) was developed for use on the Cobas Bio autoanalyser. SOD activity was expressed as units/g haemoglobin, where one unit equals the amount of enzyme required to inhibit the rate of pyrogallol autoxidation by 50%. All measurements were made over an 18 month period and an in-house quality control was used within each run. The intra batch coefficient of variation was 7.12%.

Postnatal age (d) . . .		Superoxide dismutase activity (IU/g Hb)							
		0		1		2		7	
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Survivors	41	4038	2093	4152	1576	4089	1936	4255	1684
Non survivors	9	2860	1403	4040*	862	4218*	1145	4834**	978
Survivors with lung disease	19	3625	1343	4248	1154	4301*	1050	4890*	1678

Significantly different from day 0 (paired *t* test) *$P<0.05$, **$P<0.01$.

A repeated measures analysis of variance was performed. SOD activity was significantly associated with gestational age ($r=0.319$, $P<0.01$). There was a significant increase in SOD activity with increasing postnatal age ($P=0.002$). A paired *t* test showed that this was only true for the non survivors and survivors with lung disease. There is evidence for a group effect ($P=0.065$) on the magnitude of the postnatal increase, which was significantly greater among non-survivors than well survivors ($P=0.05$).

Following the relative hypoxia of gestation, the SOD activity increases after birth, but only if the baby is likely to die or develop bronchopulmonary dysplasia. Interestingly, it is the non-surviving group which show the greatest increase in SOD activity in the first 7 d after birth, which might be a response to greater oxidant stress in this period.

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Erythrocyte lipid peroxidation in smokers compared with non-smokers: dose response of vitamin E supplementation. **By K.M.BROWN and G.G.DUTHIE,** *Division of Biochemical Sciences Rowett Research Institute, Aberdeen,AB2,9SB*

Free-radical-mediated lipid peroxidation is associated with the pathogenesis of many degenerative diseases. Consequently increased intakes of antioxidant nutrients may inhibit the oxidative processes contributing to morbidity. The requirement for antioxidant nutrients depends in part on the level of exposure to exogenous and endogenously produced free radicals. Smokers for example, incur an additional sustained free-radical load directly and indirectly from the gas and tar phase of tobacco smoke. Vitamin E. (d-α-tocopherol) is a lipophilic antioxidant present in the lipid core of cell membranes where it protects polyunsaturated fatty acids against free-radical-mediated peroxidation. We have previously shown that erythrocytes of smokers are more susceptible to peroxidation than those of non-smokers, and that increasing the erythrocyte vitamin E concentration will decrease this susceptibility (Brown *et al.* 1994). Whether the improved resistance to erythrocyte lipid peroxidation can be achieved by increased dietary intakes of vitamin E is not known. To determine the optimum vitamin E intake required to improve erythrocyte resistance to peroxidation, we examined the effect of five different doses of α–tocopherol on erythrocyte vitamin E concentration and associated susceptibility to peroxidation.

One hundred males with no current disease, fifty of whom had never smoked and fifty who smoked >10 cigarettes/d, were allocated to ten treatment groups in a 2 x 5 factorial design (smokers *v.* non-smokers and placebo *v.* 4 vitamin E supplementations). For 20 weeks each subject took one capsule/d, of either 70, 140, 560, 1056 mg d-α-tocopherol or placebo (hydrogenated coconut oil with negligible vitamin E content). Ethical permission was granted by the Joint Ethical Committee of Grampian Health Board and University of Aberdeen.

Erythrocytes of smokers showed a greater susceptibility to H_2O_2 induced peroxidation than those of non-smokers ($P<0.001$). Erythrocyte vitamin E concentration increased in a dose-dependent manner during vitamin E supplementation. This was associated with a significant decrease in susceptibility of erythrocytes to peroxidation by 20-30% in non-smokers (NS), and 40-60% in smokers (S). Table below.

Dose ...	Placebo		70 mg		140 mg		560 mg		1056mg	
	NS Mean SD	S	NS Mean SD	S	NS Mean SD	S	NS Mean SD	S	NS Mean SD	S
Vit.E μg/gHb										
week 0	9.5 1.2	9.3 0.8	8.5 1.1	8.0 1.3	7.4 1.8	10.4 1.6	9.1 2.1	7.9 1.5	8.9 0.9	8.9 1.4
week 10	10.51.5	10.4 1.8	12.4 0.8	11.4 1.1	12.8 0.7	11.5 1.4	12.3 1.4	12.5 0.8	13.5 1.2	15.3 0.5
week 20	9.3 1.3	9.6 1.5	14.6.1.0	14.0 2.1	18.3 1.0	14.4 2.8	17.2 2.1	20.3 1.8	23.1 3.8	18.0 2.1
ELP nmol/gH										
week 0	32.2 5.1	64.4 8.3	35.6 7.8	65.2 5.6	37.1 9.4	62.5 7.5	41.4 9.2	59.8 4.1	31.8 5.1	54.4 7.8
week 10	34.8 6.7	68.8 1.9	24.9 5.9	46.5 8.1	28.8 4.2	23.4 5.5	39.8 8.1	48.0 3.8	29.5 7.3	31.4 5.6
week 20	39.4 6.4	66.6 2.3	25.2 4.9	27.9 3.4	26.3 7.1	27.7 1.9	33.7 9.4	32.2 6.4	34.7 1.7	27.3 5.1

ELP, erythrocyte lipid peroxidation

An erythrocyte vitamin E concentration of 14 - 18 μg/g Hb was associated with a decrease in the susceptibility of cells to peroxidation. The 70, 140 and 560 mg doses of d-α-tocopherol were equally effective in causing this vitamin concentration in erythrocytes. However, a supplement of 1056 mg d-α-tocopherol had no effect on the susceptibility of erythrocytes of non-smokers to peroxidation, indicating that lower doses of the vitamin may be more biologically relevant.

Brown, K.M., Morrice, P.C. & Duthie, G.G. 1994. American Journal of Clinical Nutrition 60; 383-387.

Plasma concentrations of carotenoids and antioxidant vitamins in smokers and non-smokers. By MARION A. ROSS, L. KATIE CROSLEY, SUSAN J. DUTHIE, KATRINA M. BROWN, ANDREW R. COLLINS, JOHN R. ARTHUR AND GARRY G. DUTHIE, *Biochemistry Department, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Smokers, a group at increased risk of developing certain cancers and coronary heart disease, inhale large quantities of reactive free radicals from the gas and tar phases of tobacco. To assess whether this increased oxidant load reflects changes in antioxidant status, plasma concentrations of vitamins C, E, A and carotenoids were measured in a group of fifty fasted asymptomatic male smokers (age 50-59 years) and fifty age-matched males who had never smoked.

	Smokers		Never smokers	
	Mean	S E	Mean	S E
α -Carotene (μmol/l)	0.078	0.006	0.114***	0.007
β-Carotene (μmol/l)	0.484	0.035	0.596*	0.032
β-Cryptoxanthin (μmol/l)	0.076	0.011	0.114*	0.013
Lycopene (μmol/l)	0.510	0.039	0.520	0.045
Lutein/zeaxanthin (μmol/l)	0.420	0.028	0.506	0.033
Phytofluene (units)	2.429	0.451	2.152	0.187
α-Tocopherol (μmol/l)	26.42	0.873	27.16	0.768
α-Tocopherol/cholesterol(μmol/mmol)	4.596	0.175	4.856	0.117
γ-Tocopherol (μmol/l)	2.124	0.108	2.033	0.161
Retinol (μmol/l)	1.934	0.017	1.871	0.063
Ascorbic acid (μmol/l)	25.62	3.35	37.64**	2.37

Significantly different from smokers, *$P<0.05$, ** $P<0.005$, ***$P<0.001$ (2-tail).

Plasma vitamin E concentrations were unaffected by smoking. Significantly lower concentrations of ascorbate, α-carotene, β-carotene and β-cryptoxanthin were found in the plasma of smokers compared with the never-smokers, which may reflect dietary differences and/or enhanced metabolic turnover resulting from increased oxidant-induced stress. Concentrations of lycopene, lutein/zeaxanthin and phytofluene were similar in smokers and never-smokers, suggesting that intakes of these carotenoids were unaffected by smoking. Trials are now needed to assess whether increased intake of carotenoids from dietary sources or a supplement would be of any benefit to smokers. This however would be approached with caution, since in some groups of smokers increased intakes of β-carotene have been associated with adverse consequences (Heinonen *et al*, 1994).

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Heinonen, O. P., Huttunen, J. K. & Albanes, D. (1994). *New England Journal of Medicine*, **330**, 1029-1035.

Differential susceptibility of respiratory chain enzymes and lipids to peroxidation in liver mitochondria. Use of compounds generating free radicals within aqueous or hydrophobic regions. By F. BEAUSEIGNEUR[1], M. GOUBERN[2], J. GRESTI[1], M.-F. CHAPEY[2], M. TSOKO[1], J. DEMARQUOY[1] and P. CLOUET[1], 1 *Nutrition Cellulaire et Métabolique, Faculté des Sciences Mirande, BP 138, 21004 Dijon Cedex, France,* 2 *Nutrition et Sécurité Alimentaire, INRA, 78352 Jouy-en-Josas, France*

The presence of particular substances contained in certain wines probably gives rise to favourable effects observed in moderate wine-drinkers at the cardiovascular level, reported as French paradox. Among them, antioxidants may play an essential role and our goal was to check the properties of resveratrol or *trans*-3,4',5-trihydroxystilbene, which has been found in wine. The present preliminary study was designed to ascertain the effects of free radical attacks on mitochondrial enzyme activities by peroxidants, taken as models, resveratrol being probably to prevent or reduce some of these effects.

Mitochondria freshly isolated from rat liver were exposed either to cumene hydroperoxide (CuOOH), which generates free radicals in aqueous and lipid regions, or to 2,2'-azobis-(2-amidinopropane) dihydrochloride (AAPH), which generates free radicals preferentially in aqueous regions. The activity of the respiratory chain and the level of phosphorylation were examined. CuOOH was shown to cause a maximal apparent malondialdehyde production for about 1 mM concentration and above (400 nmol/min per mg), while this production was far lower with 40 mM-AAPH (30 nmol/min per mg). With increasing concentrations of AAPH, state 3 respiration was progressively depressed (up to -57% under our conditions), whereas the ratio of phosphorylation : molecular oxygen consumption (P:O) kept steady and the dinitrophenol(DNP)-stimulated respiration remained at a high level. In contrast, all the preceding variables were diminished with increasing concentrations of cumene hydroperoxide (state 3 -56%; DNP-stimulated respiration -50%; P:O -28%).

AAPH: the similar inhibition of ATP/ADP translocase by carboxyatractyloside in control and AAPH-treated mitochondria discarded the possibility of an exchange more difficult between ATP of the matrix and ADP added to the medium. However, a differential inhibition of F_1F_0-ATPase by tri-n-butyltin, which acts on this complex, was observed between the same mitochondrial preparations. Results suggested that AAPH, under the conditions of treatment, did not have disturb respiratory chain and its connexion to phosphorylation, and that an impairment of part of F_1F_0-ATPase may be responsible for the decrease of state 3 respiration. As the impairment due to AAPH could not be prevented by resveratrol, which is insoluble in aqueous phase, it was suggested that AAPH acted, under our conditions, above all on hydrophilic structures, such as proteins.

CuOOH: CuOOH-induced disorders involved too many and interconnected activities to allow to indicate precisely the actual damaged structures. However, resveratrol appeared to prevent lipoperoxidation of mitochondria much more than butylated hydroxytoluene, another well-known antiperoxidant. As it has been shown that CuOOH exerted deleterious effects at least at the level of membrane lipids, resveratrol may protect enzymatic activities by reducing the rate of peroxidized lipids around corresponding proteins.

The quantification of non-transferrin-bound iron in plasma samples from neonates, using high performance liquid chromatography. By R.A. KIME[1], A.T. GIBSON[2] and H.J POWERS[1], [1] *University Department of Paediatrics, Sheffield Children's Hospital, Sheffield S10 2TH, and* [2] *The Jessop Hospital for Women, Sheffield S3 7RE*

There has been increasing interest in the possibility that one important factor in the aetiology of chronic lung disease of prematurity might be the presence, in plasma, of non-transferrin-bound Fe (NTBI). Such Fe would be available to participate in reactions leading to the generation of reactive oxygen species. Progress in this area has been limited by the lack of availablity of a method that is sufficiently sensitive and precise and which can be applied to the very small samples of plasma usually available from neonates. A direct method for the quantification of NTBI has been developed by others, for use in studies of Fe overload (Singh et al. 1990); this relies on HPLC with spectrophotometric detection. We have developed this further for use on 100µl plasma samples from neonates.

The method relies on a low-affinity Fe chelator, disodium nitriloacetic acid (NTA), which binds to low molecular weight Fe and Fe non-specifically bound to plasma protein. A two-step filtration process removes all proteins of molecular weight greater than 20 000, including transferrin and ferritin. NTA-bound Fe in the filtrate is quantified by HPLC using a mobile phase containing a high-affinity iron chelator, CP22 (3-hydroxy-1-propyl-2-methyl-pyridin-4-one), which forms a $(CP22)_3$-Fe complex which can be detected by its absorbance at 450 nm. The method now runs with a within-batch coefficient of variation of 5.13 %, and can detect 0.05 µmol/l NTBI. Recovery of NTBI from plasma samples to which Fe had been added in excess of the Fe-binding capacity of the transferrin ranged from 92.7 to 99.1%.

NTBI could not be detected in adult plasma. In contrast, NTBI was detected in twenty-three of fifty-four plasma samples collected from twenty-one premature babies over the first week of life. Values ranged from 0.0 to 15.5 µmol/l. This newly-developed method will make a substantial contribution to our ability to evaluate the importance of NTBI as a factor in the aetiology of chronic lung disease of prematurity.

Singh, S., Hider, R.C. & Porter, J.B. (1990). *Analytical Biochemistry 186,* 320-323.

The effects of riboflavin depletion and repletion on the structure and cytokinetics of the small intestine. By P.A. ARYEE[1], E.A. WILLIAMS[2], R.D.E. RUMSEY[3] and H.J. POWERS[2], *[1]Centre for Human Nutrition, University of Sheffield, Northern General Hospital, S5 7AU;[2]University Department of Paediatrics, Sheffield Children's Hospital, S10 2TH and [3]Department of Biomedical Science, University of Sheffield, S10 2TN*

Human and animal studies have shown riboflavin deficiency to have significant effects on Fe absorption and loss which may be partially explained by the effects of riboflavin deficiency on the structure and cytokinetics of the small intestine (Powers *et al.* 1993). Transit rate of enterocytes along the villus is increased and villus number depressed in rats fed on a riboflavin-deficient diet from weaning when compared with weight-matched controls. The aim of the current study was to establish whether these changes could be reversed by repletion.

Forty-eight female weanling Wistar rats (Sheffield strain) were divided into two dietary groups and fed *ad lib.* on either a riboflavin-deficient diet or a control diet. The control group was weight-matched (WM) to the riboflavin-deficient group (RD) throughout the experiment. The rate of transit of enterocytes from the crypts and along the villi, was measured using bromodeoxyuridine (BrdU) a thymidine analogue which is incorporated into replicating cells and can be detected using an immunohistochemical technique. After 5 weeks, by which time activation coefficients for erythrocyte glutathione reductase (*EC* 1.6.4.2) were significantly higher in the RD group (2.4 (SEM 0.01)) than the WM group (1.3 (SEM 0.02)), six deficient animals and their WM partners were given an i.p. injection of BrdU (50 mg/kg body weight) and killed 24 h later. After killing, sections of intestine were removed for immunohistochemical and morphological analysis. The eighteen remaining riboflavin-deficient animals were given flavin mononucleotide (FMN) (10 mg/kg body weight) by oral intubation. From this point all animals were fed on the control diet and weight matching was continued. On days 2, 7 and 21 following FMN administration and having been injected with BrdU 24 h earlier, six pairs of animals were killed and tissues collected.

		Days from repletion							
		0		2		7		21	
Variable	Group	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
		n 6		*n* 6		*n* 6		*n* 6	
Villus number	RD	1322**	46.4	1236**	27.0	1373**	18.1	1327*	63.0
	WM	1662	41.0	1673	54.0	1677	69.5	1608	48.1
Leading edge (μm) †	RD	197*	26.7	185**	26.7	168*	13.3	173*	17.2
	WM	117	14.4	95	7.9	134	7.6	104	15.0

Significantly different from WM control animals (Mann Whitney U), $*P<0.05$ $**P<0.01$.
† Distance of the leading edge (i.e. cohort of cells furthest from the crypt:villus junction) from the base of the villus, 24 h after BrdU.

By day 7 after administration of FMN the activation coefficient of erythrocyte glutathione reductase had dropped to control values, confirming a correction of the riboflavin deficiency. The total number of villi within a 10 mm length duodenum was counted using a light microscope and the distance travelled by the BrdU-labelled cells along the villi was measured using image analysis. The number of villi within the experimental group was significantly lower than the control group after 5 weeks on a riboflavin-deficient diet and remained so throughout the period of repletion. The distance travelled by the enterocytes along the villi was significantly greater in the riboflavin-deficient group than in the control group at all points of measurement. The rate of transit of the enterocytes fell slightly, over the period of repletion, from 8.2 (SEM 1.12) μm/h to 7.2 (SEM 0.72) μm/h , but did not reach control values. The results suggest that the effects of riboflavin deficiency on the rate of transit of enterocytes and on villus number cannot be readily reversed within a 3-week repletion period.

The irreversibility of the effects of riboflavin depletion induced at weaning highlights the importance of dietary riboflavin during development.

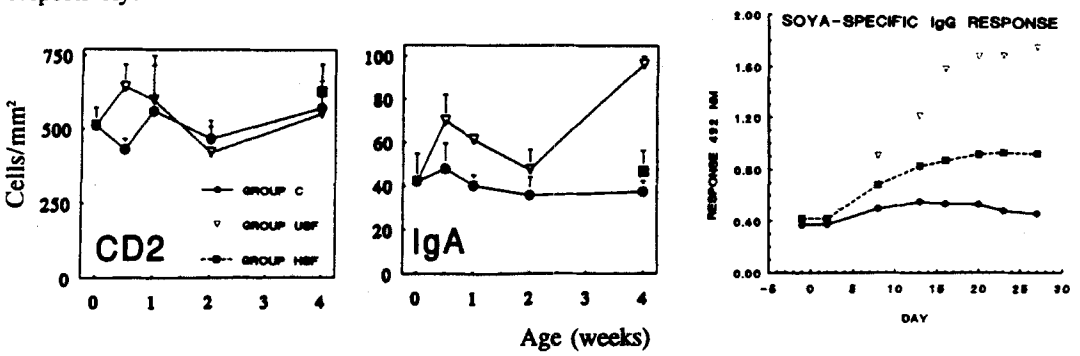
Powers, H.J., Weaver, L.T., Austin, S. & Beresford, J.K. (1993). *British Journal of Nutrition* **69**, 552-561.

Cellular and humoral immune responses in soyabean protein fed preruminant calves. By G.H. TOLMAN[1], S. SPANHAAK[2], A.T.J. BIANCHI[3], R. ZWART[3] and E. VAN ENGELEN[3], *[1]TNO Nutrition and Food Research Institute, Department of Animal Nutrition and Physiology ILOB, Wageningen, The Netherlands, [2]TNO Nutrition and Food Research Institute, Department Toxicology, Zeist, The Netherlands and [3]Institute for Animal Science and Health, Lelystad, The Netherlands.*

From an economic point of view there is a growing interest in replacing expensive skimmed milk powder (SMP) in calf milk replacers by non-milk protein sources, such as soyabean (soya) protein. However, introduction of soya protein in the diet can result in gastrointestinal disorders believed to be caused by a hypersensitivity reaction to soya antigens (Sissons & Tolman, 1991; Lallès *et al.* 1993). Sufficient knowledge about the underlying mechanism of how soya antigens stimulate the immune response and how this immune response results in gastrointestinal disorders is still lacking.

The present experiment was conducted to investigate cellular and humoral immune responses in soya fed calves. Soya naïve calves of 4 weeks old were fed on either a soya-free diet (group C), a diet containing 100g untoasted soya flour/kg (group USF) or a diet containing 100g heated soya flour/kg (group HSF). On day 0, just before the introduction of the experimental diets, three calves were killed for collecting intestinal tissue and organs. This was repeated on days 5, 9 and 14 for three calves from groups C and USF. On day 28, three calves from all three groups were killed. During the experiment, twice a week blood was sampled for measuring soya specific immunoglobulin (Ig) response.

The first week after the introduction of USF an increase in CD2-positive T-cells was present in the ileum, followed by an increase in IgM, IgA and IgG-positive cells from day 9 onwards. At the same time an increase in soya specific IgA and IgG occurred in the serum. Weight gains (day 0 - 28) were 719 (SD 46), 378 (SD 134) and 739 (SD 26) g/day for groups C, USF and HSF respectively.



Introduction of untoasted soya flour resulted in an early cellular immune response at the intestinal level, followed by a distinct soya-specific humoral immune response and a depressed performance.

Sissons, J.W. & Tolman G.H. (1991). In *Toxic factors in crop plants*, pp. 62-85 [J.P.F. D'Mello and C.M. Duffus, editors]. Edinburgh: The Scottish Agricultural College.

Lallès, J.P., Salmon, H., Bakker, N.P.M. & Tolman G.H. (1993). In *Recent advances of research in antinutritional factors in legume seeds*, pp. 253-270 [A.F.B. van der Poel, J. Huisman and H.S. Saini, editors]. Wageningen: Wageningen Pers.

Concentrations of serum cortisol, insulin and glucagon in young calves before and after receiving propionate orally. By J. A. NIKOLIĆ[1], H. A. ŠAMANC[2], Z. DAMJANOVIĆ[2], M. RATKOVIĆ[1], B. RADOJIČIĆ[2] and J. BEGOVIĆ[1], [1]*INEP - Institute for the Application of Nuclear Energy, 11080 Zemun,* [2]*Faculty of Veterinary Medicine, University of Beograd, 11000 Beograd, Yugoslavia*

The acute stimulatory effect of intravenous infusion of propionate on serum concentrations of insulin, glucagon and cortisol in adult cattle is well known. However, propionate produced in the digestive tract is taken up nearly completely by the liver, so that oral effects are presumed to occur only when the liver is overloaded (Istasse & Ørskov, 1984). In 15-d-old Holstein and Charolais cross calves the effect of peroral sodium propionate was examined by administering a drench (0.5 g/kg body weight in 250 ml milk) in the morning preprandially (Expt 1) and 3 h after feeding the morning portion of milk (3.5 litres; Expt 2). Blood was taken from the jugular vein. Insulin, cortisol and pancreatic glucagon were determined by radioimmunoassay in the separated sera.

			Expt 1						Expt 2								
			Insulin (mIU/l)			Cortisol (nmol/l)			Insulin (mIU/l)			Cortisol (nmol/l)			Glucagon (nmol/l)		
Breed	Sex		0 h	1 h	3 h	0 h	1 h	3 h	0 h	1 h	3 h	0 h	1 h	3 h	0 h	1 h	3 h
Holstein	F	Mean	14	12	13	21	15	10	46	56	18	19	18	17	32	36	34
(*n* 7)		SEM	1	1	1	5	2	1	9	13	2	7	4	3	2	2	2
Charolais	F	Mean	8	10	9	11	15	17	65	60	45	18	18	28	39	42	42
(*n* 7)		SEM	1	1	1	1	2	3	17	12	7	5	3	5	4	3	2
Charolais	M	Mean	7	7	9	22	32	21	123	117	77	19	15	20	31	36	35
(*n* 2 or 7)		SEM	2	1	0.1	12	23	-	40	59	41	4	2	3	4	2	5

The results obtained are shown in the Table. The two male calves available in Expt 1 were not included in the statistical analysis. Two-factor ANOVA showed a highly significant difference between the breeds in fasting insulin concentrations ($P<0.01$) but no effect of propionate. Concerning cortisol concentrations the marked breed×time interaction necessitated single-factor ANOVA for each group. A linear decrease in cortisol levels with time after propionate was found in the Holstein calves ($P<0.05$) and a linear increase in the Charolais cross calves ($P<0.05$). Serum hormone concentrations were not increased when propionate was administered postprandially. Postprandial insulin concentrations were significantly higher in the male calves than in each of the female groups ($P<0.05$), while postprandial glucagon concentrations were significantly higher in the female Charolais group than in the other groups ($P<0.05$).

Thus, the results showed interbreed and intersex differences in peripheral hormone levels as indicated by the different insulin profiles in the Charolais cross calves. The absence of marked increases after oral propionate suggests effective liver uptake in these young animals.

Components of the glucagon assay were from the Hammersmith Hospital

Istasse, L. & Ørskov, E.R. (1984). *Canadian Journal of Animal Science* **64**, Suppl., 148-149.

Effects of some alterations in diets for weaned piglets on serum thyroid hormone and IGF-I concentrations. By J. A. NIKOLIĆ[1] , B. ŽIVKOVIĆ[2] , G. KOSTIĆ[1] and D. STOJANOVIĆ[1] . [1]*INEP - Institute for the Application of Nuclear Energy, 11080 Zemun,* [2]*Livestock Research Institute, 11081 Zemun Polje, Yugoslavia*

With the aim of substituting imported feedstuffs with home-grown sources, the effects of replacing soyabean oilmeal with peas and fish meal with yeast were examined in four experiments between late autumn 1991 and summer 1993. After receiving isoenergetic, isonitrogenous, amino acid balanced diets *ad libitum* for 1 month, 3-month-old piglets (female and barrows) were weighed and blood was taken from the retroorbital sinus after an overnight fast. Serum total triiodothyronine (T_3), thyroxine (T_4) and insulin-like growth factor I (IGF-I) were determined by radioimmunoassay. Recovery of IGF-I was 95%.

		Standard diet				Diets with peas				Diets with yeast			
Expt		Body wt (kg)	T_3 (nmol/l)	T_4	IGF-I (μg/l)	Body wt (kg)	T_3 (nmol/l)	T_4	IGF-I (μg/l)	Body wt (kg)	T_3 (nmol/l)	T_4	IGF-I (μg/l)
1	Mean	27.2	0.68	57.0	148		-				-		
	SD	1.3	0.31	7.5	45								
2	Mean	28.0	0.40	30.4[a]	121	26.5	0.54	34.2[a]	92	27.3	0.43	14.7[b]	85
	SD	2.5	0.10	6.2	92	2.9	0.14	4.8	27	3.9	0.10	1.9	31
3	Mean	24.2	0.72	71.2	115	22.3	0.49	74.5	61		-		
	SD	2.3	0.14	11.0	65	2.4	0.29	12.7	24				
4	Mean		-				-			20.1	0.43	11.9	100
	SD									6.1	0.22	2.5	62

[ab] Values with unlike superscripts were significantly different, $P<0.05$ (ANOVA)

The Table shows that hormone levels in Expt 1 were within the normal range. The lower levels of T_3 and T_4 in Expt 2 were possibly due to lack of I in the salt supplement. This was corrected in Expt 3. Replacement of part of the maize and 75% of the soyabean oilmeal with peas led to slightly lower weight gains and poorer feed conversion but had no significant effect on feed intake and hormone levels. Substitution of fish meal by yeast was associated with even lower T_4 concentrations (Expts 2 and 4). Common salt iodinated with KI loses about one third of the I annually even in suitable storage conditions (Sinadinović *et al.* 1991). If the salt had contained no I, the diets containing fish meal would have supplied about 0.11 mg I/kg which approaches the daily requirement of 0.14 mg/kg, whereas those with yeast would have had only 0.06 mg/kg (Fëdorović-Tome *et al.* 1970).

Taken as a whole, the mean daily gain of the piglets (n 62) within the experimental periods was positively correlated with fasting T_3 levels (r 0.326; $P<0.01$) and with total IGF-I (r 0.275; $P<0.05$). The barrows were more susceptible to the presumed I deficiency than the females. At this early stage no goitres were detected but they have occurred recently on other farms in the area.

Fëdorović-Tome, M., Obradović, M. & Stošić, D. (1970). *Norms and Tables for Animal Nutrition.* Belgrade: Nolit.

Sinadinović, J., Mićić, J.B., Stojanović, D., Han, R. & Vučković, M. (1991). *Srpski Arhiv za Celokupno Lekarstvo* **119**, 63-68.

Responses in milk constituent secretion to intravascular administration of two mixtures of amino acids in dairy cows. By J. A. METCALF[1], L. A. CROMPTON[1], D. WRAY-CAHEN[1], M. A. LOMAX[1], B. J. BEQUETTE[2], J. C. MACRAE[2], F. R. C. BACKWELL[2], G. E. LOBLEY[2], J. D. SUTTON[1] and D. E. BEEVER[1]. [1]*University of Reading, Centre for Dairy Research, Hall Farm, Arborfield RG2 9HX,* [2]*Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB*

Milk constituent concentrations may be influenced by dietary manipulation but the control of these effects is unclear. In order to understand better the mechanisms affecting milk protein yield and concentration, amino acid mixtures were infused directly into the jugular vein of lactating dairy cows.

Four Holstein-Friesian cows in weeks 11 to 28 of lactation were fed on a grass silage (149 g crude protein (CP)/kg DM) and concentrates (142 g CP/kg DM) diet in a 2:3 DM ratio. Feed offered was restricted to 95% of *ad libitum* intake fed in twenty-four equal portions. Jugular vein catheters were inserted at least 24 h before infusion. Experimental design was a simple crossover of two amino acid mixtures, with a covariate saline infusion in each period. Saline infusion was for 4 d, followed immediately by amino acid infusion for 5 d with the mean of the last 3 d being used for statistical analysis. One mixture of amino acids (TAA) comprising essential and non-essential amino acids in the proportions found in milk protein, was infused at rate equivalent to 400 g protein/d. A second mixture comprising only the essential amino acids (EAA) which would be found in 400 g of milk protein was infused at a rate of 208 g of amino acid/d. Cows were milked at 06.30 and 16.30 hours daily, with milk samples taken at each milking for infra red milk analysis.

	TAA Mixture			EAA Mixture		
	Control	Infused	SED	Control	Infused	SED
Milk yield (kg/d)	23.8	24.4	0.29	22.4	23.5	0.49
Fat concn (g/kg)	46.0	43.5†	0.94	46.9	46.5	0.43
Protein concn (g/kg)	32.4	35.0**	0.29	32.5	36.9*	0.88
Lactose concn (g/kg)	48.4	47.2*	0.20	48.2	46.5	0.49
Fat yield (g/d)	1066	1046	29.4	1037	1078	19.0
Protein yield (g/d)	765	852*	14.1	726	869*	37.1
Lactose yield (g/d)	1156	1162	14.2	1084	1094	29.3

SED, standard error of difference; † $P<0.1$; * $P<0.05$; ** $P<0.01$.

Neither infusion of amino acids caused any significant increase in milk yield. Concentrations of fat and lactose decreased on TAA but not significantly on EAA whilst respective yields were largely unaffected. In contrast, protein concentration and yield increased with both TAA and EAA, with recovery of amino acids in milk protein being greater for the latter (22 *v* 36%), while the increase in milk protein yield on the EAA infusion was 69 g/100 g of infused amino acid. The recovery of TAA was similar to that previously observed with abomasal casein infusion.

It is concluded that intravascular infusion of essential amino acids can stimulate milk protein secretion to yield milk of a higher ($P<0.05$) protein content, and that this effect is unchanged by the inclusion of non-essential amino acids in the infusion mixture.

The work reported here formed part of a collaborative project funded by a consortium of the MAFF, BP Nutrition (UK), The Milk Marketing Board of England and Wales, BBSRC and SOAFD.

Effect of growth hormone administration on growth rate and plasma IGF-1 in milk- and solid-fed lambs. By K. KAMIL[1,2], J. J. GATE[1], L. A. CROMPTON[1], M. J. BRYANT[2] and M. A. LOMAX[1],
[1] *School of Animal and Microbial Sciences,* [2] *Department of Agriculture, University of Reading, PO Box 228, Reading RG6 2AJ*

It has been suggested that fetal growth is largely independent of growth hormone (GH) but during early neonatal life, an increase in hepatic somatotrophic receptors is thought to be responsible for the development of GH-dependent growth (Gluckman, 1984). We report a study investigating whether growth rate and plasma insulin-like growth factor (IGF-1) concentrations are able to respond to GH administration during the first month of life.

Sixteen lambs were divided at birth into two equal groups, control and GH-treated. Recombinant GH was injected subcutaneously (0.15 mg/kg per day) for a period of 84 d. Lambs were fed on milk replacer (Milkivit:BP Nutrition (UK) Ltd., Wincham, Northwich, Ches) from days 1-32 and then weaned onto a pelleted food (Volac Lamb Start to Finish, Volac Ltd, Orwell, Royston, Herts). Complete adaptation to solid feed was not achieved until lambs were 64 d old. Blood samples were collected at 09.00 hours at approximately 4 d intervals during days 1-32 (milk fed) and days 64-84 (solid fed).

	Milk fed			Solid fed		
	Control	GH	SED	Control	GH	SED
Growth rate (g/d)	254^{a}	255^{a}	6.4	231^{a}	326^{c}	17.3
ME intake (MJ/d per $kg^{0.75}$)	0.91^{a}	0.90^{a}	0.01	0.84^{b}	0.83^{b}	0.02
Glucose (mmol/l)	5.5^{a}	5.4^{a}	0.23	4.6^{b}	4.8^{b}	0.09
IGF-1 (ng/ml)	214.4^{a}	217.7^{a}	12.75	178.4^{b}	225.5^{a}	12.70

Different letters within each row indicate statistically significant difference from corresponding control values by Student's *t* test following ANOVA (a,b $P<0.05$; a,c $P<0.01$).

During the first month of life when lambs were milk-fed there were no effects of GH administration on growth rate, plasma glucose or plasma IGF-1 concentrations. When the same lambs were in their third month of life and fully adapted to a solid feed both growth rate and plasma IGF-1 concentrations were increased ($P<0.01$ and $P<0.05$) by GH administration. Plasma glucose concentrations were significantly ($P<0.05$) higher in milk-fed compared with solid-fed lambs, irrespective of treatment groups.

It is concluded that the somatotrophic axis may still be immature at 1 month of life despite evidence of the increase in hepatic GH receptors during the first week of neonatal life.

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The effects of meal frequency on postprandial plasma glucose tolerance, glucose dependent insulinotropic polypeptide and insulin concentrations. By C. HALLETT, S.G. ISHERWOOD, L.M. MORGAN, J.A. LOVEGROVE and M.C. MURPHY, *Nutrition and Food Science Research Centre, University of Surrey, Guildford, GU2 5XH*

The metabolic consequences of a given diet may depend partly on the frequency of meals eaten. Increasing meal frequency has been shown to decrease fasting plasma insulin and triacylglycerol levels (Jenkins *et al.* 1989), but the effect of meal frequency on postprandial hormone and metabolite reponses to a standard meal has not been investigated. Hormonal components of the entero-insular axis (the regulatory control exerted by the gastrointestinal tract on the endocrine pancreas), such as glucose-dependent insulinotropic polypeptide (GIP), play a key role in modulating the insulin secretory response to feeding (Morgan *et al.* 1988). Mean GIP levels have been found to decrease whilst consuming a nibbling diet (Jones *et al.* 1993). The present study therefore examines the effect of chronic ingestion of a nibbling (12 meals/d) or a gorging diet (2 meals/d) on fasting and postprandial insulin, GIP and glucose profiles.

Eleven female subjects (aged 22 (SD 0.89) years, BMI 23.6 (SD 2.77) kg/m^2) were studied. The subjects were allocated in random sequence to either the nibbling or gorging diet for 2 weeks, with a 3 week washout period between the diets. The study was a cross over design. Identical foods were eaten on each diet. The day before commencing the study (representing the normal diet) and after each 2 week diet, a postprandial study was performed. Subjects were cannulated at 08.30 hours after a 12 h fast and two fasting blood samples were taken before the consumption of a standard mixed meal. The meal provided 4.4 MJ energy, 82.2 g fat, 63 g carbohydrate and 20 g protein. Plasma samples were collected at 30 min after completion of the meal and then hourly for 8 h. Samples were analysed for plasma triacylglycerol (TAG), glucose, insulin and GIP. Comparisons of the total area under the plasma hormone and metabolite reponse curves (AUC) and fasting glucose:insulin molar ratios were made by ANOVA.

	Total AUC triacylglycerol min.mmol/l		Total AUC glucose min.mmol/l		Total AUC insulin min.pmol/l		Total AUC GIP min.pmol/l	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Baseline	752	80	2572	96	92096	9394	78708	12671
Gorging	756	68	2635	117	93869	8921	84143	18630
Nibbling	787	86	2598	112	92836	7654	83230	16612

There were no significant differences seen in either fasting levels or total AUC for TAG, glucose, insulin or GIP when the three diets were compared. The mean ratios for fasting glucose:insulin were 0.11 (SD 0.03), 0.10 (SD 0.02) and 0.09 (SD 0.02) for the baseline, gorging and nibbling groups and these were not significantly different from one another. Previous meal frequency therefore does not appear to affect postprandial responses as assessed by a standard test meal. We also failed to detect any changes in fasting hormone or metabolite levels, or insulin sensitivity as expressed by glucose:insulin ratios. Although these results are contrary to those previously reported, they may be explained by the use of younger female subjects in the present study. This group is likely to have a higher basal insulin sensitivity than the older men previously studied.

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Post-weaning dietary manipulation reduces blood pressure in rats with hypertension of fetal origin. By C.L.PICKARD, M.K.DEVOTO and H.D.McCARTHY. *Department of Human Nutrition, University of Southampton, Southampton SO16 7PX*

Previous studies have demonstrated that nutritional inadequacy *in utero* induces hypertension in rats maintained on standard laboratory chow (SLC) post-weaning (Langley & Jackson, 1994). The mediators of this hypertension are unknown, but dietary and/or endocrine factors could be involved. The present study was designed to determine the influence of post-weaning dietary manipulation on the maintenance of hypertension in this model.

Male and female offspring, (*n* 16 in both sexes) were studied from rat dams fed on semi-purified diets for 2 weeks before mating and throughout pregnancy with a casein content of either 90 or 180 g/kg. At age 4 weeks systolic blood pressure (SBP) was measured using the tail-cuff method. These rats were then weaned onto a macronutrient self-selection (MSS) diet based on casein, starch/sucrose and lard/maize oil (McCarthy *et al.* 1994). After 10 d, SBP was remeasured and the rats were transferred onto SLC for 14 d. This transference of diets was continued over the ensuing 5 weeks, with SBP measured at the change-over periods.

SBP values for rats of both sexes are shown in the Table below.

		Systolic blood pressure after feeding of diet (mm Hg)									
Age		29 d		39 d		53 d		68 d		90 d	
		Weaning		MSS		SLC		MSS		SLC	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Males	90 g/kg	125*	7.1	119	3.9	139	6.2	122	5.0	142	6.1
	180 g/kg	105	4.5	122	11.5	128	4.5	127	4.3	124	6.7
Females	90 g/kg	123*	2.8	116	5.6	127	5.5	124	2.8	131*	3.0
	180 g/kg	106	2.4	120	3.1	121	3.4	121	5.4	114	2.3

*Significantly higher than 180 g/kg group, $P \leq 0.05$ (2-tail).

SBP was significantly elevated in the 90 g/kg group upon weaning, but this difference was only sustained when rats were maintained on SLC. MSS lowered SBP in rats from the 90 g/kg group without affecting SBP of rats from the 180 g/kg group. The relative hypertension of the 90 g/kg group was expressed again when SLC was consumed. The effects appeared to be more pronounced in male offspring although the trend was clearly visible in female offspring.

These results indicate that diet during adulthood is critical for the expression of hypertension in this model. Since the major difference between SLC and MSS is the carbohydrate : fat ratio ingested, it is likely that the metabolic handling of these macronutrients may, in part, underlie the expression of hypertension in this model.

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The effects of environmental temperature and photoperiod on the body composition and properties of the pelt in the Djungarian hamster, *Phodopus campbelli*. By D. A. SADLER and C. M. POND, *Department of Biology, The Open University, Milton Keynes MK7 6AA*

The effects of photoperiod and ambient temperature on body mass, composition and properties of each adipose depot and pelt were investigated in *Phodopus campbelli*, a small hamster native to southern Siberia and Mongolia. Adult hamsters were studied over 102 d between November and March under four different regimes of photoperiod and environmental temperature. Specimens were caged individually, food (rat chow) and water were available *ad lib.*

Photoperiod	16 h L/ 8 h D		16 h L/ 8 h D		8 h L/ 16 h D		8 h L/ 16 h D	
Ambient temperature	24 ±2 ° simulated summer		<10 ° long day cold		24 ± 2 ° short day warm		<10 ° simulated winter	
Sex	M	F	M	F	M	F	M	F
n	19	20	9	11	14	11	17	18
Change in body mass (initial = 100%)	106.1	90.4	98.4	85.5	61.9	75.6	55.8	63.2
Food intake (g/d)	2.5	2.2	5.8	4.8	2.4	2.4	3.6	3.5
Final body composition								
Adipose tissue (g/kg final body mass)	203.2	153.0	126.0	87.1	112.6	131.8	67.8	70.2
Lipid in adipose tissue (g/kg)								
Interscapular	372.4	355.0	210.3	196.6	203.9	213.2	81.1	84.3
Inguinal	431.1	411.0	278.3	219.3	218.3	183.0	174.5	140.8
Protein in adipose tissue (g/kg)								
Interscapular	35.7	44.2	32.2	41.7	29.9	27.6	127.3	120.9
Inguinal	15.1	18.9	08.5	12.4	10.9	10.0	26.5	26.8
Lipid in pelt (μg/mg fur)								
Over rump	8.7	4.9	7.6	4.1	4.4	4.4	2.5	2.8
Behind ears	14.3	9.4	11.3	7.2	6.7	7.0	4.5	4.7
Pelt colour	dark	dark	dark	dark	paler	paler	paler	paler

Many of the changes in body mass and body composition that occurred under simulated winter were also observed in short day warm experimental conditions, and those changes seen in simulated summer, also occurred in long day cold experimental conditions. In both long day conditions, the males maintained their body mass and were fatter than the females. When corrected for body size, females ate significantly more food under similar conditions, except for short day warm. Both sexes ate much less in simulated winter than in long days at the same temperature. Males on the short photoperiods lost most weight. Changes in body mass were due almost entirely to changes in adipose tissue mass. Lipid content decreased in adipose depots under cold conditions and/or short days, and protein content increased in the depots only under short day cold conditions. The changes in lipid and protein content were much greater under all regimes in the brown adipose tissue depots than in the inguinal and all intra-abdominal depots. Changes in colour of the pelt may be related to camouflage; changes in its lipid content may be related to insulative properties for both cold resistance and heat tolerance.

We conclude that photoperiod and temperature interact to affect nearly all of the areas studied, but in most cases, photoperiod was the dominant influence. Season rather than weather is thus probably the chief cue for physiological adaptations to the Arctic climate in wild *Phodopus*.

Animal or vegetable protein intakes (100, 200 or 300 casein or soyabean proteins/kg) differentially alter plasma distribution of cholesterol and triacylglycerols in rats. By J. PROST and J. BELLEVILLE, *Unité de Nutrition Cellulaire et Métabolique, Université de Bourgogne, Dijon, France*

The aim of the present study was to investigate whether protein origin (animal or vegetable) and levels affect plasma cholesterol and triacylglycerol (TG) distribution in rats.

Twenty-four male Wistar rats weighing 80 (SD 5) g were divided into six groups. Rats were fed for 4 weeks on different diets with (g/kg) 100, 200 or 300 casein (C10, C20, C30) or highly purified soyabean proteins (98% purity, SP10, SP20, SP30), 50 soyabean oil, 50 cellulose, 20 vitamins, 40 minerals, and starch to make up to 1 kg. Blood was collected after overnight food deprivation. Plasma VLDL+LDL and HDL were isolated. Total cholesterol (TC), unesterified cholesterol (UC), cholesteryl esters (CE) and TG concentrations in plasma and lipoproteins were determined.

Plasma- and VLDL+LDL- TC values were similar in C and SP groups. HDL-TC concentrations were higher in the SP than in the C groups. VLDL+LDL-UC concentrations were higher in the SP30 than in the C30 group, VLDL+LDL-CE concentrations were more elevated in SP10 and SP30 than in C10 and C30 groups. In the SP30 group, HDL-UC concentrations were higher and HDL-CE concentrations were lower than in the C30 group (Table). The highest TG concentrations were obtained in C20 and C30 groups. The modifications in VLDL-TG were correlated with those of plasma -TG.

Distribution of TC, UC, CE and Tg in plasma and lipoproteins

	C 10		C 20		C 30		SP 10		SP 20		SP 30	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma												
TC (mmol/L)	2.50^{a}	0.37	2.46^{a}	0.36	2.69^{a}	0.41	2.30^{a}	0.50	2.52^{a}	0.42	2.50^{a}	0.38
TG (mmol/L)	0.25^{b}	0.04	0.77^{a}	0.15	0.71^{a}	0.05	0.27^{b}	0.05	0.25^{b}	0.11	0.34^{b}	0.06
VLDL + LDL												
TC (mmol/L)	0.768^{b}	0.082	0.667^{b}	0.166	0.671^{b}	0.177	1.357^{a}	0.498	0.525^{b}	0.197	0.827^{ab}	0.237
UC (mmol/L)	0.046^{c}	0.004	0.346^{a}	0.135	0.422^{a}	0.055	0.089^{c}	0.064	0.174^{ab}	0.049	0.174^{bc}	0.085
CE (mmol/L)	0.722^{b}	0.178	0.321^{b}	0.095	0.249^{b}	0.145	1.268^{a}	0.470	0.351^{b}	0.151	0.653^{b}	0.158
TG (mmol/L)	0.18^{b}	0.05	0.66^{a}	0.06	0.59^{a}	0.09	0.20^{b}	0.04	0.22^{b}	0.03	0.30^{b}	0.02
HDL												
TC (mmol/L)	0.983^{a}	0.125	1.055^{a}	0.134	1.216^{a}	0.155	0.950^{a}	0.124	1.243^{a}	0.150	0.923^{a}	0.121
UC (mmol/L)	0.347^{b}	0.024	0.450^{a}	0.061	0.380^{b}	0.044	0.431^{ab}	0.060	0.504^{a}	0.065	0.549^{a}	0.074
CE (mmol/L)	0.633^{ab}	0.088	0.608^{a}	0.065	0.836^{a}	0.061	0.519^{b}	0.064	0.739^{a}	0.143	0.373^{c}	0.048

^{a,b,c} Means values within a row with unlike superscript letters were significantly different. $P < 0.05$.

Therefore, when soyabean proteins were highly purified (98%) and added at 200 and 300 g/kg to a diet containing 50 g soyabean oil/kg without cholesterol, a decrease in plasma- and VLDL+LDL-TG concentration was observed, but plasma cholesterol was unaffected. This hypolipaemic effect may perhaps be explained by lower hepatic VLDL-TG synthesis and as we have previously shown by a higher VLDL uptake by the liver.

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Effect of animal or vegetable protein intakes (100, 200 or 300 g casein or soyabean proteins/kg) on VLDL, HDL and apolipoproteins in the rat. By J. PROST and J. BELLEVILLE, *Unité de Nutrition Cellulaire et Métabolique, Université de Bourgogne, Dijon, France*

The aim of the present study was to investigate whether protein origin (animal or vegetable) and levels have an effect on lipoprotein amounts and their apolipoprotein composition.

Twenty-four male Wistar rats weighing 80 (SD 5) g were divided into six groups. Rats were fed for 4 weeks on diets containing (g/kg) 100, 200 or 300 casein (C10, C20, C30) or soyabean proteins (SP10, SP20, SP30), 50 soyabean oil, 50 cellulose, 20 vitamins, 40 minerals, and starch to make up to 1 kg. After an overnight fast following the dietary period, blood was collected. Plasma lipoproteins (VLDL and HDL) were isolated by a single-spin discontinuous gradient (Redgrave *et al.* 1975 modified by Meghelli-Bouchenak *et al.* 1989). Apolipoproteins (apo) were assayed by electrophoresis according to Irwin *et al.* (1984).

	C 10		C 20		C 30		SP 10		SP 20		SP 30	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
VLDL												
mass (g/l)	0.310^{c}	0.035	1.131^{a}	0.105	1.188^{a}	0.110	0.322^{c}	0.017	0.518^{b}	0.055	0.635^{b}	0.100
Apo B100 (mg/l)	51^{e}	5	192^{b}	6	218^{a}	16	29^{f}	3	76^{d}	6	106^{c}	8
Apo B48 (mg/l)	41^{e}	4	85^{b}	5	138^{a}	20	26^{f}	3	52^{d}	6	71^{c}	5
Apo A-I (mg/l)	1.46^{d}	0.21	12.1^{a}	0.4	6.5^{b}	0.4	1.96^{d}	0.4	3.6^{c}	0.4	4.3^{c}	0.7
Apo A-VI (mg/l)	2.06^{d}	0.07	6.32^{b}	0.21	10.10^{a}	0.75	1.13^{d}	0.30	6.75^{b}	0.25	6.50^{c}	0.27
Apo C (mg/l)	4.37^{c}	0.51	18.00^{b}	1.22	43.55^{a}	3.21	2.15^{d}	0.23	22.20^{b}	2.03	22.94^{b}	2.2
Apo E (mg/l)	0.89^{d}	0.12	5.38^{b}	0.23	9.90^{a}	0.32	0.53^{d}	0.07	2.54^{c}	0.20	12.50^{a}	2.2
HDL												
mass(g/l)	1.915^{b}	0.220	2.595^{a}	0.360	2.847^{a}	0.160	2.655^{a}	0.350	3.123^{a}	0.220	2.672^{a}	0.390
Apo A-I (mg/l)	451^{c}	40	648^{ab}	48	593^{b}	41	606^{b}	58	819^{a}	65	582^{b}	44
Apo A-II (mg/l)	96^{a}	9	90^{a}	12	22^{c}	4	44^{b}	6	17^{c}	1	44^{b}	4
Apo A-IV (mg/l)	368^{a}	40	282^{ab}	18	322^{a}	20	201^{b}	10	219^{b}	15	307^{a}	12
Apo C (mg/l)	103^{a}	18	74^{bc}	8	87^{c}	10	97^{a}	5	48^{c}	9	46^{c}	9
Apo E(mg/l)	191^{c}	18	226^{b}	14	266^{a}	27	293^{a}	30	289^{a}	30	271^{a}	21

VLDL and HDL masses were the sum of triacylglycerols, unesterified cholesterol, cholesterol esters and proteins.

^{a,b,c} Means values within a row with unlike superscript letters were significantly different, $P < 0.05$.

The Table shows lower mass of VLDL in SP20 and SP30 compared with C20 and C30, but the mass of HDL was similar in all groups. Compared with the rats fed on casein, the distribution of VLDL- and HDL-apolipoproteins was modified in rats fed soyabean proteins.

Variable effects on lipoproteins and their apolipoprotein composition are observed with different levels of animal and vegetable proteins. These alterations may explain the hypolipaemic effect observed with highly purified soyabean proteins compared with casein.

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Limitation of the hypertrophy of adipose tissues by fish-oil *n*-3 fatty acids during high-fat feeding in the rat: eicosapentaenoic acid *v.* docosahexaenoic acid. By R. GROSCOLAS, T. RACLOT, E. MIOSKOWSKI, H. OUDART, A. C. BACH and I. MINELLO, *Centre d'Ecologie et Physiologie Energétiques, CNRS, 23 rue Becquerel, 67087 Strasbourg, France*

We have recently demonstrated that fish-oil feeding limits the hypertrophy of adipose tissue in rats fed on high-fat diets (Belzung *et al.* 1993). In addition, this effect was shown to be (1) specific to *n*-3 polyunsaturated fatty acids (*n*-3 PUFA), (2) selective of abdominal (retroperitoneal, RP; epididymal, EPI) fat depots and (3) due to a limitation of fat-cell hypertrophy. Eicosapentaenoic (EPA, 20:5*n*-3) and docosahexaenoic (DHA, 22:6*n*-3) acids are the two major *n*-3 PUFA simultaneously present in fish oil. The present study was therefore designed to determine whether the limitation of the hypertrophy of abdominal adipose tissues is due to EPA or DHA alone, or to a combination of both fatty acids.

Forty male Wistar rats (250 g) were distributed into four groups and fed for 4 weeks on isoenergetic amounts of a semi-purified high-fat diet (200 g fat/kg) the lipids of which contained 0 (reference "obesifying" diet, REF group) or 280 g/kg *n*-3 PUFA as pure ethyl ester of EPA or DHA alone (EPA and DHA groups) or in combination (MIX group, DHA/EPA: 3/2). The content of other fatty acids in each of the four diets was very similar (saturated 32, monounsaturated 33 and *n*-6 PUFA 7 g/100 g total fatty acids), additional monounsaturated fatty acids (mainly oleic acid) replacing *n*-3 PUFA in the REF diet. Daily *n*-3 PUFA intake was 0.8 g. At slaughter, RP, EPI, mesenteric (MES) and subcutaneous (SC) adipose tissues were thoroughly dissected, and their total lipid mass, fat-cell size and number were determined (Belzung *et al.* 1993). The remaining carcass was ground in liquid N_2 and lyophilized. Representative portions were used in the determination of (1) the lipid mass (triacylglycerols) of all minor fat depots, and (2) the total mass of body protein.

Final body mass (376–388 g) was not significantly different according to the dietary treatment. RP lipid mass was 30, 40 and 50% lower ($P < 0.05$) in the EPA (7.4±0.7 g), DHA (6.3±0.5 g) and MIX (5.4±0.4 g) groups, respectively, than in the REF group (10.5±0.8 g). EPI lipid mass was 20, 35 and 40% lower ($P < 0.05$) in the EPA (6.9±0.5 g), DHA (6.5±0.5 g) and MIX (5.6±0.2 g) groups, respectively, than in the REF group (9.3±0.4 g). Compared with the REF group (6.5±0.2 g), MES lipid mass was not significantly different in the EPA and DHA groups but it was 25% lower ($P < 0.05$) in the MIX group (4.9±0.3 g). Total abdominal (RP plus EPI plus MES) and body adiposity were also, respectively, 35 and 20% lower ($P < 0.05$) in the MIX than in the REF group. The mass of SC as well as that of total body protein, and the number of fat cells in fat depots, were not significantly affected by *n*-3 PUFA feeding.

We conclude that under controlled feeding conditions (same intake of energy, same content of the diets in fatty acids other than *n*-3 PUFA), EPA as well as DHA limits the accretion of lipid in abdominal fat depots in growing rats fed on high-fat diets. However, the most efficient limitation of abdominal fat cell hypertrophy, and a significant limitation of total adiposity, are obtained by feeding a combination of EPA and DHA (MIX). This suggests a synergic effect of EPA and DHA at one or several of the metabolic steps that control the partitioning of nutrients between storage or oxidation.

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Effects of eicosapentaenoic acid and docosahexaenoic acid alone or in combination on plasma lipids and hepatic fatty acid oxidation in rats fed on high-fat diets. By R. GROSCOLAS, C. LERAY, E. MIOSKOWSKI, T. RACLOT and A. C. BACH, *Centre d'Ecologie et Physiologie Energétiques, CNRS, 23 rue Becquerel, 67087 Strasbourg, France*

Dietary *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) contained in fish oil are known to lower plasma lipids and to activate hepatic fatty acid oxidation. The present study examines whether these effects (*i*) are specific to eicosapentaenoic (EPA) or docosahexaenoic (DHA) acids, the two major *n*-3 PUFA in fish oil, and (*ii*) are similar when EPA and DHA are fed alone or in combination.

Thirty male Wistar rats (250 g) were distributed into five groups (six rats/group) and fed for 4 weeks on isoenergetic amounts of a semi-purified high-fat diet (200 g fat/kg) the lipids of which contained 0 (reference diet, REF) or 280 g/kg of *n*-3 PUFA as (1) EPA, (2) DHA, (3) EPA+DHA (MIX, DHA/ EPA: 3/2) and (4) EPA+DHA (FO, DHA/EPA: 3/4). The *n*-3 PUFA were fed (0.8 g/d) as pure ethyl esters (a generous gift from Hoffman-La Roche, VFMH, Basel, Switzerland) (EPA, DHA and MIX) or as triacylglycerols (FO). The content of other fatty acids in each of the five diets was very similar (saturated 32, monounsaturated 33 and *n*-6 PUFA 7 g/100 g fatty acids), additional monounsaturated fatty acids replacing *n*-3 PUFA in the REF diet. At slaughter in the postprandial state, blood and liver samples were quicky removed for further analyses. Plasma triacylglycerols, free fatty acids and free cholesterol were determined using commercial kits. The following variables were measured in liver: wet, lipid, and protein mass; peroxisomal and mitochondrial oxidation of fatty acids as assessed by fatty acyl-CoA oxidase (FAO, *EC* 1.3.99.3) and carnitine palmitoyltransferase (CPT, *EC* 2.3.2.1) activity respectively, in crude homogenate.

When compared with the REF diet, feeding EPA or DHA, alone or in combination, resulted in a similar lowering of plasma triacylglycerols and free cholesterol by 70–80% ($P < 0.001$) and by 40–50% ($P < 0.001$) respectively, but in no effect on the plasma concentration of free fatty acids.

	REF		EPA		DHA		FO		MIX	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Protein mass (g/liver)	3.2^a	0.1	3.2^a	0.1	3.2^a	0.0	3.7^b	0.1	4.0^b	0.1
FAO (nmol/min per mg protein)	1.7^a	0.1	3.9^b	0.4	3.2^b	0.1	4.0^b	0.4	4.2^b	0.2
CPT (nmol/min per mg protein)	1.8^a	0.5	5.9^b	0.6	6.0^b	0.5	9.8^c	1.1	11.6^c	0.6

Mean values within a row not sharing the same superscript letter were significantly different, $P < 0.01$ (n 6). Peritz F-test for multiple comparisons.

The wet, lipid and protein masses of liver were significantly higher ($P < 0.001$) in the MIX and FO groups than in the three other groups. Liver FAO specific activity was significantly 1.8–2.4-fold higher in the four groups fed on *n*-3 PUFA than in the REF group. CPT specific activity was significantly 3–6-fold higher in rats fed on *n*-3 PUFA than in the REF group, this activity being almost 2-fold higher in rats fed on a combination of EPA and DHA in comparison with those fed on EPA or DHA alone.

In conclusion, our results show that feeding EPA or DHA is similarly efficient in lowering plasma triacylglycerols and free cholesterol, and in stimulating hepatic FAO and CPT activities in rats fed on high-fat diets. For plasma lipids and FAO these effects are similar when EPA and DHA are fed alone or in combination. In contrast, at the same dosage a combination of EPA and DHA is more efficient than EPA or DHA alone in increasing liver CPT activity, suggesting a synergic effect of these two fatty acids. Moreover, a combination of EPA and DHA, but not EPA or DHA alone, induces hepatomegaly without steatosis.

Involvement of brown adipose tissue in the limiting effect of *n*-3 polyunsaturated fatty acids on obesity induced by a high-fat diet in the rat. By H. OUDART, R. GROSCOLAS, C. CALGARI and A. MALAN, *C.E.P.E./C.N.R.S., 23 rue Becquerel, 67087 Strasbourg, France*

Recent studies have suggested that fish oil has a limiting effect on the obesity induced by a high-fat diet. To examine the possible role of brown adipose tissue in this limitation, we studied the influence of the two fatty acids characteristic of fish oil, i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on indices of the interscapular brown adipose tissue (IBAT) thermogenic activity. Five groups of six rats (240-260 g) were fed for 4 weeks on either a low-fat/high-carbohydrate diet (control or C group, fat content: 40 g/kg diet, 9% energy) or one of four high-fat diets (fat content: 200 g/kg diet, 39% energy) containing respectively, EPA alone (EPA group, 28% of dietary lipids as EPA), DHA alone (DHA group, 28% of dietary lipids as DHA), a mixture of EPA and DHA (MIX group, 9.4% and 15% of dietary lipids as EPA and DHA respectively), or none of these two fatty acids replaced by monounsaturated fatty acids (REF group). The amounts of *n*-6 polyunsaturated and of saturated fatty acids were similar in all diets and the *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) were incorporated into the diet under the form of ethyl ester (a generous gift from Hoffman-La Roche, VFMH, Basel, Switzerland).

The energy intake was 12% higher in the C group compared with the other groups ($P<0.05$) and did not differ between the groups fed on the high-fat diets. The *n*-3 PUFA intake (about 0.8 g/d) did not differ between the EPA, DHA, and MIX groups. The gross food efficiency was 20% lower in the C group compared with the groups fed on high-fat diets and did not differ between the REF group and the groups fed on diets enriched with *n*-3 PUFA. The body lipids assessed by the masses of the retroperitoneal plus the epididymal white fat pads were lower in the MIX and DHA groups compared with the C and REF groups. The mitochondrial IBAT activity was increased by high-fat diet feeding compared with the C group. The enrichment of the high-fat diet with *n*-3 PUFA, particularly with a mixture of EPA and DHA, induced a stimulation of IBAT activity. The total IBAT activity was increased in the groups fed on high-fat diets compared with the C group and was 1.6-fold higher in the MIX and EPA groups than in the REF group.

Diets	C		REF		MIX		EPA		DHA	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Gross food efficiency (g/100 kJ)	1.06^{a}	0.07	1.40^{b}	0.06	1.38^{b}	0.05	1.39^{b}	0.04	1.25^{b}	0.02
White fat pads mass (g)	19.1^{a}	0.9	18.0^{a}	1.0	12.6^{b}	0.6	15.8^{ac}	0.9	14.1^{bc}	0.6
IBAT mass (mg)	446.2^{a}	23.7	501.2^{ab}	30.6	455.7^{a}	28.4	578.9^{b}	20.9	536.7^{ab}	22.0
Mitochondrial GDP binding (pmol/mg protein)	202.3^{a}	23.2	334.3^{b}	14.7	438.4^{c}	54.4	363.1^{bc}	21.8	410.6^{bc}	28.1
Total GDP binding (nmol/pad)	2.46^{a}	0.44	3.87^{b}	0.45	6.20^{c}	0.85	6.04^{c}	0.72	4.94^{bc}	0.46

Mean values within a row bearing different superscript letters are significantly different, $P<0.05$.

These results indicate that a diet rich in *n*-3 PUFA, particularly in a mixture of EPA and DHA, induces a marked stimulation of the thermogenic activity of BAT. This diet-induced thermogenesis may be implicated in the limiting effect of *n*-3 PUFA on the obesity induced by a high-fat diet.

Size and clearance of chylomicrons after different fat loads in young women. By NEBIL ATTIA[1], SANA SAKR[1], JEAN-LOUIS PAUL[2], THEOPHILE SONI[2] and ANIK GIRARD-GLOBA[1]. *[1]Groupe Lipoprotéines, Faculté de Médecine X. Bichat and [2]Laboratoire de Biochimie Hôpital Broussais, Paris, France*

In the rat, polyunsaturated fatty acid (PUFA)-chylomicrons are larger than saturated fatty acid (SFA)-chylomicrons, exhibit a lower phospholipid:triacylglycerol (PL:TG) ratio, compatible with larger particles and are removed faster. In human subjects, few studies have compared the effect of ingested fatty acid composition on chylomicron metabolism as characterized by composition, size and clearance after fat load. We report data relative to size, composition and clearance of chylomicrons and their remnants after single loads of different fats.

Eleven healthy normal weight young women [25.0 (2.9) years], with normal fasting lipid concentrations (cholesterol < 5.8; TG < 1.2 mmol/l) ingested four consecutive fat loads at weekly intervals given in the form of an emulsion containing 60 g fat as sunflowerseed oil (SO), oleic-sunflowerseed oil (OO) (72 % 18: 1n-9, 18 % 18: 2n-6), mixed oil (MO) (38 % 18: 1n-9, 48 % 18: 2n-6), or beef tallow (BT) plus 35 g sucrose and 25 g casein. Retinyl palmitate (RP) was added to the meal as a marker of chylomicrons and their remnants. Serum was collected before the fat load and 2, 4, 6 and 8 h thereafter. Chylomicrons were removed by ultracentrifugation and their diameter was estimated by agarose gel filtration. Fatty acid composition of chylomicron-TG closely reflected that of the corresponding meal.

	Postprandial TG (iAUC)				Chylomicron diameter					
	Chylomicrons		Chylomicron-free serum		4 h		6 h		8 h	
	mmol. l^{-1} .h				nm					
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
SO	1.36	0.60	1.08	0.80	278[ab]	135	209	103	164	54
OO	1.32	1.19	1.02	1.01	168	75	164	46	150	23
MO	1.47	0.68	1.66	1.51	192	62	160	50	151	69
BT	0.41[*†¶]	0.30	0.48	0.57	151	42	118[c]	33	113	46

iAUC, incremental area under the curve.

[*]$P < 0.05$, for differences from OO, [†]$P < 0.001$, for differences from SO, and [¶]$P < 0.001$, for differences from MO.

[a]$P < 0.05$, for differences fromOO and MO, [b]$P < 0.01$, for differences from BT at 4 h.

[c]$P < 0.05$, for differences from all other fats at 6 h.

All fats induced the same chylomicronemia (see Table) except BT which was lower in the postprandial state. In chylomicron-free serum (containing the remnants) the differences were not significant. Chylomicron diameters were larger with SO than with all other fats at 4 h and smaller with BT than those from vegetable oils at 6 h. A strong positive correlation was evidenced between diameter of chylomicrons produced 6 h after SO load and their TG concentration ($r = +0.79$, $P < 0.01$). No such correlation could be evidenced with other fats. Differences in RP paralleled those of TG concentrations: 4 h after the load, the increase in serum RP concentration was lower for BT than for SO, OO or MO ($P < 0.05$). Reduced serum RP concentrations after BT reflected reduced chylomicron-RP concentrations ($P < 0.01$ with respect to SO or OO, or $P < 0.05$ with respect to MO). Chylomicron-remnants were not affected by the fatty acid composition of different meals and no significant difference was evidenced between BT and other fats in the RP concentration of the chylomicron-free serum fraction.

It is concluded that PUFA (SO) are rapidly absorbed and yield large chylomicrons which are rapidly lipolysed and cleared so that chylomicron-TG are correlated with diameter. By contrast, oleic acid although absorbed just as fast yields smaller chylomicron which are taken up more slowly so that their TG content is not directly related to their size. SFA (BT) are absorbed more slowly but also, in view of chylomicron-free serum RP concentrations comparable to those of other fats propably yield very small chylomicrons which either are small enough to remain in the chylomicron-free fraction or chylomicrons that are rapidly lipolysed without being cleared.

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Detection of enriched [1-^{13}C]palmitic acid in human stool samples by gas chromatography-isotope ratio mass spectrometry. By M. STOLINSKI, J.L. MURPHY and S.A.WOOTTON, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

Attempts to determine the gastrointestinal handling of ^{13}C-labelled substrates have been restricted to measurements of the level of total ^{13}C enrichment within the stool and assume that the label within stool is present in the form in which it was ingested (Murphy *et al.* 1995). On-line separation of compounds by GC before analysis of ^{13}C abundance by gas chromatography-isotope ratio mass spectrometry (GC-IRMS) would make it possible to identify the compound bearing the label. The aim of the present study was to examine the precision and repeatability of ^{13}C measurements in (I) fatty acid methyl ester (FAME) standards and (ii) FAME prepared from fatty acids present within stool lipid.

Samples of hexadecanoic acid (16:0) and pentadecanoic acid (18:0) FAME (Sigma Chemical Co. Ltd., Poole; 2 mg total FAME) were dissolved in 1 ml hexane and 2 µl injections were loaded onto the gas chromatograph (split injection mode). The ^{13}C abundance of ten repeated injections of both FAME was determined using an ORCHID gas chromatograph interface module and ANCA-*NT* isotope ratio mass spectrometer (Europa Scientific Ltd, Crewe) against an internal standard (undecanoic acid 11:0; -27.45‰ δ^{13}C v. Pee Dee Belemnite (PDB)) spiked into each sample. The levels of ^{13}C abundance in 16:0 and 18:0 FAME were -34.01 (SE 0.19)‰ and -23.62 (SE 0.3)‰ with coefficients of variation of 1.8 and 2.2% respectively. FAME were then prepared from two stool samples from the same subject from a previous study (Murphy *et al.* 1995) before and 3 d following a single oral dose of [1-^{13}C]palmitic acid (10 mg/kg body weight). Lipids were extracted by a modification of the method of Folch *et al.* (1957), saponified, methylated, evaporated to dryness and taken up in hexane for GC-IRMS analysis to give comparable peak areas as the FAME standards. FAME were identified by comparison of their retention times with a standard mixture. The levels of enrichment in the principal FAME within the sample of stool lipid of triplicate injections are given in the Table.

		Enrichment (‰ δ^{13}C v. PDB)							
	Total dry stool	C16:0		C18:0		C18:1		C18:2	
Stool	Mean	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Before	-25.25	-32.66	0.30	-33.96	1.44	-35.43	0.49	-37.30	1.10
Day 3	-18.68	+268.61	4.56	-35.76	0.18	-32.27	0.37	-38.56	1.25

These results indicate that (I) the coefficients of variation in ^{13}C abundance for each fatty acid in both the unenriched and enriched samples were low ranging from <1 to 7.3% and (ii) the enrichment was primarily restricted to the labelled species consumed by the subject (16:0) suggesting that there is minimal metabolism of the administered fatty acid within the gastrointestinal tract with no carry-over of the highly elevated signals detected in neighbouring FAME. It appears that this methodology using GC-IRMS offers good precision and repeatability for the determination of ^{13}C abundance in long-chain fatty acids present in stool lipid.

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A specific enzyme-linked immunosorbent assay for the measurement of apolipoprotein B-48 in triacylglycerol-rich lipoproteins. By K.G. JACKSON[1], J.A. LOVEGROVE[1], A. ZAMPELAS[1], A. KAFATOS[2], M. KAPSOKEFALOU[2], C.M. WILLIAMS[1] and B.J. GOULD[1],[1]*Nutrition and Food Safety Research Centre, University of Surrey, Guildford GU2 5XH* and [2]*Department of Social Medicine and Nutrition, University of Crete, Iraklion, Greece*

Recent evidence has suggested that intestinally derived triacylglycerol-rich (TAG-rich) lipoproteins may be important in the development of coronary artery disease (CAD) either directly or via their effect on other lipoprotein particles (Patsch. 1994). Apolipoprotein (apo) B-48 is the unique structural protein of chylomicrons (CM) and this protein is colinear with 48% of the amino terminal of apo B-100 which is present in VLDL, the TAG-rich hepatically derived lipoproteins.

An antiserum has been raised in our laboratory to the C-terminal residues of apo B-48 which has been shown to be specific for apo B-48, using sodium dodecyl sulphate polyacrylamide electophoresis (SDS-PAGE) and Western blotting analysis (Peel *et al.* 1993). This has been used in the development of a competitive enzyme-linked immunosorbent assay (ELISA) for the measurement of apo B-48 in TAG-rich lipoproteins. The ELISA has a sensitivity of 10 ng/ml and intra- and interassay coefficients of variation of 3.8 and 6.5% respectively. There was no cross-reactivity of the antiserum with human serum albumin, ovalbumin, thyroglobulin or apo B-100. Twelve postprandial profiles from diabetic and normal subjects were analysed using SDS-PAGE Western blot analysis and the ELISA technique and the methods were found to have a mean correlation of $r \# 0.74$.

Responses of postprandial apo B-48 to a standard test meal were investigated in subjects who habitually consume varying amounts of monounsaturated fatty acids (MUFA) in their background diet. The percentage dietary energy derived from MUFA (calculated from three-day diet diaries) was 11% for the UK, 16.5% for mainland Greece (GNC) and 21% for Crete (GC). Forty five subjects (aged 18-30 years, BMI 20-25 kg/m^2) were recruited, fifteen from the UK, fifteen from GC and fifteen from GNC. After a 12 h overnight fast, subjects were given a test meal which contained 150 g carbohydrate, 24 g protein and 42 g of fat (17% energy derived from saturated fatty acids (SFA), 12% from MUFA and 5% from polyunsaturated fatty acids (PUFA)). Two fasting blood samples were taken before consumption of the test meal and then hourly samples were collected for 9 h. The TAG-rich lipoprotein fraction was separated by ultracentrifugation and analysed for apo B-48 using the competitive ELISA. The response and shapes of the apo B-48 response curves differed between the U.K. and the Greek subjects. The postprandial lipaemic response calculated from the incremental area under the curve (IAUC) expressed as mean μg/ml per min were for the UK 285 (SE 47.8), GNC 48.9 (SE 8.8) and GC 66.4 (SE 14.3). The IAUC was significantly greater for the UK than the Greek subjects, $\underline{P} < 0.001$. In addition the postprandial apo B-48 concentration in the Greek subjects returned rapidly to baseline following the initial peak apo B-48 concentration, whereas in the UK subjects, apo B-48 remained elevated above baseline even at the end of the 9 h postprandial period.

This study has shown that an antiserum raised to apo B-48 has been successfully used in an ELISA for the measurement of apo B-48 in TAG-rich lipoproteins after a standard test meal. The attenuated apo B-48 response to a standard test meal observed in Greek subjects suggests marked differences in the metabolic processing of dietary fat in this population.

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Peel, A. S., Zampelas, A., Williams, C. M. & Gould, B. J. (1993). *Clinical Science* **85**, 521-524.

Potentially atherogenic chylomicron remnants are present in the low-density lipoprotein fraction as detected by a specific antibody to apolipoprotein B-48. By S. G. ISHERWOOD, B. A. GRIFFIN, C. M. WILLIAMS and B. J. GOULD, *Nutrition and Food Safety Research Centre, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

It has been suggested that dietary-derived lipoproteins are involved in the development of coronary artery disease (CAD) (Zilversmit, 1979). Small, dense chylomicron remnants (CMR) present throughout the postprandial phase and persisting in the postabsorptive state may have an impact on the metabolic fate of endogenously-derived lipoproteins, creating pro-atherogenic abnormalities in fasting lipoproteins. The aim of the present study was, therefore, to investigate the size and density distribution of CMR in fasting and postprandial samples by two ultracentrifugation methods.

A blood sample was taken from four subjects after a 12 h overnight fast. The subjects were then given a high-fat meal (80 g fat) and postprandial blood samples were taken 4 h and 8 h (3 subjects only) after the meal. Plasma was used to isolate lipoprotein fractions by sequential flotation ultracentrifugation (SFU) and also by discontinuous, cumulative, density gradient ultracentrifugation (DGU). In SFU lipoproteins of increasing density were separated by consecutive spins at densities *(d)* of 1.006 g/ml (VLDL), 1.019 g/ml (intermediate density lipoproteins, IDL) and 1.063 g/ml (LDL). In DGU lipoproteins were separated by their rate of flotation in the ranges Sf >60 (CM and VLDL1), 20-60 (VLDL2), 12-20 (IDL) and 0-12 (LDL). The presence of CMR was demonstrated by separation of the proteins in each lipoprotein fraction using denaturing polyacrylamide gel electrophoresis, followed by immunoblotting with a specific antibody to apolipoprotein B-48 (apo B-48) (Peel *et al.* 1993), the apolipoprotein found uniquely in dietary-derived lipoproteins. Relative amounts of apo B-48 in each fraction were determined using densitometric scanning. The mean results for four subjects are expressed in the Table as the optical density values at 550nm (OD_{550}) as a percentage of an internal standard .

DGU	Apo B-48 level [mean OD_{550} as % standard (SEM)]			SFU	Apo B-48 level [mean OD_{550} as % standard (SEM)]		
fraction	Fasting n 4	4 h n 4	8 h n 3	fraction	Fasting n 4	4 h n 4	8 h n 3
Sf >60	2.8 (1.0)	24.9 (6.1)	12.2 (3.8)	*d* <1.006 g/ml	10.3 (4.5)	16.7 (5.3)	19.0 (8.5)
Sf 20-60	11.4 (4.5)	8.9 (3.5)	9.3 (1.8)	*d* <1.019 g/ml	16.7 (1.5)	19.9 (7.0)	16.6 (0.2)
Sf 12-20	2.8 (0.9)	2.5 (1.7)	3.4 (1.5)	*d* <1.063 g/ml	4.8 (2.2)	2.3 (0.9)	3.3 (0.2)
Sf 0-12	0.6 (0.2)	1.6 (0.7)	0.9 (0.5)				

These results provide qualitative evidence that small, dense, dietary-derived lipoproteins, similar in size and density to IDL and LDL particles and thereby potentially pro-atherogenic, are present throughout the postprandial and postabsorptive states. Analysis of apo B-48 levels by a specific ELISA developed in our group will provide a quantitative assessment of the distribution of CMR and will give additional information on the metabolic fate of these cholesterol-enriched dietary-derived lipoproteins.

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Placental transfer of fatty acids from the maternal to the fetal circulation studied in the perfused human placenta. By P. HAGGARTY[1], K. PAGE[2], D. ABRAMOVICH[3], J. ASHTON[1] and D. BROWN[1]. *[1]Rowett Research Institute, Greenburn Rd, Aberdeen AB2 9SB, [2]Department of Biomedical Sciences, Aberdeen University, Aberdeen and [3]Department of Obstetrics and Gynaecology, Aberdeen University, Aberdeen AB9 2ZD.*

The developing fetus requires a supply of pre-formed long chain polyunsaturated fatty acids (LCPUFA), the absence of which is associated with reduced concentrations in the tissues and measurable impairment of visual and intellectual development. The n-3 and n-6 fatty acid structure available to the growing fetus is ultimately derived from the maternal circulation via the placenta, either as preformed LCPUFA or in the form of the two essential fatty acids (linoleic and α-linolenic acid) which can then be used to synthesize the LCPUFA.

The role of the placenta in controlling the supply of fatty acids to the fetus was investigated in four term placentas from "normal" pregnancies. Binding studies indicate that human placental fatty acid binding protein has a greater affinity for the essential fatty acids than the non-essential fatty acids (Campbell *et al.* 1994). The hypothesis tested here was that the human placenta selectively transfers linoleic and α-linolenic acids from the maternal to the fetal circulation in preference to non-essential fatty acids such as oleic acid. In addition, the capacity of the human placenta to chain-elongate and desaturate the essential fatty acids was determined by monitoring the appearance of ^{14}C from the linoleic and α-linolenic acid in arachidonic acid and docosahexaenoic acid in both the maternal and fetal perfusates.

The maternal side of the placenta was perfused with a modified Krebs Ringer solution (as described by Abramovich *et al* 1987) containing a physiological mixture of fatty acids and fatty acid free human albumin. The circulating fatty acid profile and the free fatty acid : albumin ratio were determined in pregnant mothers in Aberdeen Maternity Hospital in the last trimester and reproduced in the maternal side perfusate. No exogenous fatty acids were added to the fetal perfusate. There was no recycling of the venous return on either the maternal or fetal side. The rate of transfer of [^{3}H] oleic acid was compared with that of [^{14}C] linoleic and [^{14}C] α-linolenic acids. The placenta was perfused for 90 min and the perfusate extracted for neutral lipids and phospholipids and the fatty acid species of interest isolated by reverse phase HPLC. The radioactivity in each of the fatty acid fractions was determined by scintillation counting. Rates of transfer across the placenta were calculated relative to the concentration present in the maternal perfusate. Statistical significance was evaluated by a one tailed t test.

When compared with the reference fatty acid (oleic acid) the rates of transfer from the maternal to the fetal side were +43 (SE 19) % (P=0.053) for linoleic acid and +75 (SE 18) % (P=0.013) for α-linolenic acid. There was no detectable ^{14}C in the LCPUFA, arachidonic acid or docosahexaenoic acid, in either the maternal or fetal perfusate. The results indicate that the placenta is able to transfer selectively the more important essential fatty acids to the fetal circulation in preference to the non-essential fatty acids and that α-linolenic acid is transferred in preference to linoleic acid. No placental chain elongation and desaturation of the essential fatty acids was detected in this study.

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Liver and adipose tissue stearoyl-CoA desaturase activity in the growing Meishan pig. By M. KOUBA and J. MOUROT, *INRA, Station de Recherches Porcines, Saint-Gilles 35590, France*

Our laboratory is interested in the study of lipogenesis in the pig. Among the lipogenic enzymes, the stearoyl-CoA desaturase (Δ9 desaturase, *EC* 1.14.99.5) generates monounsaturated fatty acids (16:1 and 18:1) which are major fatty acids in the depot fat of pigs. Therefore, the aim of the present work was to study the stearoyl-CoA desaturase activity in the growing Meishan pig. This Chinese breed of pig is well known for its great propensity to fatten, and so it is a good model for the study of adipose tissue development in the pig.

The pigs were reared in individual pens. After weaning, the animals were fed on a conventional swine diet (lipid 40, crude proteins 170 and lysine 8 g/kg), according to a feeding scale based on live weight. The pigs were slaughtered in the fed state at 20, 40 or 60 kg live weight (10, 8, 7 pigs per weight group respectively). Stearoyl-CoA desaturase activity was measured in the liver and in two subcutaneous adipose tissue (backfat and neck) homogenates; the relationship between desaturase activity and the fatty acid composition of the lipids in the three tissues was determined.

Table: *Stearoyl-CoA desaturase activity (nmol oleic acid / h per mg protein)*

Weight (kg). . .	20		40		60	
	Mean	SD	Mean	SD	Mean	SD
Backfat	21.06[a]	6.87	23.55[a]	9.15	9.54[a]	2.29
Neckfat	11.45[b]	4.85	12.24[b]	3.51	5.45[a]	1.27
Liver	4.88[bc]	1.56	3.96[bc]	0.97	4.82[a]	1.10

[a,b,c] Values within the same column with unlike superscripts were significantly different at $P<0.05$.

The desaturase activity was lowest in the liver, highest in the backfat and intermediate in the neck, whatever the weight group, except at 60 kg live weight, where the activity in both neck and liver was similar. The lower stearoyl-CoA desaturation in the liver than in the adipose tissue confirms previous results obtained in the pig by Ho & Elliot (1973). The much lower activity of the enzyme in the neck than in the backfat confirms results of other lipogenic enzyme activities obtained in our laboratory in Large White pigs (Mourot *et al.* 1995) and in Meishan pigs (Mourot, unpublished results) showing that lipogenesis is paradoxically lower in a very thick adipose tissue such as the neck. In both adipose tissues, the Δ9-desaturase activity was constant between 20 and 40 kg and decreased thereafter, whereas the activity did not vary in the liver. This decrease after 40 kg live weight is also described for acetyl-CoA carboxylase (*EC* 6.4.1.2) activity in Meishan pigs (Mourot, unpublished results). We found a positive and significant relationship ($0.6 < r < 0.8$) between the desaturase activity and the proportion of monounsaturated fatty acids in the lipids of the three tissues, whatever the weight group.

The desaturation of stearic acid (18:0) to oleic acid (18:1) is of particular interest, since these fatty acids constitute a major portion of porcine adipose tissue and the relative ratio of them influences the pork meat quality. However, few studies concern the activity of Δ9-desaturase in the pig; therefore, the study of this enzyme needs further research.

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The organisation of adipose tissue surrounding lymph nodes in guinea-pigs and methods of studying local interactions between adipose and lymphoid tissue. By C.A. MATTACKS and C.M. POND. *Department of Biology, The Open University, Milton Keynes MK7 6AA*

In adult guinea-pigs white adipose tissue is partitioned into discrete depots, lymph nodes are found embedded in many of these depots (Cooper & Schiller, 1975). This study was carried out to investigate the functional basis for the anatomical relationship between these two tissues.

Mixed lymphoid cells from the retropharyngeal and axillary lymph nodes were co-cultured with 1 mm^3 pieces of adipose tissue from nine adipose depots. Samples of adipose tissue were taken from adjacent to and about 1 cm from lymph nodes in depots with lymph nodes, and near to and 1 cm from blood vessels in depots without lymph nodes. The adipose tissue explants and lymphoid cells were incubated in culture medium (RPMI 1640 + 25 mM Hepes) plus 100 ml/L foetal calf serum, 2 mM glutamine, antibiotics, and with or without the mitogens, 40 μg/ml concanavalin A (con A) or 50 μg/ml lipopolysaccharide (LPS). Adipose tissue viability was tested using ^{14}C-glucose uptake. Lymphocyte proliferation was measured as the incorporation of ^{3}H-thymidine (Calder *et al.* 1991) and lipolysis as glycerol release (McGowan *et al.* 1983).

After incubation for 48 h under these conditions, living explants take up ^{14}C-glucose from the incubation medium at 98% of the rate measured after 1 h. Explants killed by freezing to -20° take up no glucose from the medium.

In a detailed study of site-specific differences in the capacity of adipose tissue to interact with lymphoid cells (Pond & Mattacks, 1995), it was found that in the absence of mitogens, ^{3}H-thymidine incorporation into lymphoid cells averaged 1933 (SE 149) dpm. Stimulated with con A, incorporation averaged 40617 (SE 626) dpm and with LPS was 17541 (SE 470) dpm. Adipose tissue explants inhibited proliferation of unstimulated lymphocytes to between 87.9 and 60.2% of control values, depending on the anatomical site from which the adipose tissue was taken. Inhibition of proliferation was 74.5-57.6% of the control with con A and 68.7-33.7% with LPS. Adipose explants from intermuscular, mesenteric and omental depots inhibited proliferation more than those from superficial depots. Samples from near to lymph nodes were always more effective at inhibition than those from 1 cm away from nodes. Adipose tissue from the perirenal depot, that contains no nodes, was the least inhibitory.

Adipose explants incubated alone under these conditions, released between 31-81 μmol/ml of glycerol into the medium, the lowest from the intermuscular depots and the highest from the perirenal, there were no within-depot differences. In the presence of lymphoid cells, the glycerol released increased by less than 5% for the perirenal samples, but by more than 245% for the near to node samples from the intermuscular depots, to produce a maximum concentration of 132 μmol/ml after 48 h incubation.

The functional relationship between adipose tissue explants and lymphoid cells can be studied using this method. The data obtained demonstrate that there are site-specific differences in the interactions between lymphoid cells and adipose tissue. These interactions provide evidence for structural and physiological organisation of adipose tissue in relation to lymph nodes (Pond, 1996).

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The effect of dietary lipid manipulation upon fuel utilization by rat lymphocytes. By N.M. JEFFERY, P. YAQOOB, P.C. CALDER and E.A. NEWSHOLME, *Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU*

Lymphocytes have the ability to utilize glutamine, glucose and long-chain fatty acids as fuels (see review by Calder, 1995); the supply of glucose and glutamine appears to be essential for optimal lymphocyte functioning. Dietary lipid manipulation has been shown to alter the fatty acid composition of the phospholipids of lymphocytes (Yaqoob *et al.* 1995) and to modify the activities of membrane-bound enzymes including substrate transporters (Stubbs & Smith, 1984). The transport of fuel molecules across the lymphocyte membrane and their subsequent utilization may therefore be affected by changes in the membrane fatty acid composition.

In the present study, weanling male Lewis rats were fed for 10 weeks on a low-fat diet (LF; 20 g/kg) or on high-fat diets containing 200 g/kg of either hydrogenated coconut oil (HCO), olive oil (OO), safflower-seed oil (SO), evening primrose oil (EPO) or menhaden oil (MO). Spleen lymphocytes were prepared and were incubated with either 5 mM-[1-^{14}C]glucose, 2 mM-[U-^{14}C]glutamine or 1 mM-[U-^{14}C]palmitic acid for 1 h at 37°. Oxidation was halted by the addition of 250 ml/l perchloric acid. The CO_2 produced was collected in a subsequent 1 h incubation with a 1:1 mix of phenylethylamine and methanol. The amount of $^{14}CO_2$ produced was determined by liquid scintillation counting. The cellular protein content was measured, as was the concentration of lactate in the medium (only for incubations with glucose).

Diet	Rate of lactate production (nmol/h per mg protein)		Rate of oxidation (nmol CO_2 produced/h per mg protein)					
			Glucose		Glutamine		Palmitic acid	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LF	0.36	0.03	0.17	0.05	1.08	0.22	0.36	0.12
HCO	0.23*	0.01	0.17	0.01	2.30*	0.29	0.33	0.09
OO	0.31†	0.03	0.16	0.02	0.68†	0.13	0.32	0.09
SO	0.22*‡	0.02	0.14	0.02	2.43*‡	0.44	0.65	0.20
EPO	0.25	0.03	0.13†	0.01	1.54	0.47	0.74†‡	0.13
MO	0.24	0.03	0.09†	0.02	1.12†	0.31	0.68	0.13

Values are means for four animals fed on each diet with their standard errors.
Mean values were significantly different from: *LF, †HCO, ‡OO (P<0.05, ANOVA).

Whatever the diet fed, the rate of CO_2 production from glutamine was greater than that from glucose or palmitic acid. The rates of oxidation of the fuels investigated were affected by the type of lipid present in the diet. Lymphocytes from HCO- or SO-fed animals had higher rates of glutamine oxidation than those from LF- or OO-fed animals. The rate of CO_2 production from glucose was lowest in the lymphocytes from EPO- or MO-fed animals. The rate of CO_2 production from palmitic acid was greater in lymphocytes from the SO-, EPO- or MO-fed animals compared with those from animals fed on the other diets. Lymphocytes isolated from LF-fed animals had higher rates of lactate production from glucose than those from rats fed on the high-fat diets. Lymphocytes from HCO-, SO- or MO-fed animals had significantly lower rates of lactate production than those from the LF- or OO-fed animals.

Thus the oxidation of key fuels is affected by the type of lipid fed to rats; in particular feeding the MO diet suppresses glucose oxidation and feeding the OO diet suppresses glutamine oxidation. Impairment of the utilization of glucose or glutamine could affect the ability of lymphocytes to respond to stimuli. This may partly explain the immunosuppressive effects seen following the feeding of fish-oil or olive-oil-rich diets (Yaqoob *et al.* 1994*a,b*)

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The influence of triidothyronine and/or noradrenaline on perirenal adipose tissue composition in lambs delivered near to term by Caesarean section. By J. A. BIRD, S.J. LYKE, M. A. LOMAX and M. E. SYMONDS, *School of Animal and Microbial Sciences, University of Reading, PO Box 228, Reading,RG6 2AJ*

Brown adipose tissue (BAT) plays a vital thermoregulatory role in the neonatal lamb. A delay in feeding in newborn lambs is associated with an appreciable decline in BAT mass, DNA and protein content during the first few hours of life (Bird *et al*, 1994). The post-partum surges in catecholamines and thyroid hormones have been suggested to play a role in the control of body temperature after birth, which may be impaired following Caesarean section delivery (Symonds *et al.* 1994). The present study therefore investigated the effect of bolus administration of triidothyronine (T_3) and noradrenaline (NA) on BAT in lambs delivered by Caesarean section.

Fifty twin lambs were delivered by Caesarean section at 144-147 d (term=147 d) of gestation into either a warm (30°) or a cool (15°) ambient temperature. Before clamping of the umbilical circulation, lambs were given a 5 ml injection via the umbilical vein, at random, of either saline (S), T_3 (2 nmol), NA (50 μg) or NA+T_3. Caesarean section was performed using paravertebral anaesthesia using 20 g/l lidocaine hydrochloride followed by an intravenous injection of 4 ml ketamine (100 mg/ml). Lambs were humanely slaughtered at 2-2.5 h of age, when body temperature had stabilized and their perirenal BAT was sampled for measurement of thermogenic activity (guanosine diphosphate (GDP) binding), protein and DNA content as described by Symonds *et al.* (1992).

			BAT weight (g)		GDP binding (pmol/mg mitochondrial protein)		Total protein (g)		DNA content (mg/g)	
Treatment		*n*	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
S	15°	6	24.1	1.64	155	8.8	2279	1.76	2.79	0.25
S	30°	6	24.1	1.64	124	9.2	2098	1.76	2.50	0.32
T_3	15°	7	24.8	1.52	140	9.1	2378	1.63	3.61*	0.39
T_3	30°	7	27.8	1.52	146	10.6	2143	1.63	3.24*	0.32
NA	15°	6	26.2	1.64	128	9.0	2241	1.76	3.22	0.28
NA	30°	6	24.4	1.64	154	8.6	1981	1.86	3.60*	0.27
T_3+NA	15°	7	23.3	1.52	135	8.4	1965	1.70	3.38	0.12
T_3+NA	30°	5	24.9	1.80	116	9.1	2099	1.86	3.22	0.19

Significantly different from "S" group at the same ambient temperature, (analysis of variance) *P<0.05.

Ambient temperature or hormone treatment had no effects on either BAT weight, or its protein content and thermogenic activity. Administration of T_3, NA or a combination of both, resulted in lambs having a higher DNA content of BAT than in the saline treated animals. This response occurred independently of ambient temperature.

In conclusion bolus administration of T_3, NA or a combination of both hormones before cord clamping does not influence the thermogenic activity of BAT, but may act to reduce BAT cell loss during the initial 2.5 h of life in lambs delivered near to term by Caesarean section.

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Factors influencing final consumption of fruit and vegetables in Scotland: some preliminary results. By MARIA G. PIACENTINI[1], PAMELA TURNER[1], TERRY KIRK[2] and RICHARD C. PRENTICE[3], [1]*Department of Applied Consumer Studies,* [2]*Department of Dietetics and Nutrition,* [3]*Department of Hospitality and Tourism Management, Queen Margaret College, Edinburgh EH12 8TS*

Mortality rates from diseases such as coronary heart disease and certain cancers in Scotland are among the highest in the world. The recently published Scottish Diet Report (SOHHD, 1993) emphasizes the importance of diet in Scotland's poor health record, recommending fruit and vegetable consumption (FVC) be increased from 181g/day to 400g/day by the year 2000. Fruit and vegetable intake is considerably lower in Scotland than in the rest of the UK (NFS, 1994), and barriers to FVC in Scotland have already been identified (Anderson *et al*, 1994). Theories of consumer behaviour within the health context suggest that it is also important to investigate enabling factors in order to obtain a sufficiently comprehensive explanation of consumption patterns (Moorman *et al*, 1993).

The aim of this research was to make a preliminary investigation into the full range of constraining and enabling factors affecting FVC in Scotland, and to gain some insight their relative importance. A qualitative approach using focus group discussions was chosen, with consumers in high and low socio-economic groups in Scotland. Four discussion groups of 6-8 people were conducted in August and September 1994. Analysis was at two levels: initially the various factors that were revealed were listed. This material was then used with existing models of consumer behaviour to construct a framework describing the various factors and their relationship to each other. The factors that appeared to be most important are listed below. These were judged in terms of frequency of occurence and intensity of discussion surrounding them.

	Constraining factors	**Enabling factors**
Related to: Food characteristics	Poor perceived quality generally	Perceived good quality in supermarkets; General acceptability and convenience of processed forms
Economic, social and cultural environment	Inappropriateness of F&V for many contexts; Inadequate time, money and poor access to shops; Lack of knowledge of F&V preparation and use; Negative family preferences relating to F&V	Habit of traditional meal structure (i.e. meat and two vegetables); Responsibilty for family's health; Culinary skills regarding F&V
Individual	Confused/negative attitudes and beliefs in relation to health; Subjective norms (to consume other foods); Hedonic (experiential) consumption of other foods deemed more attractive	Motivations relating to preventive health (long term), personal appearance (medium term) and hedonic (immediate or short term).

From the focus group discussions it became apparent that constraining factors emanating from the economic, social and cultural context in which the individual consumes fruit and vegetables are important in trying to explain low consumption in Scotland. However, enabling factors (in particular a sense of responsibility for family's dietary habits and motivations pertaining to appearance and weight reduction) provide further insight into consumption of fruit and vegetables. Some progress was made in conceptualising the inter-relationship of these factors, representing the first stage in establishing the relative importance of the factors to FVC in Scotland.

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'Fruit instead of cakes?': an evaluation of a fat-lowering dietary intervention. By M. E. BARKER[1], K. OGDEN[1] AND H. J. POWERS[2], [1]*Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Herries Rd., Sheffield S5 7AU and* [2]*Department of Paediatrics, Children's Hospital, University of Sheffield, Sheffield S10 2TH.*

A reduction in population fat intake, particularly saturated fatty acid intake, is integral to British public health policy (Department of Health, 1992). In order to achieve these targets Bingham (1991) advocates, *inter alia*, cutting population consumption of cakes, biscuits and confectionery by a half. The current study was designed to evaluate the effectiveness of dietary advice to substitute fruit for cakes, biscuits and confectionery. The evaluation encompassed the effects on food and nutrient intake, levels of plasma cholesterol and ascorbic acid and ease of compliance to the advice.

A total of thirty-two subjects (thirty women and two men), aged between 18 and 54 years, took part in the study. Of these, twenty-five were recruited through advertising in general practice surgeries, while seven subjects volunteered as a result of personal contact. Of the thirty-two subjects, twenty-seven were from the non-manual social classes (Office of Population, Censuses and Surveys, 1990). All subjects consumed cakes, biscuits and confectionery at least twice a week. At the study's outset, subjects completed a 3d estimated dietary record, a general questionnaire on sociodemographic variables and food habits, and provided a 5 ml, fasting, venous blood sample. All subjects were given information (oral and written) on food sources of fat, the nutritional benefits of fruit and specific instruction to substitute fruit for cakes, biscuits and confectionery for a 3-week period. At the end of the intervention food recording and blood sampling were again carried out and all subjects completed a compliance questionnaire. Nutrient intakes were calculated using the FOODBASE (Institute of Brain Chemistry, London) dietary program. Plasma cholesterol and ascorbic acid concentrations were measured using established spectrophotometric and fluorimetric techniques respectively. Statistical testing was by paired *t* tests. A control group of subjects (*n* 12) provided blood samples to assess temporal changes in plasma cholesterol and ascorbic acid concentrations. The changes in nutrient intake and in biochemical markers are shown in the Table.

	Pre-intervention mean	Post-intervention mean	Difference	SE of mean difference
Food energy (MJ/d)	8.58	6.57***	-2.00	0.338
Fat (g/d)	90.4	57.0***	-33.4	5.23
Saturated fatty acid (g/d)	33.6	19.4***	-14.2	2.40
Added sugar (g/d)	45.2	23.1***	-22.0	2.88
Vitamin C (mg/d)	80.7	126.8**	46.0	14.73
Cholesterol (mmol/l)	4.58	4.04***	-0.54	0.136
Ascorbic acid (μmol/l)	46.2	58.1***	11.8	3.11

Values were significantly different from pre-intervention mean ** $P<0.01$, *** $P<0.001$.

There were no significant changes in mean concentrations of plasma cholesterol and plasma ascorbic acid in the control group. Three-quarters of subjects reported that it was easy to keep to the fruit substitution. The remainder reported difficulty due to craving for sweet foods, although the majority had been able to adhere to the intervention. Ninety percent of subjects said they would continue with this type of diet. In conclusion, 'fruit instead of cakes' appears to be an effective method of achieving nutrient intake targets and reducing coronary risk in non-manual female population groups.

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Consumption of cakes, biscuits and confectionery by British schoolchildren: association with nutrient intakes. By SIGRID A. GIBSON, *Leatherhead Food Research Association, Randalls Rd, Leatherhead, KT22 7RY, (present address: 21 Orchard Rd, Burpham, Guildford, GU4 7JH)*

Children in the UK today have a greater freedom to choose their own foods than in previous generations. This has generated concern about certain foods diluting the nutritional quality of children's diets. Evidence for an association between nutrient intake (fat, protein, Ca and Fe) and consumption of four frequently-criticized foods (biscuits, cakes, sweets and chocolate) was examined using data from the 1983 dietary survey of 2705 schoolchildren aged 10-11 and 14-15 years (Department of Health, 1989). From their 7 d weighed records the children in each age and sex group were classified in tertiles of consumption of each food. Results shown below are for the total sample, calculated from the average of the age/sex subgroups for parity of distribution. Differences in mean daily intake of nutrients between the tertiles were assessed by the Duncan multiple range test at the 5% significance level.

	Tertile	Energy (kJ)		Fat (g)		Fat (% of food energy)		Protein (g)		Calcium (mg)		Iron (mg)	
Cakes	Low	7941	*a*	80	*a*	37.4	*a*	58	*a*	735	*a*	9.3	*a*
	Med	8357	*b*	86	*b*	38.0	*b*	59	*b*	774	*b*	9.6	*b*
	High	9194	*c*	95	*c*	38.3	*b*	63	*c*	851	*c*	10.4	*c*
Biscuits	Low	7854	*a*	80	*a*	37.7	*a*	58	*a*	730	*a*	9.2	*a*
	Med	8483	*b*	87	*b*	37.9	*a*	60	*b*	793	*b*	9.7	*b*
	High	9152	*c*	94	*c*	38.0	*a*	62	*c*	837	*c*	10.3	*c*
Sweets	Low	8205	*a*	85	*a*	38.4	*c*	60	*a*	779	*a*	9.6	*a*
	Med	8365	*a*	86	*a*	37.9	*b*	59	*a*	786	*a*	9.6	*a*
	High	8940	*b*	90	*b*	37.2	*a*	61	*a*	794	*a*	10.0	*b*
Chocolate	Low	8085	*a*	83	*a*	37.8	*a*	59	*a*	752	*a*	9.6	*a*
	Med	8414	*b*	86	*b*	37.8	*a*	60	*a*	786	*b*	9.7	*b*
	High	8990	*c*	92	*c*	38.0	*a*	62	*b*	822	*c*	10.0	*c*

a,b,c, Mean values within a category not sharing a common letter were significantly different, $P<0.05$.

There was the expected positive relationship between consumption of these four foods and energy intake. Intakes of protein, fat, Ca and Fe mostly rose in parallel to energy. Absolute intakes of these nutrients did not therefore appear to be compromised by high intakes of sugary snack foods, because children who ate these foods tended to eat more food in general. Fat (as a percentage of food energy) was positively associated with cake consumption, inversely with sweet consumption, and showed no association with consumption of biscuits and chocolate. We conclude that, from these data, above-average intakes of cakes, biscuits or confectionery do not appear to have an adverse effect on intakes of those nutrients examined, nor a marked effect on fat energy intake. We attribute the latter mainly to the inverse relationship between sugar and fat energy that is evidenced in many dietary surveys (Gibney, 1990).

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Relationship of biscuit, cake and confectionery consumption to body mass index and energy intake in Scottish women. By SUSAN A. NEW[1] and DAVID A. GRUBB[2], [1]*Osteoporosis Research Unit, Woolmanhill Hospital, Aberdeen AB9 8AU,* [2]*Computing Department, Rowett Research Institute, Aberdeen AB2 9SB*

The relationship of frequency of consumption of biscuits, cakes and confectionery to weight, BMI and energy intake was investigated in women aged 45-49 years who had been randomly selected from the Community Health Index and had participated in a screening study which has been reported previously (Garton *et al.* 1992). Dietary intake was assessed using a postal food-frequency questionnaire (FFQ) which had been developed and validated against 7 d weighed records (Lanham *et al.* 1993); 1008 FFQ were returned (82% response rate with one reminder letter). Results are presented for 994 women as fourteen FFQ were excluded due to incorrect completion. Frequency of consumption (times/d and number d/week) for savoury biscuits (SAB), sweet biscuits (SWB), cakes (C), sugar confectionery (SC) and chocolate confectionery (CC) were divided into low, medium and high categories as follows: SWB and SAB (low 1 t/wk or less; medium 2-6 t/wk; high 7 t/wk or greater): C, SC and CC (low <1 t/wk; medium 1-4 t/wk; high 5 t/wk or greater).

High frequency consumption of SWB, C or CC was not associated with increased weight or BMI. Women who consumed medium intakes of SC were heavier and had a higher BMI ($P<0.01$). High frequency of consumption of SAB was associated with increased body weight ($P<0.01$). Total energy intake was found to be significantly increased in the high frequency consumption categories for the five food groups as shown in the Table below.

Relationship of body weight, BMI and energy intake to frequency of consumption

Food group	Consumption frequency	n	Weight (kg) Mean	Weight (kg) SD	BMI (kg/m^2) Mean	BMI (kg/m^2) SD	Energy intake (MJ) Mean	Energy intake (MJ) SD
SWB	Low	220	63.9	9.8	24.6	3.6	6.7^a	3.6
	Medium	318	64.2	11.0	24.7	4.2	7.7^b	4.2
	High	457	64.2	11.8	24.5	4.1	9.1^c	4.1
SAB	Low	374	62.7^a	10.7	24.1	3.9	7.6^a	2.2
	Medium	440	64.5	10.6	24.7	4.0	8.1^b	2.1
	High	181	66.1^b	12.9	25.0	4.4	9.3^c	2.5
C	Low	241	64.0	10.6	24.4	3.8	7.1^a	1.9
	Medium	569	64.1	11.0	24.6	3.9	8.0^b	2.1
	High	185	64.6	12.3	24.7	4.6	9.8^c	2.5
CC	Low	338	63.7	10.2	24.4	3.6	7.3^a	2.0
	Medium	540	64.5	12.0	24.7	4.3	8.4^b	2.2
	High	117	63.4	9.6	24.4	3.5	9.4^c	2.6
SC	Low	697	63.2^a	10.4	24.2^a	3.7	7.8^a	1.9
	Medium	234	67.0^b	12.9	25.6^b	4.7	8.7^b	2.1
	High	64	63.9^a	10.0	24.5^a	3.9	9.7^c	2.5

a,b,c Values with unlike superscripts within a food group were significantly different, $P<0.01$

These preliminary results indicate that high frequency of consumption of sweet biscuits, sugar confectionery and chocolate confectionery is not associated with obesity.

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Low sugar intakes are associated with high body mass index in the Dietary and Nutritional Survey of British Adults. By SIGRID A.GIBSON, *21 Orchard Rd, Burpham, Guildford, GU4 7JH.*

Restriction of both dietary fat and extrinsic sugars is standard advice for the treatment of excess weight (Department of Health, 1994), and it is often claimed that diets (and foods) high in both fat and sugars particularly contribute to the maintenance of the obese state. Evidence for this was examined using data from the Dietary and Nutritional Survey of British Adults (Gregory *et al.* 1990). Extrinsic sugars were calculated as the sum of sugars from all food groups except milk and fruit (Ministry of Agriculture, Fisheries and Food, 1992). Men and women were divided into four exclusive groups (LSLF, HSLF,LSHF, HSHF); low or high in extrinsic sugars as a percentage of food energy (LS <=15% , HS >15%) and low or high in fat (LF <=40%, HF > 40%). Mean BMI was compared across groups using ANOVA and the Bonferroni multiple range test .

	Total sample						Excluding dieters/unwell					
	Men			Women			Men			Women		
	n	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
LS LF	154	25.1 ab	0.3	189	25.7 a	0.4	130	24.9 ab	0.3	119	24.5 ab	0.4
LS HF	370	25.6 a	0.2	421	24.9 ab	0.2	316	25.5 a	0.2	324	24.6 a	0.3
HS LF	320	24.1 c	0.2	309	24.0 bc	0.3	284	24.1 b	0.2	240	23.5 b	0.3
HS HF	230	24.7 bc	0.2	182	23.5 c	0.3	211	24.8 ab	0.3	144	23.4 ab	0.4

a,b,c, Mean values in each column not sharing the same letter were significantly different $P<0.05$.

In the total sample, at each level of fat intake, mean BMI was significantly higher in the low-sugar group. The mean difference in BMI between LS and HS groups was 4% for men and 6% for women. Since dieting may potentially explain the high BMI in the LSLF group (notable in women), we examined the effect of excluding dieters, (and the unwell), from the analysis. This reduced the size of the differences in mean BMI (to 3% for men and 4% for women) and their statistical significance, so that only the LSHF and HSLF groups were significantly different at the 5% level. In combination, however, the two low-sugar groups still had higher mean BMI than the two high-sugar groups (*t*-test, 2-tailed significance: $P<0.0001$ for men; $P<0.001$ for women). These results suggest that restricting sugar intake to prevent obesity may be counter-productive. Further studies are required to separate the effects of fat and sugar and identify a possible mechanistic basis for relationships with body weight.

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The effect of sucrose- and aspartame-sweetened drinks on energy intake, hunger and food choice of female, slightly restrained eaters. By J. H. LAVIN, N. W. READ and S. J. FRENCH, *Centre for Human Nutrition, Northern General Hospital, Sheffield S5 7AU*

To compare the effects of aspartame-sweetened and sucrose-sweetened soft drinks on food intake and appetite ratings, fourteen female students, shown to have a slight eating restraint, were given drinks of aspartame-sweetened lemonade (ASL), sucrose-sweetened lemonade (SSL) and carbonated mineral water (CMW) on three separate days. The drinks (330 ml) were consumed at 09.30, 11.30, 14.00 and 16.00 hours. Seven of the subjects were informed of the drink type they were consuming on each occasion. Appetite ratings were recorded on visual analogue scales (VAS) at hourly intervals from 09.00 to 19.00 hours. Energy and macronutrient intakes were measured from consumption of snacks, a self-selection lunch and evening meal provided in the department and from foods and beverages consumed during the remainder of the evening and following day after leaving the department, recorded in food diaries.

	Energy intake (kJ)							
	Study day		Study day + test drinks		Following day		Total 2d	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ASL	13263*	941	13263	941	8368†	978	22480‡	870
SSL	11627	866	13008	866	6372	653	20138	816
CMW	12406	787	12406	787	6816	489	19786	791

*Significantly different from SSL, $P < 0.005$

†Significantly different from SSL, $P < 0.01$ and CMW, $P < 0.05$

‡Significantly different from SSL and CMW, $P < 0.01$

During the study day, energy intake from the snacks and test meals was higher whilst drinking the aspartame-sweetened lemonade compared with the sucrose-sweetened lemonade (13263 (SE 941) *v.* 11627 (SE 866) kJ; $P < 0.005$), although neither differed significantly from energy intakes during the day they drank water (12406 (SE 787) kJ). When the energy from the sucrose-sweetened lemonade was included (1381 kJ), energy intake did not differ between treatments suggesting that the additional energy was compensated for by a reduction in intake from the foods. The following day, energy intake was significantly higher after the aspartame-sweetened lemonade (8368 (SE 678) kJ) compared with the sucrose-sweetened lemonade (6372 (SE 653) kJ; $P < 0.01$) and the water (6816 (SE 489); $P < 0.05$), resulting in a higher total energy intake over the 2d studied (ASL 22480 (SE 870) kJ, SSL 20138 (SE 816) kJ, CMW 19786 (SE 791) kJ; $P < 0.01$). Moreover, the percentage energy consumed as protein was significantly lower during the day following ingestion of the aspartame-sweetened lemonade (12.3 (SE 0.9)) compared with the sucrose-sweetened lemonade (16.1 (SE 0.8); $P < 0.01$) and water (15.1 (SE 1.0); $P < 0.02$). Knowledge of the drink types had no effect on energy intake or macronutrient intake. Appetite ratings did not differ between drinks and were not affected by knowledge of the drink types.

These results suggest that in females of slight eating restraint, substituting sucrose-sweetened drinks for diet drinks does not reduce total energy intake and may even result in a higher intake during the subsequent day. Knowledge of the drink types had no effect.

Responses to weight-reducing diets including and excluding sucrose as a sweetener. By J.A.WEST and A. E. DE LOOY, *Department of Dietetics and Nutrition, Queen Margaret College, Clerwood Terrace, Edinburgh EH12 8TS*

Westenhoefer et al. (1993) suggest that the general avoidance of sucrose and sucrose-containing foods offers no general advantage in a weight-reducing diet. The present study investigated the effects of the omission of sucrose-containing foods, a traditional feature of weight-reducing diets, on weight loss and carbohydrate and fat intakes.

Research indicates that the removal of sucrose from diets may cause an increase in fat intake as a compensatory measure (Black, 1991). The removal of sugar and sugar-containing foods from weight-reducing diets has similar potential (Bolton-Smith & Woodward, 1994). Fat is more energy dense and less satiating (Lawton et al. 1992) than carbohydrate and as such is less desirable in weight-reducing diets. By incorporating sucrose in the diet carbohydrate is easily increased and may have potential to spontaneously reduce fat and promote weight loss.

Two isoenergetic diets were provided, a low sugar diet (LSD) had <5 g/d added sucrose and the sugar-containing diet (SD) was of equal energy and fat content but contained 45 g/d sucrose, incorporated as sweet foods. Sixty-eight subjects were randomly allocated to either LSD or SD for 8 weeks and then switched to the alternate diet for 8 weeks.

Weight loss over the first 8 weeks was 3.0 kg (SD) and 2.1 kg (LSD). During weeks 9-16 minimal weight loss was recorded. The diet records of subjects during weeks 9-16 indicated that the diet was being followed but as weight loss was minimal in most subjects it was concluded that these were unreliable. This is not unknown in weight reducing patients (Prentice et al. 1986). Mean initial body mass index (BMI kg/m^2) on the LSD was 29.2 and on the SD 30.1 and at week 8, 28.2 and 28.8 respectively.

Mean initial energy for subjects on the LSD was 9317 kJ (2227 kcal) and 9351 kJ (2235 kcal) on the SD. At week 8 these values were 5819 kJ (1391 kcal) and 5761 kJ (1377 kcal) respectively. Mean initial sucrose intakes were not significantly different for each of the groups. For those commencing on the LSD the mean initial sucrose intake was 47 g/d (providing 8% energy), and for those on the SD 57 g/d (providing 10% energy). At week 8 mean sucrose intakes on the LSD were 18 g/d (providing 5% energy), and on SD 33 g (providing 10% energy). This was significant at $P<0.01$. Mean initial carbohydrate on the LSD was 230 g/d (providing 40% energy) and similar levels were found on the SD at 237 g/d (providing 40% energy). During the first 8 weeks mean carbohydrate intake on LSD was 158 g/d (providing 44% energy) and on SD 167 g/d (providing 46% energy). These were not significantly different. Mean initial intake of fat for those on the LSD was 88 g/d (providing 36% energy) and on the SD 89 g/d (providing 36% energy). Reported intake of fat was lower on the SD (49 g/d, providing 32% energy) during the first 8 weeks than on the LSD (54 g/d , providing 36% energy). This was significant at $P<0.05$ and would seem to support the theory of an inverse relationship between fat and sugar. Percentage energy from fat included alcohol.

Weight loss can be achieved when sucrose is included in weight-reducing diets and it seems hard to justify the removal of sugar or sugar-containing foods from such diets.

Financial support for this study is acknowledged from the Sugar Bureau.

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Energy expenditure during heavy work and its interaction with body weight. By P. HAGGARTY[1], M. E. VALENCIA[2], G. M[C]NEILL[1,3], N.L. GONZALEZ[2], S. Y. MOYA[2], A. PINELLI[2], L. QUIHUI[2], M.S. SAUCEDO[2], J. ESPARSA[2], J. ASHTON[1] and E. MILNE[1], *[1]Rowett Research Institute, Greenburn Rd., Aberdeen AB2 9SB, [2]CIAD A.C., PO Box 1735, Hermosillo, Sonora, Mexico, [3]Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen AB9 2ZD*

The use of BMR multiples to estimate energy requirements has been employed in both the FAO/WHO/UNU (1985) and Department of Health (1991) reports and it is widely used to express energy expenditure as a physical activity level or physical activity ratio. The BMR multiple approach has the great advantage that it is easy to understand and simple to use but its validity across a wide range of body weights has yet to be tested.

Energy expenditure was determined during a 10 d highly controlled work programme in thirteen adult male construction workers in Mexico with a wide range of body weights (mean weight 63.9 (SE 3.5) kg, range 46.7-80.1, mean BMI 22.5 (SE 1.2) kg/m^2, range; 16.7-28.9). Each working day (8 of the 10 d of the study) lasted 7 h including a 1 h break for lunch. Within each hour of work, subjects carried out three highly controlled activities for 15 min on each activity: activity I, walking at 112 steps/min; activity II, walking on a treadmill at 5.6 km/h; activity III, wall construction using 7.4 kg concrete blocks, followed by 15 min rest.

Total energy expenditure (mean 12.68 (SE 0.66) MJ/d or 1.78 (SE 0.07) x BMR) was determined using doubly-labelled water and the energy costs of work activities by Oxylog (both as described by Haggarty *et al* 1994). The energy expenditure during occupation (mean 5.75 (SE 0.29) MJ/d or 3.48 (SE 0.09) x BMR) was estimated from the energy costs of the individual activities and the time spent in those activities. The average measured energy costs of these activities were 20.17 (SE 1.12) kJ/min for activity I, 20.94 (SE 1.08) kJ/min for activity II and 21.6 (SE 1.23) kJ/min for activity III. The energy expenditure during discretionary time was 4.37 (SE 0.58) MJ/d or 1.49 (SE 0.17) x BMR. When expressed as a BMR multiple (see Table) there was no significant relationship between the total expenditure or discretionary expenditure and the body weight. When the energy costs of the individual activities were summed to give a total value for occupational expenditure the correlation between the BMR multiple and body weight gave a P value of 0.051 suggesting that the assumed constancy of BMR multiple across a wide range of body weights may not be valid, at least for the activities studied here. If this observation is confirmed for other types of activity, the BMR multiple approach may have to be modified when using this method to estimate energy requirements, particularly at the extremes of body weight.

Regression Equation	r	P
Total energy expenditure (x BMR) = 1.55 + 0.0036 x Body weight (kg)	0.19	0.540
Occupational energy expenditure (x BMR) = 2.56 + 0.0143 x Body weight (kg)	0.55	0.051
Discretionary energy expenditure (x BMR) = 1.69 - 0.0033 x Body weight (kg)	-0.07	0.825

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First measurements of resting metabolic rate (RMR), diet-induced thermogenesis (DIT) and respiratory quotient (RQ) in lithium-treated patients. By R.A. HOLT[1] and E.M.W. MAUNDER[2], *[1]Nutrition and Dietetic Department, Dewsbury District Hospital, Dewsbury WF13 4HS and [2]Faculty of Health and Social Care, Leeds Metropolitan University, Leeds LS1 3HE*

Weight gain (5-11 kg) is a common side-effect of Li therapy, used for relapse prevention in manic-depressive disorders. The mechanism for Li-induced weight gain is still not fully understood, although disturbances of thirst, appetite, carbohydrate metabolism, fat metabolism, fluid balance and thyroid hormones have been described (Vendsborg *et al*, 1976). However, the energy expenditure of Li-treated patients has not previously been measured. A prospective controlled trial was performed to establish whether changes in body weight in female Li-treated patients were due to changes in energy consumption or energy expenditure. Estimated 3 d food and drink intake, fasting RMR and 1 h post prandial DIT (induced by 284 g *Formance* nutritional supplement) were measured using a ventilated hood (Deltatrac II) in five psychiatric inpatients receiving Li (LiG), five psychiatric inpatients not receiving Li (PCG) and five healthy control subjects (HCG).

	Energy Intake (EI) (MJ/d)		RMR (MJ/d)		RMR (kJ/kg FFM)		EI:RMR	Non-protein RQ (fasting)		Peak DIT(kJ/min)	
	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD
LiG	6.6	1.3	5.75	1.2	112	26	1.25	0.96	0.10	0.76	0.3
PCG	6.8	1.5	5.77	0.8	133	25	1.19	0.88	0.04	0.44	0.1
HCG	-	-	6.00	0.6	130	14	-	0.84	0.07	0.69	0.3

Energy intake from recorded drinks contributed 21% to total energy in LiG, but only 8% in PCG, indicating a greater thirst in LiG, but this did not result in a greater energy intake. A low energy intake:RMR ratio was found in both groups, so, the defect in energy balance in LiG did not appear to be due to an increase in the energy intake. There was no significant difference in RMR, peak DIT or 1 h DIT, between LiG, PCG and HCG. However, RMR in LiG was 5% below predicted RMR, from standard equations (Harris & Benedict, 1919) and LiG had a 15% lower RMR when expressed per kg fat-free mass (FFM) compared with PCG and HCG, suggesting a difference in metabolism in Li-treated patients. In this respect it is interesting to note that fasting non-protein RQ was greater in LiG than HCG and PCG, indicating that carbohydrate was utilized in preference to fat. Patients with the lowest Free thyroxine and highest thyroid stimulating hormone levels had the lowest RMR. The above trends were not statistically significant but this was probably due to the small group size and high variability in the data. However, this pilot study has shown new important findings, which are currently being investigated in a larger study.

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The metabolic disposal of [1-^{13}C]ethanol in relation to habitual ethanol consumption in healthy men. By J.S. KATHURIA, J.L. MURPHY and S.A. WOOTTON, *Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD*

The rate of oxidation of ethanol is governed by many factors, most notably habitual ethanol intake. Studies have shown that the hepatic metabolism adapts to high intakes of ethanol by showing increased rates of clearance of ethanol from the circulation which is assumed to reflect increased rates of oxidation (Whitfield & Màrtin, 1994). An alternative approach utilizing stable-isotope tracer methodologies may provide a useful non-invasive tool for direct examination of ethanol oxidation. The purpose of the present study was to examine the metabolic disposal of [1-^{13}C] labelled ethanol in healthy men in relation to habitual ethanol intakes.

Following an overnight fast, eleven healthy men aged 20-22 years (BMI 20.5-26.1 kg/m^2) ingested 50 mg of [1- ^{13}C]ethanol with 0.5 g/kg body weight ethanol in the form of calvados which has a low natural ^{13}C abundance. Breath samples were collected before and during a fasting period for at least 6 h and each hour until 9 h and at 24 h after administering the label. Whole-body breath CO_2 excretion was measured by indirect calorimetry (Deltatrac, Datex Instrumentarium Corp., Helsinki, Finland) before and at hourly intervals for at least 6 h after label administration.by indirect calorimetry (Deltatrac, Datex Instrumentarium Corp., Helsinki, Finland). Enrichment of $^{13}CO_2$ on breath was analysed by mass spectrometry (ABCA systems, Europa Scientific Ltd., Crewe). Habitual intake of ethanol was determined as units (1 unit = 8g ethanol) by self-completed questionnaire. The results shown are as medians and ranges.

	Breath $^{13}CO_2$ (% administered dose over 9 h)	Peak breath $^{13}CO_2$ (% administered dose)	Units of alcohol (units/week)	Ethanol oxidation (g/h over 9 h)
Median	45.2	9.0	20	1.80
Min	32.3	7.3	10	1.55
Max	53.6	10.1	62	2.15

Peak excretion of ^{13}C on breath $^{13}CO_2$ occurred 5 h (range 3.5-6 h) after ingesting the label. Enrichment of breath $^{13}CO_2$ returned to baseline values within 9 h in three of the subjects whilst the remaining subjects exhibited baseline values by 24 h. There was no relationship between the total proportion of label excreted on breath $^{13}CO_2$ over 24 h and number of units of ethanol consumed each week (r 0.08; P=0.83). However, a trend was observed towards an association between the number of units of ethanol consumed each week and both the time (r 0.66, P=0.03) and the proportion (r 0.58, P=0.06) of label excreted on the breath at peak excretion. These results suggest that the rate of ethanol oxidation calculated from the excretion of breath $^{13}CO_2$ following oral administration of [1- ^{13}C] ethanol may be substantially lower than those calculated from the clearance of ethanol from the blood (Wagner *et al.* 1976) and was not influenced by the reported habitual alcohol intake.

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Energy expenditure of adult cats estimated using the doubly-labelled-water method, indirect calorimetry and activity monitoring. By O. P. BALLEVRE[1], J. AMBROSE[2], C. PIGUET[1], H. SCHIERBEEK[1] and F. CHAUFFARD[1]. *[1]Nestec Ltd. (Friskies Research) PO Box 44, CH 1000 Lausanne 26, Switzerland, [2] Westreco Inc. (Friskies Research) Pettis Road, St Joseph, Missouri, USA*

The doubly labelled water (DLW) method could be an important tool to define accurately the energy requirements of pets in a free living situation. A preliminary evaluation of its application (Ballevre *et al.* 1994) has shown that the current estimate of energy requirement for adult cats (National Research Council (NRC) 1986) might be excessive. The objective of the present study was to compare the DLW method with either energy balance or indirect calorimetry (IC) in a similar activity situation in order to validate the DLW method in cats.

Nine domestic short-haired cats were housed in metabolic cages and fed on a standard commercial dry food (14 kJ metabolizable energy/g) for 6 weeks . After 3 weeks, energy expenditure was evaluated with DLW as previously described (Ballevre *et al.* 1994). ^{18}O and deuterium enrichment of serum samples obtained at baseline, 2 h and 14 d after the dose was measured with head space analysis of equilibrated CO_2 and Zn reduction methods respectively. CO_2 production was calculated from the standard equation for small animals (Lifson & McClintock, 1966). Indirect calorimetry measurements (24 h record of gas exchanges) were performed two to three times during the DLW period (Columbus Instruments, Columbus Ohio, USA). Activity was recorded on six cats with collar activitimeters (Gaehwiler Electronic) for the 3 weeks. Energy balance was evaluated during the 6-weeks period using food intake records and body weight changes. Energy expenditure was calculated as energy intake minus energy stores assuming 30.1 kJ/g of body weight change (Webster *et al.* 1984).

	Body-weight gain (g/d)		Energy intake (kJ/kg per d)		CO_2 production (mmol/kg per d)		Energy expenditure (kJ/kg per d)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
DLW	10	2	251[a]	15	318[a]	16	162[a]	8
IC	ND	ND	163[b]	20	277[b]	11	125[b]	5
Balance	9	1	238[c]	16			174[a]	12

ND, not determined.

[a,b,c] Mean values within a column with unlike superscript letters were significantly different, $P<0.05$ (paired t test

CO_2 production with DLW was 15% higher than with IC ($P<0.05$). This can be explained by a significantly higher energy intake (+44%) and activity (+64%) during the DLW test period than during the IC test period. A strong linear correlation was found between activity index and CO_2 production ($r = 0.876$, $P<0.01$) when data from DLW and IC were pooled. By contrast, energy expenditure measured with DLW was lower than energy intake ($P<0.01$). This was explained by body weight gain during the period of measurement. The slope ($r = 0.91$, $P<0.01$) of the relation between body weight gain and energy balance (intake-expenditure) as measured with DLW had a value of 24 kJ/g. Furthermore DLW energy expenditure was not significantly different from the value obtained by 6 weeks energy balance. The energy expenditure in cats measured by DLW (162 (SE 8) kJ/kg per d) was 44% lower than the NRC recommendation for adult caged cats (293 kJ/kg per d, NRC 1986).

In this study, variations in food intake and activity, despite similar housing conditions, occurred during the DLW and IC measurements. Corrections for these variations indicate that DLW might be valid in cats. Nevertheless a direct comparison of DLW and IC data cannot be made. Further studies in which food, activity and environment are identical will be necessary for full validation.

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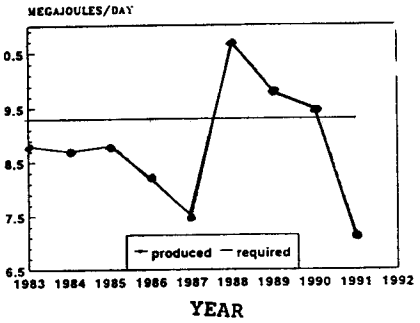
A comparison of potential energy supply from smallholder production with population's energy requirement in Malawi by M.O. MPOMA[1], B.M. MARGETTS[1,2], and A.A. JACKSON[1], [1]*Institute of Human Nutrition and* [2]*Wessex Institute of Public Health Medicine University of Southampton, Southampton SO16 7PX*

Maize is the main food crop in Malawi upon which the population depend for sufficiency. Thus the adequacy of food has been identified with the supply of maize and maize has been targeted for achieving food self sufficiency. The country's policy for agricultural practice has been to achieve national food self sufficiency by satisfying the requirement of the market. With this policy between 1983 and 1889 the country appeared to meet the requirement of the market and also exported 18 000 tonnes of maize, 30 321 of groundnuts, 11 126 of groundnuts and 4 272 tonnes of rice to neighbouring countries. However, 56% of children in Malawi are stunted (height for age -2 standard deviations of the National Centre for Health Statistic reference) (Chiligo 1985, Centre for social research 1988). The high rates of childhood malnutrition raise a question about the adequacy of the food supply to the market to satisfy food requirement of all people in Malawi.

In the present investigation we have related food production in Malawi to the food requirements for population, using energy requirements as the currency of reference.

Fig.1 POTENTIAL PER CAPITA ENERGY SUPPLY MATCHED WITH REQUIREMENT



More than 85% of the population are small holder farmers who depend on agricultural production and farming for survival. Therefore, it was assumed that the quantity of their food intake depends on the agricultural produce within the area. For this reason the level of food production within districts is important. Crop production information was abstracted from 1983 to 1991 and converted into joules. Energy supply for the population was estimated for each food crop correcting for exports, imports, seed, storage and processing losses and converted into joules. Daily per-capita energy production was estimated and a weighted per- capita energy requirement was derived with account being taken of differences in age, sex and activity, and allowance being made for infection.

Fig. 1 shows that in each year except 1988 and 1990 for the past decade, energy availability, and hence food production has failed to satisfy the requirement for food at national level. On average the shortfall was 10% over the period. Young children are most vulnerable to nutritional deprivation. It may be that the high rates of stunting among the young children in Malawi are a direct consequence of adjusting to low levels of food and energy intake. Although other factors may be important and need to be considered, young child growth should be considered as an important factor in determining the adequacy of national food supplies.

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The effects of ambient temperature on substrate utilization during prolonged moderate intensity exercise in man. By S.D.R. GALLOWAY and R.J. MAUGHAN, *Department of Environmental and Occupational Medicine, University Medical School, Aberdeen AB9 2ZD*

The available literature describing the effects of heat and cold exposure on substrate utilization during prolonged exercise is at best inconclusive. The present study specifically examined the effects of exposure temperature on substrate utilization. Eight healthy males performed four rides to exhaustion on an electrically braked cycle ergometer at approximately 70 % of maximum O_2 consumption. Dietary intake was the same on the 2 d before each ride. The four rides were performed 1 or 2 weeks apart after an overnight fast and at the same time of day. No fluid was ingested in any of the trials. Ambient temperature (T_a) was maintained at 3.6 (SD 0.3), 10.5 (SD 0.5), 20.6 (SD 0.2) and 30.5 (SD 0.2) ° dry bulb with a relative humidity of 70 (SD 2) % and an air velocity of approximately 0.7 m/s. Expired air was collected over a 2 min period every 15 min during the tests for determination of O_2 consumption (VO_2), and estimation of fat and carbohydrate (CHO) oxidation.

		Exercise time (min)							
		15		30		45		Total Oxidation (g/ride)	
	Temperature (°)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
VO_2	3.6	2.89^{b}	0.11	3.10^{c}	0.12	3.22^{c}	0.11		
(l/min)	10.5	2.84^{b}	0.15	2.92^{b}	0.13	2.98^{b}	0.13		
	20.6	2.66^{a}	0.10	2.67^{a}	0.12	2.72^{a}	0.11		
	30.5	2.65^{a}	0.13	2.69^{a}	0.13	2.61^{a}	0.14		
Fat oxidation	3.6	0.48	0.06	0.50^{b}	0.10	0.66^{ab}	0.12	51.1^{abc}	16.8
(g/min)	10.5	0.49	0.02	0.61^{ac}	0.06	0.73^{b}	0.06	66.3^{c}	9.0
	20.6	0.43	0.03	0.52^{ab}	0.03	0.58^{ab}	0.05	42.5^{b}	5.4
	30.5	0.42	0.08	0.59^{ab}	0.05	0.55^{ac}	0.07	22.4^{a}	4.1
CHO oxidation	3.6	2.28	0.15	2.53^{b}	0.28	2.30	0.32	168.2^{a}	20.0
(g/min)	10.5	2.26	0.19	2.06^{a}	0.24	1.83	0.19	166.1^{a}	18.8
	20.6	2.19	0.14	1.98^{a}	0.16	1.88	0.13	149.3^{b}	11.0
	30.5	2.19	0.16	2.02^{a}	0.19	1.82	0.13	89.6^{c}	6.3

$^{a, b, c}$ - Mean values within a category at each time point bearing unlike superscript letters were significantly different ($P<0.05$, ANOVA).

Time to exhaustion was significantly influenced by T_a ($P<0.01$): exercise duration (min) was 51.55 (SE 3.72) at 30.5°, 81.15 (SE 5.74) at 20.6°, 93.52 (SE 6.19) at 10.5° and 81.39 (SE 9.60) at 3.6°. VO_2 was not different during exercise at 20.6 and 30.5° but on both trials was higher during exercise at 3.6 and 10.5°. In addition, VO_2 on the 3.6° trial was higher than that during the 10.5° trial from the 30 min sample onwards. Fat oxidation rate was higher during the trial at 10.5° than that during exercise at 3.6° at the 30 min sample time. Differences in fat oxidation rate were also observed between the 10.5° trial and the 30.5° trial at 45 min. CHO oxidation rate was higher during the trial at 3.6° compared with all other trials at 30 min. No differences were observed in CHO oxidation rates between the other three trials. Total CHO oxidation was similar on the 3.6 and 10.5° trials but was lower on the other two trials. These data demonstrate that during prolonged moderate intensity exercise cold exposure (3.6°) can elevate metabolic rate and CHO oxidation. This is most likely due to non-observable shivering in inactive muscle groups. In the heat (30.5°) CHO depletion does not appear to be limiting exercise capacity as total CHO oxidation is much less.

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Resting metabolic rate and excess weight gain during maintenance chemotherapy for childhood acute lymphoblastic leukaemia. By J.J. REILLY[1], C.J. BLACKLOCK[1], I. ODAME[2], M. DONALDSON[3], and B.E.S. GIBSON[2], *University of Glasgow Departments of Human Nutrition[1] and Child Health[3] and Department of Haematology[2], Yorkhill Hospitals, Glasgow G3 8SJ*

Obesity is a common and important late effect of therapy for acute lymphoblastic leukaemia (ALL; Odame *et al* 1994). The precise mechanism which predisposes children with ALL to obesity is unclear. There is some evidence that children treated for ALL on MRC therapeutic protocol UKALL-X (1985-1991) had RMR and energy intakes (EI) similar to controls (Bond *et al,* 1992) during maintenance chemotherapy. Since 1991 UK therapy for ALL has been altered and the changes made may be of relevance to the development of excess weight gain (Odame *et al* 1994). The present study had two aims: to describe excess weight gain in patients during the first two years of therapy; to test for differences in RMR between patients treated on the current MRC protocol (UKALL-XI) and controls matched for sex and fat-free mass (FFM).

In twenty-nine consecutively diagnosed patients treated for ALL who remained in continuous complete remission mean change in BMI expressed as a SD score relative to French reference data was +1.2 (95% CI + 0.7 to + 1.7). for the first two years after diagnosis.

RMR was measured on one occasion after an overnight fast in sixteen patients and sixteen controls (eleven boys, five girls) using a ventilated hood indirect calorimeter (Deltatrac). FFM was estimated from bioelectrical impedance in each subject using a validated prediction equation (Reilly *et al*, 1995). Measurements on patients were made at least 3 weeks after prednisolone/vincristine therapy, at least 5 weeks post intensification, and while children were well and in first remission. Mean age of patients was 9.2 (SD 3.3) years and controls 9.3 (SD 2.5) years.

Paired differences in RMR (kj/Kg) between patients and controls were not statistically significant (95% CI for difference, patients minus controls -7 to +23). The relationship between RMR and FFM (slope, intercept) was not significantly different between the two groups (analysis of covariance).

In conclusion, the present study does not support the hypothesis that RMR is affected by maintenance chemotherapy for ALL . It would appear that rates of excess weight gain on the current treatment protocol for ALL are similar to those previously reported (Odame *et al* 1994).

The work was supported by the Wellcome Trust and Royal Society.

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Negative energy balance in patients hospitalized with exacerbation of chronic obstructive lung disease. By M.K. SRIDHAR, S.W. BANHAM [1] and M.E.J. LEAN, University Department of Human Nutrition and [1] Respiratory Medicine, Glasgow Royal Infirmary, Glasgow G31 2ER

Although weight loss is a well recognized adverse prognostic feature of patients with chronic obstructive pulmonary disease (COPD), the reason for its occurrence in these patients is not known (Sridhar et al, 1994). It has been suggested that the negative energy balance suffered by the patients during acute infective exacerbations of their illness may be one reason, but there are no data on the subject (Bates, 1973). We therefore measured resting energy expenditure (REE) within 48 h of admission and before discharge in twenty patients (ten male; ten female; mean 66 SD 6.8 years) who were admitted to hospital acutely unwell with an infective exacerbation of COPD. REE was measured by indirect calorimetry (Deltatrac Metabolic Monitor ™). Height, body weight, mid-upper arm circumference and skinfold thickness at four sites were measured and body composition estimated. Dietary energy intake was measured on the day before discharge by a computer based technique (Food Meter UK ™) from semi-weighed records kept by nursing staff.

Median duration of stay in hospital was five days (range 4 - 12 days). Seven of the twenty patients had a BMI of < 18.5. REE was elevated in all patients at the time of admission (mean REE : 115 SD 11.6 % predicted; range : 104 -131), compared with values predicted by the Harris-Benedict equation, but fell to a mean of 106.1 SD 4.5 % predicted before discharge. Patients on maintenance corticosteroid therapy at the time of admission (n 8) had a lesser elevation of REE to 112.8 SD 5.9% predicted, whilst patients not on steroid therapy (n 12) had a REE of 117.1 SD 7.4% predicted (retrospective analysis of data). All patients were in a state of negative energy balance (mean net energy balance -3720 (SD 1238) kJ/D; range : - 2272, -7368) even at the time of discharge. There was no relation between fat free mass or BMI and the degree of negative energy balance.

We conclude that during an acute exacerbation, COPD patients suffer a state of considerable negative energy balance which contributes to their cachectic state. The influence of steroid therapy on energy balance and body composition of these patients merits further study.

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The effects of alterations in dietary carbohydrate intake on running performance during a 10 km treadmill time trial. By Y.P. PITSILADIS, C. DUIGNAN and R.J. MAUGHAN, *Department of Environmental and Occupational Medicine, University Medical School, Aberdeen AB9 2ZD*

Since early studies demonstrated the effects of carbohydrate (CHO) availability on capacity to perform exercise, attention has been directed towards the improvement of exercise performance by dietary manipulation. The present study examined the effects of diet on performance during a 10 km treadmill time trial. With local ethics committee approval, six endurance-trained male runners (maximum O_2 consumption (VO_2 max) mean 67 (SD 5) ml/kg per min) ran two 10 km time trials after a 7 d period of dietary manipulation. Before the two experimental trials subjects completed at least two familiarization trials. All trials were run on a treadmill set at a constant 4 % gradient. The treadmill speed was set to elicit an intensity of 80 % VO_2 max for the first 2 km after which subjects manually controlled the speed for the remainder of the run using a hand-held microswitch. The two experimental diets were a low-CHO diet (40 % CHO) to be consumed for 7 d and a high-CHO diet containing 55 % CHO for the first 4 d followed by 70 % CHO for the remaining 3 d. Both diets were isoenergetic with the subjects' normal diets which contained 52 (SD 6) % CHO, and were administered in a randomized order. Normal training was continued throughout the periods of dietary control and involved running 64 (SD 30) km/week. All subjects consumed a standard breakfast (2.5 (SD 1.4) MJ, with the same composition as the experimental diet) 4 h before each experimental trial. Expired gases were collected at 1.5, 5.0 and 9.0 km and O_2 consumption (VO_2) and respiratory exchange ratio (R) were determined. Heart rate (HR) was monitored at rest, at 0.5 km and at every km thereafter. Blood samples were obtained before and immediately following each performance trial. Statistical analysis of the results was carried out using a two-factor ANOVA for repeated measures followed by Student's *t* test for paired data where necessary. Performance times following the high (48.8 (SD 2.7) min) and low (48.6 (SD 2.3) min) CHO diets were not different (P=0.72), nor were there any differences in running speed for each successive km of the 10 km treadmill time trial between conditions. No differences were found between conditions in any of the metabolites measured (blood lactate, glucose, glycerol and plasma free fatty acids (FFA)). Blood glucose, lactate, and glycerol did however increase at the end of both trials. In contrast, plasma FFA concentration did not change during the run following the high-CHO diet, while following the low-CHO diet a reduction in FFA concentration occurred. Although R tended to be higher during the run following the high-CHO diet (P<0.10) no statistical difference was found. However, the rate of CHO oxidation was greater on the high-CHO diet compared with the low-CHO diet (P<0.05). No differences were found between conditions in VO_2 and HR at any of the measured time points. There was no change in plasma volume on either of the performance trials (0.1 (SD 5.5) % and (-0.2 (SD 5.3) % following the high- and low-CHO diets respectively). The results of this study indicate that moderate changes in the composition of the diet do not affect 10 km running performance in endurance-trained subjects.

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Intra individual variation in the basal metabolic rate of women: the effect of the menstrual cycle.

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The validity of using BMR to calculate an individual's energy requirements is based upon the assumption that the intra individual variation in BMR is small. However, the majority of studies that have fuelled this assertion were carried out on male subjects. Early studies on BMR in women have illustrated that the menstrual cycle may have a profound effect and may be responsible for producing high levels of intra individual variation (Hafkesbring & Collett, 1924; Benedict & Finn, 1928; Conklin & McClendon, 1930).

Two independent studies were undertaken in which the BMR of twelve weight-stable women aged 21-23 (not taking the contraceptive pill) was measured every day (excluding weekends) for a period of 5 weeks. The six women participating in the first study were measured using a Douglas bag, while the six subjects in study 2 were measured with a Deltatrac (Datex, Helsinki). Nine of the twelve subjects measured demonstrated a peak in BMR during the late luteal phase of the menstrual cycle, while eight of the twelve subjects exhibited a fall in BMR after the onset of menstruation. Group analysis of results indicated that BMR during the early follicular phase was significantly lower than BMR during the late luteal phase (Wilcoxon's signed rank test: $P<0.01$).

The level of intra individual variation was assessed by calculating the coefficient of variation (CV) for the measurement period. The CV found in serial BMR measurements of men are in the region of 2-4% (Henry *et al.* 1989). BMR in six of the women in this study exhibited a similar level of variation as in the above quoted male study. However, the other six women had up to three times the level of intra individual variation, with CV as high as 12%. In these six women therefore, BMR may not be considered a biological constant.

Data from this study were also combined with data collated from the literature (Hafkesbring & Collett, 1924; Benedict & Finn, 1928; Conklin & McClendon, 1930) in which serial BMR measurements were made in women of reproductive age. When these were subjected to a statistical analysis, BMR during the early follicular phase was again found to be significantly lower ($P<0.05$), than during the late luteal phase. Thus a pattern of variation similar to those observed in the experiment emerged. Intra individual variation in BMR in 42% (eleven cycles from a total of twenty-six) of the cycles, was twice that observed in male subjects. The remaining cycles (57%) exhibited intra individual variations similar to those reported in male BMR.

Further work on the constancy of BMR in women and the role of the menstrual cycle is urgently required. This area is of particular importance given the fact that the energy requirements of both individuals and populations are now calculated using values of energy expenditure.

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Basal metabolic rate (BMR) in pre-adolescent and adolescent children. By SARAH DYER, C.J.K. HENRY and A. GHUSAIN-CHOUEIRI, *School of Biological and Molecular Sciences, Oxford Brookes University, Oxford OX3 0BP*

Adolescence is a time of great change in body size, composition and function. Existing equations for the prediction of energy requirements for adolescents tend either to extrapolate adult data (Harris & Benedict, 1919; Cunningham, 1980) or combine wide age ranges together (FAO/WHO/UNU, 1985). In addition, a database of only 450 data points from 10-15-year-old children was used by the FAO/WHO/UNU to predict energy requirements.

We report 782 measurements of BMR in 10-15-year-old children from a mixed-longitudinal study. These measurements were used to assess the adequacy of three of the most frequently used predictive equations. A portable indirect calorimeter with a rigid transparent canopy (Datex Deltatrac) was used for all measurements.

Years	Sex (*n*)	Measured BMR (kJ/d)		Predicted (kJ/d)							
				FAO/WHO/UNU 1		Harris & Benedict			Cunningham		
		Mean	SD	Mean	SD	Mean	SD		Mean	SD	
All 10-15	Female (498)	5421	721	5470	562 **	4418	550	‡**	5116	526	‡
	Male (325)	5727	918	5845	774	5534	750	†	5141	627	‡
10-11	Female (70)	5182	697	5155	446 *	4138	349	‡**	4759	392	‡
	Male (36)	5314	671	5346	517	5001	507	†	4691	388	‡
11-12	Female (97)	5367	695	5315	462 **	4248	362	‡**	4991	428	‡
	Male (69)	5517	773	5666	684	5333	642	†	4925	464	‡
12-13	Female (85)	5484	737 *	5520	527 **	4390	413	‡**	5227	468	‡
	Male (52)	5759	873	5919	736	5621	683		5209	537	‡
13-14	Female (49)	5538	753 *	5756	603 **	4623	540	‡**	5533	507	
	Male (28)	6015	1023	6291	807	6018	765		5598	652	†
14-15	Female (20)	5631	712 *	6102	578 †	5521	740	†**	5777	450	
	Male (14)	6524	1011	6691	676	6339	663		6019	598	†

Significantly different from measured BMR (ANOVA): † $P<0.05$, ‡ $P<0.001$.
Significantly different from male BMR in the same age group using the same equation:* $P<0.05$, ** $P<0.001$.

The measured BMR was not affected by sex between 10 and 12 years. However, after this age the girls' BMR was significantly lower than the boys'. This suggests that differences between the onset of male and female puberty may influence BMR. None of the predictive equations illustrated this pattern of changes in BMR. The FAO/WHO/UNU equation and the Harris & Benedict equations predicted a significant sex difference throughout the age range used, whilst the Cunningham prediction was designed to cover both sexes. In addition, the Harris & Benedict and Cunningham equations significantly underestimated female energy requirements .

It is apparent that of the three predictive equations tested, none accounted for the pattern of BMR observed in the current study. However, the FAO/WHO/UNU equation, which was developed from a comparable age range, predicted the observed values most closely.

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Weight loss and factor VIIc activity in overweight subjects. By CATHERINE R. HANKEY, ANNE RUMLEY[1], GORDON D. O. LOWE[1] and MICHEAL E.J. LEAN, *Departments of Human Nutrition and [1] Medicine, Glasgow Royal Infirmary, Glasgow G31 2ER*

This present study examined factor VIIc activity in overweight subjects with angina. We investigated the response of factor VIIc activity to weight loss in subjects with increased risk of heart disease. The results were compared with those in healthy overweight subjects. Previous work has shown that factor VIIc activity improves with weight loss of 4.5 kg, in healthy overweight subjects (Hankey *et al.* 1995). This present study recruited overweight subjects with elevated ischaemic heart disease risk as indicated by a history of angina pectoris.

Forty-five subjects (eighteen women and twenty-seven men), mean age 60.3 (SD 6.7) years and with a mean BMI of 29.2 (SD 4.2) kg/m^2 were recruited and continued to have angina symptoms as measured by the Rose questionaire (Rose, 1962). Forty subjects (thirty-three women and seven men), mean age 47.0 (SD 9.6) years with a BMI of 34.9 (SD 3..4) kg/m^2 comprised the overweight group. Factor VIIc activity was measured using conventional clotting assays.

At baseline, mean factor VIIc activity of the angina patients was 120.5 (SD 24.7) % which was not significantly different from that of the overweight patients 114.3 (SD 20.9) %. Dietary advice was given with nutrient profiles close to the dietary reference values (Department of Health, 1991) to all subjects with a daily energy deficit of 2510 kJ. Data were analysed using a 1 sample Student's *t test*.

Weight reduction was 3.5 (SD 2.5) kg in angina subjects and 4.3 (SD 1.9) kg in the overweight group after a 12 week dietary intervention. After weight loss in the angina patients factor VIIc activity fell significantly by 5% and was 115.3 (SD 19.8) % (P=0.045). In the otherwise healthy overweight subjects factor VIIc activity also fell significantly by 6% to 107.2 (SD 20.2) % (P=0.006). These results suggest that moderate weight loss results in a reduction in factor VIIc activity in both study groups, and is of value in improving a principle thrombotic indicator of ischaemic heart disease (Bottiger and Carlson, 1980).

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Coronary heart disease mortality in Peninsular Malaysia. By S. MOHD-YUSOF[1] and B.M. MARGETTS[1,2], [1]*Institute of Human Nutrition and* [2]*Wessex Institute of Public Health, University of Southampton, Southampton SO16 7PX*

The proportion of all hospital deaths in Malaysia attributable to coronary heart disease (CHD) differs between the Malays, Chinese and Indians (Khoo *et al.* 1991). However the differences are small compared with that seen between the three ethnic groups in Singapore (Hughes *et al.* 1990). The analysis by Khoo *et al.* (1991) did not refer to the population denominator for each ethnic group. This is because rates of medically certified deaths differ between Malays (29%),Chinese (51%) and Indians (56%) (Department of Statistics, 1992). Death rates have been recalculated for urban areas (and excluding rural areas) only, because:

(1) virtually all certified deaths take place in hospitals, which are located in urban areas; (2) deaths in hospitals capture residents in their catchment areas (as well as some deaths of rural residents); (3) the death certification rate is similar for deaths in urban areas for each ethnic group.

Only certified deaths have been used and cause of death is based on the total number of deaths (7th revision of the International Classification of Causes of Deaths) due to arteriosclerotic and degenerative heart disease for age group 30 - 69 in the years 1968 to 1971 was divided by four to obtain the average number of deaths per year during that period (Mohamad, 1973). The age-specific mortality rate for CHD for the year 1968-1971 was calculated, using as the denominator the estimated number of urban Malays, Chinese and Indians in those age groups by sex, based on the 1970 population census figures.

The Table shows that the age-specific mortality rate (per 100 000 population) due to CHD in the age group 30-69 was highest among the Indians and lowest among the Chinese. The mortality rate among male Indians in the age group 30-69 years was 3.5 times that of Chinese and 2.6 times that of Malays. The differences in mortality rate between Indians and Chinese were highest in the 30-39 age group and fell gradually as age increased. In all ethnic groups males had a higher mortality rate than females. These trends are similar to those found in Singapore. However, the rates in Malaysia could be an overestimate because of possible inclusion of deaths of rural residents in the numerator.

Age group	Malay		Chinese		Indian	
(years)	Male	Female	Male	Female	Male	Female
30-39	30.8	12.3	13.3	4.0	97.2	15.4
40-49	123.3	19.6	69.9	16.3	298.1	50.7
50-59	273.2	73.2	164.3	47.0	538.2	139.7
60-69	279.4	69.6	257.3	92.9	684.2	301.1
30-69	142.3	33.0	105.6	31.2	367.2	74.4

By using the urban population as the population at risk it is possible to estimate more accurately the mortality rate due CHD in Malaysia. However this estimate gives an indication of the situation in the urban areas and not the overall population, and is likely to be a better reflection of the underlying CHD rate than previously published.

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Nutrition in the medical undergraduate curriculum. By M. E. J. LEAN, *Department of Human Nutrition, University of Glasgow Department of Human Nutrition, Glasgow Royal Infirmary G31 2ER*

Heightened awareness of nutrition-related ill-health in Britain (Department of Health, 1993; Scottish Office Home and Health Department, 1993), of the need for nutrition in primary care (Patel et al, 1993), and of the continuing problems with malnutrition in hospitals (McWhirter & Pennington, 1994), have stimulated new interest in Human Nutrition in the medical undergraduate curriculum. In 1993 a representative with an interest in Human Nutrition from each medical school provided information about their undergraduate curriculum, and attended a meeting at Stratford. The Nutrition Society subsequently convened the Stratford Executive Committee to investigate and guide nutrition in medical training. A further postal survey of medical school nutrition representatives and deans was conducted one year later, in 1994.

A 100% response rate from twenty-six medical schools was achieved in 1994, with replies from nineteen nutrition representatives, and from fifteen medical deans. Five schools had independent departments of human nutrition, compared with three in 1993; two further schools have advanced plans for new departments. Five other schools have nutrition as part of another department, of gastroenterology, medicine or physiology. Thirteen schools had a named person representing nutrition on their undergraduate curriculum committee, but nutrition representatives and deans agreed about the identity of this person in only four of the schools where both offered a name.

Total hours of specific nutrition teaching varied between 1 and 60 (mean 19h) for the ten medical schools who were able to quote specific hours. This compares with 15 in 1993. Nutrition was identified mainly in basic science preclinical teaching (twenty medical schools), less frequently in clinical (eleven schools) and public health (six schools). The numbers of schools teaching nutrition in each year of training were 14, 13, 8, 4, 3.

Medical curricula are currently under review (General Medical Council, 1993). Human nutrition offers an integrative theme of direct relevance to every branch of medicine (Department of Health, 1994), but currently only one medical school teaches it in every year of medical training. If it is to be learned by medical students medical schools need a named coordinator of nutrition on curriculum committees, and it must appear in examinations: only four schools currently require nutrition in medical finals, ten in class examinations.

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