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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at the University of Edinburgh on Tuesday–Friday, 27–30 August 1991, when the following papers were read.

Gastric emptying and unidirectional water absorption of glucose solutions in man. By C. S. PRIMROSE, J. B. LEIPER and R. J. MAUGHAN, *Department of Environmental and Occupational Medicine, University Medical School, Foresterhill, Aberdeen AB9 2ZD*

Gastric emptying of glucose drinks is controlled such that the higher the energy density the slower the emptying rate but the greater the energy delivery to the intestine. Few studies have examined how quickly water from ingested drinks is supplied to the gut and absorbed. We have compared the rate of gastric emptying and absorption of water from two glucose-electrolyte solutions (GES) in resting man. Eight fasted male volunteers consumed on two occasions, in randomized order, 500 ml of either a 5% (LG) or 20% (HG) glucose solution containing the same concentrations of electrolytes and 12 g of deuterium oxide. Gastric emptying was measured over 60 min using a double-sampling aspiration technique (Beckers *et al.* 1988), and unidirectional uptake of water was estimated by measuring the rate of accumulation in arterialized-venous blood of a tracer for water (deuterium) (Maughan *et al.* 1990). Mean (SD) gastric emptying rate expressed as slope values (ml/min) of LG (8·5 (1·6)) was faster ($P<0·001$) than HG (4·9 (1·0)). The amount of glucose emptied from both solutions was similar for the initial 30 min after ingestion; thereafter glucose delivery was greater from HG. By the end of the study 60·9 (SD 11·3) g of glucose was emptied from HG and 24·3 (SD 0·9) g from LG ($P<0·004$). Time to peak deuterium concentration (T_{max}, min) in the circulation was faster from LG (30 (SD 13)) compared with HG (51 (SD 11); $P<0·004$). Deuterium accumulation rate (ppm/min) expressed as slope values to T_{max} was faster from LG (13 (SD 5)) than from HG (4 (SD 1); $P<0·001$). These data do not allow us to conclude whether the faster rate of deuterium accumulation from LG was due to the difference in gastric emptying rate or the known improved intestinal water absorption rate of dilute GES compared with hypertonic GES with a high glucose content (Leiper & Maughan, 1986).

Beckers, E. J., Rehrer, N. J., Brouns, F., Ten Hoor, F. & Saris, W. H. M. (1988). *Gut* **29**, 1725–1729.
Leiper, J. B. & Maughan, R. J. (1986). *Journal of Physiology* **373**, 90P.
Maughan, R. J., Leiper, J. B. & McGaw, B. A. (1990). *Experimental Physiology* **75**, 419–421.

Effects of guar gum supplementation of breakfast cereals on small intestinal hydrolases in the rat. By J. C. MATHERS, PATRICIA A. LAWLOR and D. S. PARKER, *Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

Guar gum (GG) consumption flattens the post-prandial glycaemic response by mechanisms which are not fully understood. When fed in semi-purified diets, GG increases the viscosity of small intestinal (SI) contents, reduces convective movement adjacent to the mucosa and may alter mucosal enzyme activities (Johnson, 1990). It is not known if GG has these effects when present in normal mixed human foods.

Twenty-four male Wistar rats (initial weight 96 g) were given *ad lib.* access to diets of which breakfast cereals provided 0·45–0·49 present as corn flakes (CF), CF + GG (9%) wheat flakes (WF) or WF + GG (12%). Dried skimmed milk powder was added to attain protein contents of 150 g/kg. After 27 d, activities of brush border hydrolases (U/mg mucosa) were measured in mucosa from the proximal and distal regions of the SI; 25 and 75% of distance from the pylorus respectively.

	Corn flakes		Wheat flakes		SE of	Statistical significance		
	-GG	+GG	-GG	+GG	mean	Flakes	GG	Interaction
Proximal small intestine								
Maltase	16·3	16·5	22·6	21·6	2·15	**	NS	NS
Sucrase	1·82	1·41	2·32	1·86	0·172	**	**	NS
Tripeptidase	64·8	79·9	56·4	73·5	6·90	NS	**	NS
Distal small intestine								
Maltase	27·0	26·6	19·2	18·1	2·04	***	NS	NS
Sucrase	1·77	2·24	1·26	1·59	0·153	***	**	NS
Tripeptidase	63·6	66·1	63·8	37·8	8·83	NS	NS	NS
SI length (cm)	113	118	109	120	3·2	NS	*	NS

NS, not significant.
*$P<0·10$, **$P<0·05$, ***$P<0·01$.

Disaccharidase activities were higher in the proximal SI of WF- than of CF-fed rats whilst the reverse was observed for the ileum. Inclusion of GG had no effect on maltase activity but sucrase was reduced in the proximal SI and increased in the ileum. Up-regulation of sucrase in the ileum may result from more sucrose reaching this site. Displacement of digestion caudally could be responsible for increased SI length in GG animals.

We thank Peter Fletcher (Newtime Food Products Ltd) and Ruth Fairchild for providing the cereal products.

Johnson, I. T. (1990). In *Dietary fibre: chemical and biological aspects*, pp. 151–163 [D. A. T. Southgate, K. Waldron, I. T. Johnson and G. R. Fenwick, editors]. Cambridge: Royal Society of Chemistry.

Effects of enzyme supplementation on digestibility of wheat feed by weaned pigs. By DEVINA MCCLEAN[1] and K. J. MCCRACKEN[1,2], [1]*The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX* and [2]*Food and Agricultural Chemistry Department, Department of Agriculture for Northern Ireland*

There is evidence (Elwinger & Saterby, 1987), that enzyme supplements improve the feeding value of diets for broiler chickens, and Inborr & Graham (1991), observed improvements in faecal digestibility of wheat–barley-based diets for weaned pigs.

This experiment was done to assess the relative contributions of the small and large intestine to digestion of diets based on wheat feed, and the effect of enzyme supplementation on apparent digestibility at either site.

The experiment was done in two replicates using six four-week-old littermate pigs per replicate. Pigs, fitted with a T-piece cannula at the ileo–caecal junction, were allocated to one of six experimental diets. Diets contained wheat feed at 200 or 400 g/kg and were supplemented with cellulase (*EC* 3.2.1.4) alone (0·2 g/kg) or plus pectinase (*EC* 3.2.1.15, 0·1 g/kg) and xylanase (*EC* 3.2.1.32, 0·2 g/kg). Faecal collections (7 d) were made in conjuction with 8 h ileal collections on alternate days throughout the balance period.

Digestibility of dry matter (DM), crude protein (CP) and gross energy (GE) at faecal and ileal sites were significantly higher ($P<0·001$ and $P<0·05$ respectively) for the 200 g/kg diet (see Table). No significant effects of enzyme supplements were observed on digestibility at either site.

	Apparent ileal and faecal digestibilities (*n* 12)					
	Dry matter		Crude protein		Gross energy	
Diet (wheat feed g/kg)	Faecal	Ileal	Faecal	Ileal	Faecal	Ileal
200	0·87	0·75	0·87	0·77	0·87	0·77
400	0·81	0·66	0·81	0·69	0·81	0·67
SEM	0·012	0·021	0·016	0·032	0·014	0·024
$P<$	0·001	0·05	0·001	0·05	0·001	0·05

The difference between faecal and ileal digestibility values was greater ($P<0·05$) for the 400 g/kg wheat feed diet. However, the degree of fermentation of the residue from the terminal ileum was unchanged by level of wheat feed inclusion, averaging 43·6%.

It is concluded that there is considerable capacity for hind gut fermentation in the weaned pig and that the digestibility of wheat feed is not improved by enzyme supplementation.

The financial support of J. Bibby Agriculture Ltd., is gratefully acknowledged.

Elwinger, K. & Saterby, B. (1987). *Swedish Journal of Agricultural Research* **17**, 133–140.
Inborr, J. & Graham, H. (1991). *Animal Production* (In the Press).

Comparison of the effects of bran and ispaghula on colonic contents in the rat. By C. A. EDWARDS and M. A. EASTWOOD, *Gastrointestinal Laboratory, Western General Hospital, Edinburgh EH4 2XU*

Ispaghula and bran are both effective in bulking the stool and relieving the symptoms of diverticulosis. However, bran reduces intraluminal colonic pressure and ispaghula increases it (Eastwood *et al.* 1978). In the present study the effect of ispaghula and bran on the caecal, proximal and distal colonic contents was investigated to identify differences in the physical or chemical properties that may account for the different effects on pressure.

Forty male Wistar rats (150 g) were transferred onto a low-fibre diet (45 g non-starch polysaccharide (NSP)/kg) from a stock diet (CRMX, 133 g NSP/kg) for 4 weeks. Their diet was then supplemented with either 100 g coarse wheat bran/kg (Chancellot Mills Ltd, Edinburgh, 95% passed through 0·5–1·5 mm sieves, 463 g NSP/kg; ten rats) or 50 g ispaghula/kg (*Plantago ovata* Husk, Richardson Vicks Ltd UK, 996 g NSP/kg; ten rats) to provide equal amounts of dietary fibre. Twenty rats remained on the low-fibre diet. After a further 4 weeks the rats were killed and the contents of the caecum, proximal and distal colon (first half and second half) collected for wet and dry weight measurements, and short-chain fatty acid (SCFA) analysis. Results were compared by one-way analysis of variance and Student's *t* test.

Both ispaghula and bran increased faecal wet and dry weights but ispaghula was the more effective (wet weight (g/d): basal 3·15, ispaghula 7·9, bran 5·4 (SE 0·43); dry weight (g/d): basal 1·51, ispaghula 2·8, bran 2·10 (SE 0·13)). Ispaghula increased the basal wet content throughout the colon by over 100% (caecum: basal wet weight 3·78 g, ispaghula 7·53 g (SE 0·44); proximal colon: basal wet weight 0·5 g, ispaghula 1·92 g (SE 0·16); distal colon: basal wet weight 1·33 g, ispaghula 2·55 g (SE 0·2); $P<0·001$) but significantly increased the dry content only in the caecum, resulting in greater and more liquid colonic contents. Bran had no significant effect on wet or dry weight of colonic contents compared with the basal diet animals. Although ispaghula-fed rats had the highest SCFA content (μmol) throughout the colon (caecum: basal 334, ispaghula 532, bran 272 (SE 31·1); proximal colon: basal 47·9, ispaghula 103·6, bran 52·5 (SE 9·8); distal colon: basal 88·0, ispaghula 137, bran 76·1 (SE 11·9); $P<0·01$ ispaghula compared with basal), because of increased water the concentration was similar to the bran-fed and basal rats. There was no significant difference between the amount of colonic SCFA of the bran-fed and basal rats but faecal output of SCFA was increased in the bran-fed rats.

The SCFA profile of the bran-fed and ispaghula-fed rats was different, with a higher proportion of propionic acid (mol/mol) in the caecum of the ispaghula-fed animals (basal 178, ispaghula 216, bran 150 (SE 7·6); $P<0·01$ ispaghula compared with basal), and a higher proportion of butyric acid (mol/mol) in the bran-fed animals (basal 141, ispaghula 129, bran 232 (SE 12·8); $P<0·001$ bran compared with basal). Whereas the SCFA profile was maintained throughout the colon for ispaghula, the characteristic profile for bran fermentation was present only in the caecum. In the colon there was no significant difference between the bran- and basal diet-fed rats.

These results have indicated large differences in the luminal colonic contents of bran- and ispaghula-fed rats. The biggest differences were seen in the proximal colon. The increased liquid content of the ispaghula-fed rats may increase distension and therefore alter motility. The SCFA results suggest that most of the fermentation of bran occurs in the caecum whereas ispaghula may continue to be fermented throughout the colon, maintaining its effects on the luminal SCFA.

Eastwood, M. A., Smith, A. N., Brydon, W. G. & Pritchard, J. (1978). *Gut* **19**, 1144–1147.

The effect of supplementing elemental diet with dextran on colonic short-chain fatty acids and cellular proliferation in the rat. By C. A. EDWARDS, M. BRUCE and A. FERGUSON, *Gastrointestinal Laboratories, Western General Hospital, Edinburgh EH4 2XU*

Prolonged use of elemental diets causes colonic mucosal hypoplasia thought to be related to a decrease in short-chain fatty acid (SCFA) production. In this study, the effect of supplementing an elemental diet (E028, Scientific Hospital Supplies Ltd., Liverpool) with dextran 35 (a fermentable polysaccharide composed of glycopyranose units linked at C1 and C2 with <5% branching, mol wt 35 000; Fisons plc., Loughborough) on colonic SCFA and mucosal cellular proliferation was investigated. Sixty female Wistar rats, weaned on to a laboratory diet (CRMX, 133 g non-starch polysaccharide/kg) for 4 weeks, were divided into three groups fed either CRMX, elemental (ED) or ED plus dextran (50 g/kg). After 4 weeks, ten rats in each group were injected with colchicine. At 20, 40, 60, 80 or 110 min after colchicine the rats were killed, a sample of tissue was taken from the proximal and distal colon and processed for microdissection and measurement of number of metaphase per crypt and crypt length. The other rats were killed and samples were taken of caecal, proximal and colonic contents for wet and dry weight and SCFA content. Caecal SCFA results were used because of the lack of proximal sample available.

As expected rats fed ED had little colonic content, and lower SCFA than the rats fed CRMX. At both sites studied, for ED-fed rats the mean crypt length and crypt cell production rate (CCPR) were lower than CRMX-fed rats, although only significantly so in the proximal colon. Addition of dextran 35 increased colonic contents and the amount of SCFA in the caecum and distal colon (but not concentration), and produced similar values for CCPR and crypt length to the CRMX-fed rats, but crypt length in the proximal colon remained significantly shorter.

In conclusion, the elemental diet caused significant hypoplasia of the proximal but not the distal colon despite large changes in SCFA content. Dextran increased colonic contents and SCFA, but although there was a tendency for CCPR and crypt length to increase, they did not return to the CRMX values in all rats.

	Total SCFA (mmol/l)		Total SCFA (μmol/section)		Tissue wt (g)		Crypt length (μm)		Metaphases/ crypt per h	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Proximal colon†										
CRMX	86	3	287	28	0·43	0·03	147	26	5·09	0·82
Elemental	66**	4	63***	9	0·30**	0·03	119*	19	4·02*	0·60
Elem+dextran	73	5	112***‡	11	0·32*	0·03	121*	14	5·67	1·36
Distal colon										
CRMX	69	3	88	9	0·40	0·02	194	17	4·18	1·46
Elemental	45***	6	13***	1	0·35	0·02	122	27	3·09	0·24
Elem+dextran	60	6	34**‡	4	0·32**	0·02	199	20	4·32	1·54

Significantly different from laboratory diet (CRMX): *$P<0·05$, **$P<0·01$, ***$P<0·001$.

Crypt cell production rate was compared by analysis of variance of slope; all other variables were compared by one-way analysis of variance and *t* test.

† Caecal SCFA was measured instead of proximal colon.

‡ Dextran significantly different to elemental.

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In vitro fermentation of a galacto-oligosaccharide by human bacteria in continuous culture. By M. DURAND, C. CORDELET, G. HANNEQUART and P. BEAUMATIN, *Laboratoire de Nutrition et Sécurité Alimentaire, INRA, 78352 Jouy-en-Josas Cédex, France* and J. P. GRIVET, *Département de Physique, Université d'Orléans, 45071 Orléans Cédex, France*

A transgalactosylated oligosaccharide (TOS), (Gal)*n*-Glc (*n* 1–4), produced in Japan (Yakult Institute) is assumed to escape digestion in the small intestine and be available for fermentation in the large bowel. This study was designed to determine the effects of TOS on fermentation metabolism and balance after adaptation of the flora.

The four 1 litre vessels of a continuous culture system (see Denis *et al.* 1990) were inoculated with a mixture of homogenized and diluted (110 g/l) human faeces collected from three methane (CH_4)-producing adult volunteers (faeces kindly provided by L. Abensour, Hôpital St Lazare, Paris). The vessels were continuously infused with a complex medium. After 7 d of adaptation, two vessels (+TOS) were fed daily 10 g of TOS delivered twice, whereas the other two (controls) were run with the complex medium only; the experiment was then continued for 15 d. At the end of the experiment the flora from each control and +TOS vessels were collected and assayed for reductive acetogenesis activity measured by ^{13}C NMR (see De Graeve *et al.* 1991).

Maximum degradability of TOS (95%) was obtained after 3 d of feeding and thereafter the fermentation became stabilized. The daily production of short-chain fatty acids (SCFA) and gas* (mmol) measured after 5–6 d (*n* 4) are shown in the Table.

Vessels		Total SCFA	Acetate	Propionate	Butyrate	CO_2	CH_4
Control	Mean	49·7	26·7	11·2	6·0	13·3	9·9
	SED	4·6	3·2	0·9	0·3	1·8	2·0
+TOS	Mean	126·4	79·5	25·6	13·5	99·3	22·9
	SED	12·2	8·6	2·9	0·5	6·5	2·5

* Only traces of H_2 were detected.

In addition to the increase in SCFA and gas production, feeding TOS resulted in a significant larger acetate molar proportion (63·0 SD 1·4% *v.* 53·7 SD 3·0%). This is in keeping with the results of reductive acetogenesis activity according to which TOS had an enhancing effect on the incorporation of $^{13}CO_2$ into acetate and butyrate carbons (8·3 and 3·1 mM ^{13}C incorporated for TOS and control, respectively). These observations suggest that oligosaccharides may activate the reduction of CO_2 to acetate and butyrate by human flora even in the presence of methanogenesis. Calculation of the fermentation balance of TOS carbon (+TOS minus control data) showed that 55% was recovered in SCFA whereas 15% was recovered in the biomass. The recovery in SCFA indicates the amount of energy which could be saved for the host when feeding this oligosaccharide.

De Graeve, K. G., Grivet, J. P., Durand, M., Beaumatin, P. & Demeyer, D. (1991). *Canadian Journal of Microbiology* **36,** 579–582.

Denis, I., Durand, M., Stévani, J., Hannequart, G. & Dumay, C. (1990). *Sciences des aliments* **10,** 265–274.

Effects of galacto-oligosaccharides (TOS) on bacterial enzymic activities and metabolite production in rats associated with a human faecal flora. By HIROKO KIKUCHI, CLAUDE ANDRIEUX and ODETTE SZYLIT, *Laboratoire d'Ecologie et de Physiologie du Système Digestif, INRA, 78352 Jouy-en-Josas Cédex, France*

In man, galacto-oligosaccharides (TOS) appear well fermented by intestinal microflora (Ito *et al.* 1990). To determine the fermentative pattern, adult germ-free rats were inoculated with a human flora belonging to a methanogenic donor (see Andrieux *et al.* 1991 for details). Rats (four per group) were fed a 'human-type diet' containing either 10% of sucrose (control diet), or 5% or 10% of TOS (experimental diets). Total gas excretion was measured in a respiratory chamber (Le Coz *et al.* 1989). Bacterial glycolytic activities were measured in the faeces and end-products of bacterial metabolism were measured in caecal content.

In the faeces, increasing the amount of TOS in the diet from 0 to 5 or 10% increased β-galactosidase (*EC* 3.2.1.23) activity (mean (SD)) from 1·9 (0·3) to 8·3 (2·2) and 11·1 (2·7) U/g; did not alter N-acetyl-β-galactosaminidase (*EC* 3.2.1.53) (0·48 (0·1) U/g) and lowered β-glucosidase (*EC* 3.2.1.21) activity from 1·4 (0·3) to 0·9 (0·2) and to 0·5 (0·1) U/g. β-glucuronidase (*EC* 3.2.1.31) activity decreased only with 10% TOS (from 0·4 (0·1) to 0·2 (0·1) U/g).

	Daily gas excretion (ml/100 g rat per 10 g diet)				End-product concentration in caecal content (μmol/g wet content)					
	H_2		CH_4		SCFA		Lactic acid		Succinic acid	
Diet	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	0·033[b]	0·005	4·13	1·09	15·6[b]	3·3	0·85[b]	0·23	0·22[b]	0·16
+5% TOS	0·32[a]	0·20	5·74	0·61	29·3[a]	5·3	0·79[b]	0·37	0·19[b]	0·17
+10% TOS	0·36[a]	0·22	3·57	2·20	30·6[a]	4·3	2·34[a]	0·93	0·47[a]	0·03

[a,b] Different superscripts within a column indicate a significant difference: $P<0·05$.

In both experimental groups, H_2 production increased 10 times whereas CH_4 production was not significantly modified.

Caecal pH decrease was more marked with 10% than with 5% TOS in the diet. Caecal concentration of SCFA increased twofold with both diets but with 10% TOS the pattern of SCFA was significantly modified: acetate proportion increased whereas butyrate, valerate and isoacids decreased. Lactic and succinic acid concentrations were enhanced in rats fed a 10% TOS diet.

These results show that TOS intake induces qualitative and quantitative changes of bacterial metabolic pathways in rats associated with a human flora.

Andrieux, C., Lory, S., Dufour-Lescoat, C., de Baynast, R. & Szylit, O. (1991). *Food Hydrocolloids* **5**, 49–56.

Ito, M., Deguchi, Y., Miyamori, A., Matsumoto, K., Kikuchi, H., Matsumoto, K., Kobayashi, Y., Yajima, T. & Kan, T. (1990). *Microbial Ecology in Health and Disease* **3**, 285–292.

Le Coz, Y., Morel, M.-T., Bousseboua, H., Dufour, C. & Szylit, O. (1989). *Science Technique Animal Laboratoire* **13**, Suppl. 4, 35–39.

The effects of dietary guar gum or soya fibre on the activities of some NADPH-generating enzymes in rats. By HEATHER J. FINLAYSON, RANDI BARSTAD and J. C. MATHERS, *Department of Agricultural Biochemistry and Nutrition, The University, Newcastle upon Tyne NE1 7RU*

We have previously reported (Pedroso *et al.* 1989, 1990*a*) that replacement of sucrose by foods rich in complex carbohydrates (CC), reduced the activity of several lipogenic enzymes. Inclusion of short-chain fatty acids (SCFA), the major fermentation end-products of CC, in a sucrose-containing diet mimicked the effects of the CC (Pedroso *et al.* 1990*b*).

The current experiment investigated the individual and interactive effects of differing dietary CC and oil sources on the activities (nmol product/min per mg protein) of three NADPH-generating enzymes: glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; G6PDH), NADP-malate dehydrogenase (*EC* 1.1.1.40; ME) and NADP-isocitrate dehydrogenase (*EC* 1.1.1.42; IDH). The results reported refer only to the effects of the three CC sources (100 g/kg diet): glucose, i.e. no CC (NC), guar gum (GG) and soya fibre (SF), each of which was fed as part of a semi-synthetic diet containing no sucrose to a total of twenty rats for 21–26 d.

	Caecal SCFA				Enzyme activity			
		Molar proportions (nmol/mol)			Liver			G6PDH (adipose tissue)
CC source	Total (mM)	Ac	Pro	Bu	IDH	ME	G6PDH	
NC	45·2[a]	538[a]	284[b]	94[b]	172[b]	6·7[a]	11·5[b]	11·5
GG	77·4[b]	527[a]	372[c]	53[a]	162[ab]	24·4[b]	17·3[c]	11·7
SF	66·0[b]	633[b]	178[a]	147[c]	154[a]	7·6[a]	7·6[a]	13·1
SEM	5·6	15	11	4	3·2	0·49	0·45	0·83

Ac, acetate; Pro, propionate; Bu, butyrate.

[a,b,c] Values in the same column with different superscripts are significantly different: $P<0·01$.

Although inclusion of both GG and SF increased the total SCFA concentration relative to NC, there were marked differences in the proportions of the individual SCFA. Compared with NC, GG resulted in greatly increased propionate and reduced butyrate with the converse for SF. Activities of ME and G6PDH (which provide most of the NADPH for lipogenesis) were significantly increased by GG whilst G6PDH was reduced in SF-fed rats. There was no effect of CC source on activity of G6PDH in epididymal adipose tissue. The results suggest: (1), that the ratio of butyrate to propionate reaching the liver may influence the hepatic NADPH-generating enzymes and (2), that the effect is directly on the liver and not via a systemic mechanism, since adipose tissue G6PDH remained unchanged despite major changes in hepatic G6PDH activity.

The authors acknowledge the co-operation of Dr. R. Noble, Scottish Agricultural College, Ayr and Prof. M. Cocchi, University of Bologna, Italy in this experiment.

Pedroso, L. M. R., Finlayson, H. J. & Mathers, J. C. (1989). *Proceedings of the Nutrition Society* **48**, 54A.

Pedroso, L. M. R., Mathers, J. C. & Finlayson, H. J. (1990*a*). *Proceedings of the Nutrition Society* **49**, 52A.

Pedroso, L. M. R., Mathers, J. C. & Finlayson, H. J. (1990*b*). *Proceedings of the Nutrition Society* **49**, 212A.

The influence of chronic ethanol feeding on duodenal and hepatic monoamine oxidase (*EC* 1.4.3.4) in the rat. By N. P. KENNEDY, *Department of Clinical Medicine, TCD Medical School Building, St James's Hospital, Dublin 8* and G. T. M. HENEHAN, and K. F. TIPTON, *Department of Biochemistry, Trinity College, Dublin 2, Republic of Ireland*

Chronic ethanol intake in rats and in man is reported to cause ultrastructural alterations of the mitochondria and endoplasmic reticulum of the small intestinal mucosal epithelium and the hepatocyte. Monoamine oxidase (*EC* 1.4.3.4, MAO), an integral enzyme of the outer mitochondrial membrane, is present in both of these tissues, where it plays an important role in the first-pass metabolism of dietary amines. Two isoenzymes exist with differing substrate affinities and inhibitor sensitivities; 5-hydroxytryptamine and β-phenylethylamine being preferential substrates, clorgyline and 1-deprenyl being selective inhibitors of MAO-A and MAO-B respectively. Chronic ethanol intake is associated with decreased MAO activity in the brain of rats and humans. This study was carried out to determine the effect of ethanol consumption on intestinal and hepatic MAO.

Male Wistar rats were fed a Lieber–deCarli diet for 5 weeks. The test group (C, *n* 16) received ethanol-containing (5% w/v, 36% total calories) feed *ad lib.* and the pair-fed control group (B, *n* 16) a diet with isocaloric substitution of dextrin–maltose for ethanol. Another control group (A, *n* 16) took ethanol-free feed *ad lib.* MAO-A and MAO-B activities were determined in duodenal mucosal cell homogenates and in liver mitochondrial preparations, using a radiochemical technique. Duodenal and liver samples (*n* 8) from each group were examined histologically and by transmission electron microscopy.

	Controls (A) *Ad lib.*		Controls (B) Pair-fed		Test (C) Alcohol-fed	
	Mean	SD	Mean	SD	Mean	SD
Body-wt (g)	300·6	11·8	254	14·9	257	15·6
Duodenal mucosa						
MAO-A (cpm × 10^{-3}/mg protein)	7·5	1·8	7·5	1·4	8·2	2·4
	n 7		*n* 8		*n* 8	
MAO-B (cpm × 10^{-3}/mg protein)	5·1	1·9	5·7	2·4	9·5	2·8
	n 7		*n* 8		*n* 8	
Liver mitochondria						
MAO-A (cpm × 10^{-3}/mg protein)	84·5	8·1	85·8	12·1	95·0	14·5
	n 8		*n* 8		*n* 8	
MAO-B (cpm × 10^{-3}/mg protein)	67·0	19·3	74·2	14·8	67·3	18·0
	n 8		*n* 8		*n* 8	

Growth of the alcohol-fed rats and their isocaloric controls was significantly less than growth of the *ad lib.* controls. Fatty infiltration was present in the livers of the alcohol-fed group and to a lesser degree in their isocaloric controls, but absent in the *ad lib.* control livers. Despite alcoholic hepatitis and mitochondrial changes in intestinal epithelial cells and hepatocytes in the alcohol-fed group, liver MAO activities were similar in all three groups. However, MAO-B activity was significantly higher in the duodenal mucosa of alcohol-fed rats (A *v.* C, $P=0{\cdot}003$; B *v.* C, $P=0{\cdot}01$; A *v.* B, $P=0{\cdot}95$; Mann-Whitney U-test).

These results suggest that the first-pass metabolism of dietary amines is not compromised by chronic ethanol intake in rats.

The increase in *n*-3 fatty acids in plasma of humans consuming enriched eggs. By DAVID FARRELL, *Department of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, NSW 2351, Australia*

The beneficial effects of *n*-3 long-chain polyunsaturated fatty acids (*n*-3 LCPUFA) on the incidence of cardiovascular disease and some other diseases in humans (Galli & Simopoulos, 1989) is now well established; fish is a major dietary source of *n*-3 LCPUFA.

We offered diets with added (70 g/kg) cod liver, rapeseed (Canola) or linseed oils to hens and successfully enriched the eggs with *n*-3 LCPUFA compared to eggs from hens on a control diet (Fig. 1a). A sensory evaluation panel could not distinguish between the four egg types. Forty-four free-living volunteers consumed eggs from hens on a commercial feed for 2 weeks. The volunteers were then divided into equal groups and given two eggs daily from one of the four groups of birds for the following 7 weeks. Plasma samples were drawn and other measurements made on subjects fasted overnight and at intervals of 0, 2, 5, 7 and 9 weeks.

Consumption of two eggs daily for 9 weeks did not alter mean plasma cholesterol which ranged from 4·7 to 5·2 mmol/l. High-density lipoproteins declined ($P<0·05$) from 1·5 to 1·1 mmol/l over time. The increase in plasma *n*-3 LCPUFA of volunteers after 7 weeks on eggs from hens given the experimental diets is shown in Fig. 1b. The increase was greatest in those consuming the eggs from hens on the cod liver oil and Canola oil diets and particularly for docosahexaenoic acid (22:6) and total *n*-3 LCPUFA. It is concluded that enrichment of eggs is a practical way of providing humans with a dietary source of *n*-3 LCPUFA.

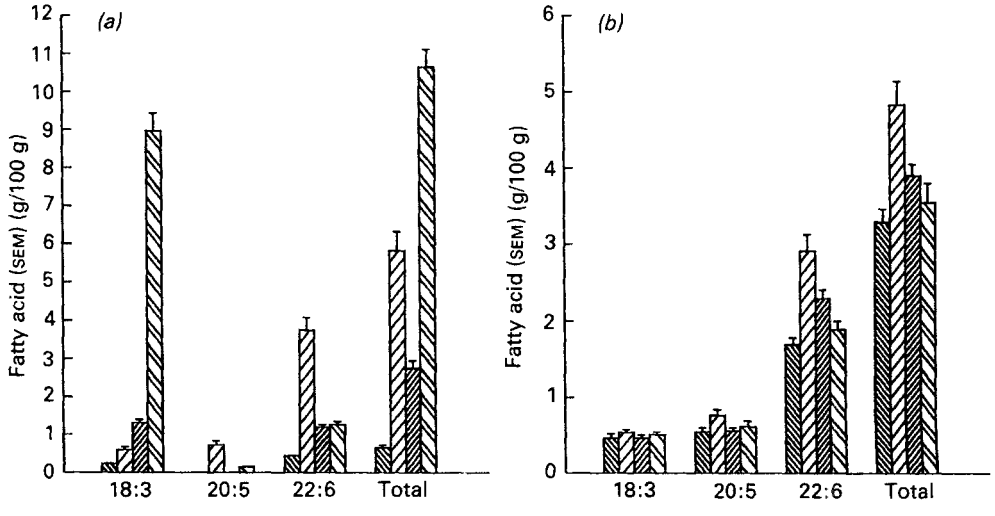

Fig. 1. The *n*-3 LCPUFA (a) in eggs from hens on diets with or without added oil after 42 d and their effect (b) on plasma levels in volunteers consuming two of these eggs daily for 49 d. ▨, Control; ▨, cod liver; ▨, Canola; □, linseed.

I thank Dr. R. A. Gibson, Flinders University Medical Centre, Adelaide, SA, for carrying out the fatty acid analysis.

Galli, C. & Simopoulos, A. P. (1989). *Dietary* n-*3 and* n-*6 Fatty Acids*. New York: Plenum Press.

Free radical oxidant generation by murine brain glial cells in vitro: potential nutritional antioxidant interactions and relevance to Alzheimer's dementia. By P. H. EVANS, *MRC Dunn Nutrition Centre, Milton Road, Cambridge CB4 1XJ* and E. PETERHANS and T. BURGE, *University Institute of Veterinary Virology, Berne, Switzerland* and J. KLINOWSKI, *University Department of Chemistry, Cambridge*

The occurrence of aluminosilicate deposits in the brains of subjects with Alzheimer's dementia (Candy *et al.* 1986) has stimulated interest in the possible role of such inorganic agents in the aetiopathogenesis of the disease (Evans *et al.* 1991).

We have examined the capacity of several model natural and synthetic aluminosilicate materials of differing particle size, shape and ionic composition, to stimulate the generation of free radical and related reactive oxidant species (ROS) by purified murine brain glial macrophage-type cells in vitro. The ROS were monitored in real time by luminol-dependent chemiluminescence. Standard soluble and particulate positive control stimulants of the oxygen burst, namely the tumour promoter phorbol myristate acetate (PMA) and zymosan, were also examined, together with phosphate saline buffer alone.

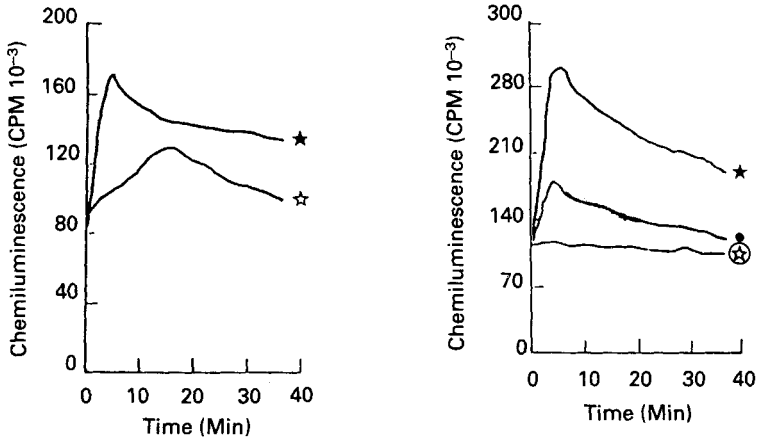

Fig. Murine glial chemiluminescent response to calcium ionic form of fibrous aluminosilicate (Turkish erionite) •, PMA ☆, zymosan ★, and phosphate-buffered saline ⊛.

The results show that the calcium ionic form of the fibrous aluminosilicate mineral erionite, exhibited a significant stimulatory reactivity.

The findings suggest that analogous deposits of fibrillary aluminosilicates in association with reactive microglial cells found within the cerebral neuritic plaques in Alzheimer's disease, may similarly contribute to free radical oxidant-mediated neurodegenerative damage. Reported changes in serum antioxidant vitamin concentrations in patients with Alzheimer's dementia (Jeandel *et al.* 1989) indicate that the role of such nutrients in the progression of the disease warrants further detailed investigation.

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The effect of dietary iron levels on iron status and antioxidant enzymes in the rat. By H. E. BRISTOW, J. J. STRAIN and R. W. WELCH, *Human Nutrition Research Group, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB*

Previous work from this laboratory has indicated that feeding rats diets either low (20 mg/kg) or high (400 mg/kg) in iron had little effect on antioxidant enzyme activities or hepatic malondialdehyde (MDA) levels. However, the high-Fe diet significantly increased plasma cholesterol (Bristow *et al.* 1991). In the current experiment a range of dietary Fe levels was used to investigate further the effect of Fe status on antioxidant enzymes, MDA and plasma cholesterol.

Four groups (*n* 6) of male weanling Sprague-Dawley rats were housed individually, and were fed *ad lib.* for 6 weeks on synthetic diets which contained Fe levels of 20 mg/kg, 35 mg/kg, 150 mg/kg and 400 mg/kg. Haemoglobin (Hb), transferrin saturation (TS), and hepatic and kidney Fe were measured as indices of Fe status. A range of antioxidant and related enzyme activities, namely plasma caeruloplasmin (*EC* 1.16.3.1), whole blood glutathione peroxidase (*EC* 1.11.1.9, GSH.Px), erythrocyte glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49, G6PDH), catalase (*EC* 1.11.1.6, CAT) and superoxide dismutase (*EC* 1.15.1.1, SOD), and hepatic SOD, G6PDH, GSH.Px, CAT, glutathione reductase (*EC* 1.6.4.2) and glutathione S-transferase (*EC* 2.5.1.18) were assayed, together with hepatic MDA, and plasma cholesterol. The data were analysed by one-way analysis of variance (ANOVA).

	Iron levels (mg Fe/kg diet)				ANOVA	
	20	35	150	400	Fe effect	LSD
Rat body-wt (g)	409	443	408	422	NS	—
Haemoglobin (g/l)	129[a]	145[b]	150[b]	135[a,b]	NS	—
Liver Fe (μg/g)	91[a]	102[a,b]	136[a,b]	158[b]	NS	—
Kidney Fe (μg/g)	84[a]	80[a]	141[b]	104[a,b]	*	43·8
Plasma cholesterol (mmol/l)	1·10[a]	1·26[a,b]	1·49[b]	1·10[a]	*	0·26
Whole blood GSH.Px (U/mg protein)	714[c]	547[a]	599[a,b]	581[a,b]	*	102
Hepatic G6PDH (U/mg protein)	3·34[a]	7·73[b]	6·75[b]	5·77[a,b]	*	2·6
Hepatic MDA (nmol/mg tissue)	14·7	21·9	21·1	22·0	NS	—

[a,b] Means within a row with different superscripts significantly different ($P<0·05$).
NS, not significant; LSD, least significant difference between means ($P<0·05$).
* $P<0·05$.

Dietary Fe levels had little effect on hepatic MDA levels or on antioxidant enzyme activities apart from an increase in whole blood GSH.Px activity, and a decrease in hepatic G6PDH activity in rats fed the diet containing a marginally-deficient Fe level.

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Influence of heated vegetable oils and α-tocopheryl acetate on susceptibility of chicken tissues to lipid peroxidation. By P. J. A. SHEEHY, P. A. MORRISSEY and A. FLYNN, *Department of Nutrition, University College, Cork, Republic of Ireland*

Susceptibility of chicken muscle and other tissues to lipid oxidation is influenced by tissue levels of α-tocopherol (α-T) (Sheehy *et al.* 1990). It has been suggested that peroxidized fats in foods accelerate α-T turnover (Kubow, 1990) and promote lipid oxidation in vivo (Izaki *et al.* 1984). The aim of this study was to investigate the effects of heated vegetable oils and α-tocopheryl acetate (α-TA) supplementation on the oxidative stability of chicken tissues.

		TBARS*											
	Incubation time	HSO		HSE		FSO		HLO		HLE		FLO	
Tissue	(min)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver (*n* 5)	0	0·93[a]	0·14	0·67[a]	0·09	0·37[b]	0·05	1·75[a]	0·17	0·95[b]	0·10	0·84[b]	0·18
	100	5·37[a]	0·72	1·61[b]	0·26	1·06[b]	0·25	14·2[a]	1·24	2·79[b]	0·24	1·96[b]	0·37
Heart (*n* 5)	0	1·14[a]	0·13	0·84[a]	0·12	0·87[a]	0·07	2·99[a]	1·29	1·26[a]	0·06	1·49[a]	0·29
	150	26·0[a]	1·80	20·1[a]	2·60	9·55[b]	2·23	32·4[a]	2·96	16·3[b]	1·21	8·81[c]	1·44
Thigh muscle	0	1·36[a]	0·17	0·88[b]	0·11	0·67[b]	0·06	1·65[a]	0·19	2·07[a]	0·59	2·12[a]	0·93
(*n* 5)	120	10·7[a]	1·06	6·54[b]	1·09	3·44[b]	0·91	14·0[a]	1·25	11·4[a]	1·94	10·6[a]	3·82
Breast muscle	0	1·08[a]	0·16	0·47[b]	0·02	0·52[b]	0·09	0·69[a]	0·09	0·37[b]	0·04	0·42[a,b]	0·13
(*n* 5)	120	2·25[a]	0·63	0·80[a]	0·10	0·50[b]	0·05	4·12[a]	0·65	1·31[b]	0·18	1·18[b]	0·15
Spleen (*n* 5)	0	2·11[a]	0·53	1·82[a]	0·28	1·33[a]	0·30	5·23[a]	1·51	3·43[a]	0·67	3·09[a]	0·50
	120	18·2[a]	1·59	3·90[b]	0·46	3·20[b]	0·61	27·3[a]	5·49	5·78[b]	0·96	6·99[b]	1·83
Lung (*n* 5)	0	1·05[a]	0·25	0·46[a]	0·05	0·73[a]	0·12	1·85[a]	0·25	0·88[b]	0·04	0·84[b]	0·10
	160	3·07[a]	0·24	2·37[a]	0·25	2·26[a]	0·35	9·54[a]	1·41	4·26[b]	0·65	2·89[b]	0·55
Plasma (*n* 6)	0	8·33[a]	1·87	7·91[a]	1·76	2·70[b]	0·79	9·66[a]	2·05	8·00[a]	0·95	4·85[a]	0·81

[a,b,c] For sunflower or linseed oil, mean values in horizontal rows not sharing a common superscript letter are significantly different (unpaired *t* test): $P<0·05$.

* nmol malondialdehyde/mg protein or nmol malondialdehyde/ml plasma.

Sunflower and linseed oils were heated at 140° for 24 h with constant aeration. Forty-eight one-day-old chicks were randomized into six groups and fed diets containing 80 g/kg fresh sunflower oil (FSO), fresh linseed oil (FLO), heated sunflower oil (HSO), heated linseed oil (HLO), α-TA-supplemented heated sunflower oil (HSE) or α-TA-supplemented heated linseed oil (HLE). Diets contained 50 mg BHT/kg, and 50 mg α-T/kg except for HSO and HLO, which were devoid of α-T. Thiobarbituric acid-reacting substances (TBARS) in plasma were determined by fluorimetry. TBARS in other tissues were measured before (basal) or after incubation with iron ascorbate for various lengths of time.

Basal TBARS were significantly elevated in several tissues in chicks fed heated unsupplemented oils compared to those in chicks fed fresh or α-TA-supplemented heated oils. The differences were more pronounced following stimulation of oxidation with Fe ascorbate. The reduced oxidative stability of tissues from chicks fed heated oils was partially offset by supplementation of the diet with α-TA. TBARS numbers in chicks fed linseed oil were usually higher than those in chicks fed sunflower oil, possibly reflecting greater unsaturation of tissue lipids. The results suggest that dietary peroxidized fats promote lipid oxidation in vivo, and increase the α-tocopherol requirement.

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The effects of dietary lipid source on lipid peroxidation and damage to cardiac tissue induced by ischaemia and reperfusion. By S. O'FARRELL and M. J. JACKSON, *Department of Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX*

Dietary fatty acids provide the ultimate source of fatty acids in tissue membranes. Consumption of long-chain *n*-3 fatty acids (derived from marine oils) has been linked to a low incidence of heart disease (Herold & Kinsella, 1986), but recent work suggests that the consumption of fish oils renders tissues more susceptible to free radical-mediated lipid peroxidation (Hu *et al.* 1989). Damage to cardiac tissue, induced by ischaemia and subsequent reperfusion, may be mediated by oxygen radical species (Badylak *et al.* 1987; Ytrehus *et al.* 1987) with subsequent peroxidation of cardiac membrane lipids (Ferrari *et al.* 1988). We have therefore studied the effect of different dietary lipid regimes on tissue peroxidation and the response of the isolated heart to ischaemia–reperfusion-induced damage in the rat.

Groups of female Wistar rats were fed diets containing 100 g/kg corn oil, menhaden oil or lard for 82 (SD 3) d. The response of isolated hearts to 30 min ischaemia and 120 min reperfusion was examined using the Langendorff preparation (Langendorff, 1895). The extent of damage was assessed by the measurement of creatine kinase (*EC* 2.7.3.2, CK) efflux at 15 min intervals following reoxygenation. Lipid peroxidation was assessed by measurement of thiobarbituric acid-reactive substances (TBARS) in cardiac tissue.

The peak release of CK from reoxygenated hearts of the three groups of animals was not significantly different (mean (SEM) values (*n* 9) were corn oil 336 (83); menhaden oil 272 (76); lard 406 (190) mU/30 min per mg heart). The TBARS content of the cardiac muscle was significantly ($P<0{\cdot}05$) higher in rats fed the menhaden-oil diet (mean (SEM) values were corn oil 1·03 (0·04); menhaden oil 1·21 (0·03); lard 1·08 (0·06) A_{532}/mg muscle). A similar pattern was obtained for TBARS produced by incubation with $FeSO_4$ (50 μM) and ascorbate (50 μM), (mean (SEM) values were corn oil 5·18 (0·57); menhaden oil 12·98 (1·21); lard 3·98 (0·49)).

Therefore it appears that dietary supplementation with menhaden oil (high in *n*-3 polyunsaturated fatty acids) has a dramatic effect on the susceptibility of cardiac tissue to free radical-mediated lipid peroxidation. However, in this experimental model, this has no influence on the susceptibility of the heart to damage induced by ischaemia and reperfusion.

The authors gratefully acknowledge financial support from the Nutritional Consultative Panel of the UK Dairy Industry.

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Effect of *trans* fatty acids on skeletal muscle membrane metabolism and viability. By M. J. JACKSON, *Department of Medicine, University of Liverpool, Liverpool L69 3BX* and R. NEALE and G. NORTON, *Department of Applied Biochemistry and Food Science, University of Nottingham School of Agriculture, Sutton Bonington LE12 5RD*

The fatty acid composition of skeletal muscle membranes appears to be susceptible to modification by long-term variations in dietary fatty acid composition (Neudoerffer & Lea, 1967; Jackson *et al.* 1988). Modification of muscle fatty acid composition to increasing unsaturation may influence muscle eicosanoid metabolism and exacerbate the damage response of muscle to a standardized damaging stress (Jackson *et al.* 1988). *Trans* fatty acids (*t*FA) derived from partially-hydrogenated oils and ruminant fats form a variable part of the human diet and have been recognized to influence linoleic acid and eicosanoid metabolism (Kinsella *et al.* 1988). We have therefore studied the effect of *t*FA supplements on skeletal muscle membrane function and viability in response to a standardized stress.

Groups of female Wistar rats (50–60 g) were fed either a casein-based diet containing 25% of dietary energy as *trans* 18:1 (*t*18:1), or a control diet with equivalent fat, but no *t*18:1. Following mating, female offspring of these animals were fed the same diet until sacrificed at approximately 60 g body-weight. Soleus muscles were rapidly removed and placed in an in vitro incubation system as previously described (Jones *et al.* 1983). Prostaglandin E_2 (PGE_2) production was assessed following treatment with the calcium ionophore A23187 (20 μM), and the effect of excess contractile activity on muscle damage was assessed by measurement of creatine kinase (*EC* 2.7.3.2, CK) release following 30 min of repetitive tetanii, stimulated electrically (at 30 V for 0·5 sec every 2 sec) via platinum electrodes.

Mean peak release of PGE_2 from muscles from *t*FA-fed animals was 2020 (SEM 632) pg/30 min per muscle, which was not significantly different from control muscles (2400 (SEM 906)). Muscle weights did not differ between the two groups. Mean peak CK release following stimulation (204 (SEM 42) mU/30 min per muscle) from *t*FA-supplemented rats was not significantly different from control animals (215 (SEM 73)). Analysis of the muscle fatty acid composition revealed that up to 10% of the fatty acids in polar lipids was *t*18:1, but this was also associated with an increase in the 18:2 content in the *t*FA-supplemented muscle.

Results therefore indicate that supplementation of diets with *t*FA causes substantial modification of skeletal muscle membrane fatty acid composition, but had no effect on muscle membrane function or viability as assessed by prostaglandin production or response to excess contractile activity.

The authors are grateful to Unilever Research, Vlardingen, the Netherlands for supplying the fats used in this study.

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Milk choices in Saskatchewan, Canada: consumer behaviour and reasons for milk choice.

By ALISON M. STEPHEN and BRUCE A. REEDER, *Division of Nutrition and Dietetics and Department of Community Health and Epidemiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*

Four different types of milk are available to the consumer in the province of Saskatchewan, Canada: homogenized (4% fat), 2% (fat), 1% (fat), and skimmed (<0·1% fat). Two independent studies have been used to assess consumer choice in type of milk consumed and the reasons for that choice.

The Saskatchewan Heart Health Survey (SHHS) investigated risk factors for heart disease in a randon sample of 2168 Saskatchewan residents in 1989. Included in the survey questionnaire were twelve questions on consumption of foods which are thought to influence total intake of fat or dietary fibre, including type of milk consumed. In the spring of 1991, a survey was conducted on 271 residents of Saskatoon, Saskatchewan to investigate changes in milk consumption in the last 5 years, and the reasons for change. The primary goal in this survey was to determine whether health issues influence milk choice. Both surveys were conducted on adults aged 18–74 years, and on equal numbers of males and females. Results were analysed using the Chi-square test.

The number of subjects consuming each type of milk in the two surveys is as follows:

	SHHS 1989			Saskatoon 1991		
	% of subjects			% of subjects		
Type of milk	M & F (*n* 2168)	M (*n* 1078)	F (*n* 1090)	M & F (*n* 271)	M (*n* 135)	F (*n* 136)
Homogenized (4% fat)	12·8	14·7	11·2	12·9	16·3	9·6
2% fat	50·0	55·0	45·2	44·6	48·9	40·4
1% fat	14·2	12·8	16·8	21·8	14·8	28·7
Skimmed	13·1	9·4	17·2	16·6	14·1	19·1

These results demonstrate a significant difference between males and females in their milk consumption patterns, more males consuming homogenized and 2% milk and more females consuming 1% and skimmed milk in both surveys. A significant difference between the two surveys is probably due to the period between them, in that 1% milk was newly introduced in 1989, when the SHHS was conducted.

In the Saskatoon survey, respondents were asked if they had changed their milk type in the last 5 years and, if so, why. They were given nine possible reasons, ranging from taste, cost and availability to health reasons such as body-weight control, control of serum cholesterol or because of the fat content of the milk. Of the 121 individuals who had changed in the last 5 years, 83% changed to a lower fat milk, the main reason for which was health, particularly mentioning fat content of the milk. Males and females differed in reasons for change, males being more concerned with control of serum cholesterol, females more with weight control. Fourteen per cent of those changing to low-fat milk were previously skimmed-milk drinkers, supporting the opinion that 1% milk is easier for many individuals to consume than skimmed milk.

These studies indicate that the population of Saskatchewan is consuming mainly 2% or lower fat milk, that 1% milk has a growing popularity and that health concerns are the major motivator of change.

The influence of type of milk and breakfast cereal consumption on daily intake of fat and non-starch polysaccharide in university students. By ALISON M. STEPHEN, WENDY J. DAHL and GLYNIS M. SIEBER, *Division of Nutrition and Dietetics, College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*

In Canada, four different types of milk are available to the consumer: homogenized (4% fat), 2% (fat), 1% (fat) and skimmed (<0·1% fat). One per cent milk was introduced into the province of Saskatchewan in January 1989. In order to assess whether consumption of a certain type of milk influences daily fat intake, university students in a first year class have carried out a 24 h recall of their diet in 1988, 1989 and 1990. This dietary information was also used to assess whether intake of breakfast cereal had an influence on daily intake of non-starch polysaccharide (NSP) (dietary fibre).

Twenty-four hour recalls (452) were analysed for nutrients using the NUTS computer program (Quilchena, Vancouver) which is based on the Canadian Nutrient File. No North American nutrient database includes NSP; hence a new computer program, FIBREFIND, was developed using values from the UK food tables and supplemented with values from analysis of Canadian foods in our laboratory. Data were analysed using analysis of variance.

The average intake of the 452 students was: energy 8·34 MJ (1996 kcal); fat 34·4% energy; protein 14·8% energy; carbohydrate 50·4% energy. NSP intake was 12·2 g/d; 5·2 g soluble, 7·0 g insoluble. Data were subdivided by type of milk, giving the following nutrient intakes for each group:

Type of milk	*n*	Energy (MJ)	% Energy			NSP (g/d)
			Fat	Protein	CHO	
Homogenized (4% fat)	14	9·10[a]	38·4[a]	15·1[a]	44·8[a]	9·7[a]
2% fat	139	9·04[a,b]	35·9[a]	15·0[a]	48·4[a]	11·8[a,b]
1% fat	58	8·33[b,c]	32·5[b]	14·9[a]	52·7[b]	13·1[b,c]
Skimmed	96	7·92[c,d]	31·0[b]	15·9[a]	52·8[b]	14·9[c]

[a–d] Values in vertical columns with different superscript letters are significantly different.

Data were also subdivided by whether or not the subject had eaten breakfast cereal on the day of assessment. Intake of nutrients were as follows:

	n	Energy (MJ)	% Energy			NSP (g/d)
			Fat	Protein	CHO	
Cereal	178	8·48	31·8	15·2	53·0	14·4
No cereal	274	8·26	36·1**	14·5	48·7**	10·8**

**$P<0·001$.

Of the total NSP intake for those who ate cereal, 5·9 g/d was soluble (41·0%), 8·5 g insoluble (59·0%). For those who did not eat cereal, soluble NSP intake was 4·7 g/d (43·5%), insoluble 6·1 g/d (56·5%).

Consumption of low-fat milk or breakfast cereal is associated with a daily intake which is lower in fat and higher in fibre.

The effect of variation in sources of energy intake on the nutritional quality of the diet of pre-school children. By JESSICA A. PAYNE, *Department of Child Life and Health, University of Edinburgh EH9 1UW*

The suitability of modified diets for pre-school children, aimed at reducing morbidity and mortality in adulthood, is a controversial issue. Little information is available on the effect of modification of the intake of fat or sugars on the intake of energy and other nutrients.

Children aged 2–5 years were randomly selected from the Lothian Child Health Register of six areas of Edinburgh. Of 600 approached by letter, 205 were recruited. Forty-six subsequently withdrew for 'social' reasons and six surveys were unsuitable for use. The remaining 153 children had a slight bias towards non-manual groups. Fifty-four children repeated the survey after 12 months. Data were collected by the 7-d weighed inventory method between May 1988–April 1990. One researcher visited all mothers at home to explain the study and use of the digital scales. Crockery was weighed before meals, and with any remaining food after meals. Leftovers were also described, and spillages recorded, in the food diary. Food data were analysed using the nutritional analysis system COMP-EAT 3.

Comparison of energy intake and sources of energy

	2 Years		3 Years		4 Years	
Group	Mean	SD	Mean	SD	Mean	SD
Girls						
Number	42		38		30	
Energy intake (kJ/d)	4390	830	4757	715	5062	885
% Energy from: Total fat	36	5	36	4	36	5
Total sugars	31	6	30	6	27	7
Starch	19	4	21	4	21	4
Boys						
Number	31		31		35	
Energy intake (kJ/d)	4504	755	5008	888	5300	790
% Energy from: Total fat	35	5	34	4	34	4
Total sugars	30	7	29	5	29	6
Starch	21	6	23	4	23	4

The effect of variation in sources of energy intake on the nutritional quality of the diet was assessed by correlating the % of energy from fat, sugars, and starch with total daily energy intake and nutrient intake per 4·2 MJ (1000 kcal).

Using the Pearson correlation test, no significant correlation was found between total daily energy intake and % of energy from fat, sugars or starch. A strong negative relationship of −0·62 ($P<0·001$) was found between % energy from sugars and % energy from fat. % Energy from fat was negatively correlated ($P<0·001$) with intake of fibre, iron, thiamin, total folic acid, vitamin C and ($P<0·01$) vitamin B_6, and positively correlated ($P<0·001$) with intake of protein, vitamin A and vitamin B_{12}. % Energy from sugars was negatively correlated ($P<0·001$) with intake of protein and ($P<0·01$) vitamins D, B_6 and B_{12} and positively correlated ($P<0·001$) with vitamin C, due to a strong relationship between intake of sugars and fruit juice. % Energy from starch was positively associated ($P<0·001$) with intake of fibre, Fe, thiamin, vitamin B_6, and total folic acid and negatively correlated ($P<0·001$) with intake of calcium and ($P<0·01$) vitamin A.

In conclusion, the above results imply that the diets of pre-school children taking high intakes of fat or high intakes of sugars could be improved by encouraging a higher intake of starchy foods, whilst ensuring an adequate intake of Ca and vitamin A.

This study was supported by the British Heart Foundation and the Nutritional Consultative Panel of the UK Dairy Industry.

Intake and sources of sugar in the diet of pre-school children. By JESSICA A. PAYNE, *Department of Child Life and Health, University of Edinburgh, Edinburgh EH9 1UW*

The mean intake of total sugars of UK adults aged 16–64 years was recently reported as being 19% of food energy intake (OPCS, 1990). Very little information is available on the total sugars intake of pre-school children though current policy is to recommend avoidance of food and drink rich in added sugars (DHSS, 1988).

By means of the 7-d weighed inventory technique, intake of total sugars was assessed in children aged 2–5 years from Edinburgh.

	2 Years		3 Years		4 Years	
Group	Mean	SD	Mean	SD	Mean	SD
Girls						
Number	42		38		30	
Age (months)	31	3	42	3	54	4
Energy intake (kJ/d)	4390	830	4757	715	5062	885
Intake of sugars (g/d)	88	28	90	23	91	20
% Energy from sugars	31	6	30	6	29	7
Boys						
Number	31		31		35	
Age (months)	30	3	43	4	54	4
Energy intake (kJ/d)	4504	755	5008	888	5300	790
Intake of sugars (g/d)	85	27	91	19	98	27
% Energy from sugars	30	7	29	5	29	6

The mean total daily energy intake of 2- and 3-year-old children was within the margin of error of the recently reported mean total daily energy expenditure of 2- and 3-year-old children, as measured by the doubly-labelled water method (Prentice *et al.* 1988; Davies *et al.* 1991).

Sources of sugars were examined in the diet of those children with an exceptionally high intake of total sugars of 40%–53% of energy from sugar (*n* 10). The main sources of sugars were (mean values as % of total intake): Ribena 20, pure fruit juice 18, chocolate 6, fresh fruit 5, fruit yoghurt 5, orange squash 5, other sweets 4. In individual children Ribena or pure fruit juice contributed up to 50% of sugar intake.

In conclusion, young children tend to have a high intake of total sugars. Ribena and pure fruit juice were found to contribute to the exceptionally high intake of some children.

This study was supported by the British Heart Foundation and the Nutritional Consultative Panel of the UK Dairy Industry.

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The absorption of retinol in Bangladeshi children with ascariasis. By F. AHMED, *Institute of Nutrition and Food Science, University of Dhaka, Dhaka-1000, Bangladesh* and B. MARGETTS and A. A. JACKSON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

Vitamin A deficiency, one of the most common nutritional deficiencies in the world, is thought to be caused primarily by a dietary deficiency. However, infection may also contribute to the disorder and an association has been drawn between deficiency and infection with intestinal helminths, especially ascariasis. Ascariasis has been said to cause malabsorption of vitamin A, based upon the change in the concentration of retinol in peripheral blood following an oral loading dose. In very few studies have direct measures been made of the losses of vitamin A in stool. We were interested to know whether ascariasis caused increased losses of retinol in the stool, and whether these losses could be attributed to accumulation of the vitamin by the parasite.

In twenty-four children aged 5–10 years from an urban slum area, a quantitative count for ascaris eggs in a 24 h stool was used to determine worm burden. In ten children with a range of worm burdens, a vitamin A absorption test was carried out: a single oral dose of retinol (as palmitate, 12 mg) was given and stools were collected for the next 48 h. The retinol content of the total stool was determined by high performance liquid chromatography (HPLC) (Ahmed *et al.* 1990).

For the ten children the median worm burden was 804 eggs/g (range nil to 8353). The median total excretion of retinol in the stool over a 48 h period was 31 μg (range 10 to 108 μg). Hence the median apparent absorption of retinol was 99·7% (range 99·05 to 99·91%). Long transformation of the worm burden excluded one individual with no eggs in the stool and a relatively-low apparent absorption for retinol (99·24%). For the other nine cases, there was a highly significant inverse relationship between the log worm burden and the apparent absorption of retinol ($P = 0{\cdot}017$, $r -0{\cdot}76$). In children being treated for ascaris, male and female worms were isolated from stool and the retinol content determined by HPLC. Retinol could not be detected in any of the worms isolated.

A significant relationship has been demonstrated between retinol in stool and the burden of infection with ascaris, but in all children less than 1% of the oral retinol load was recovered in the stool. Therefore, it cannot be said that these children malabsorbed retinol. Retinol could not be detected in the asacarids and, therefore, there was no evidence that the worms preferentially accumulated available retinol. All of the children in this study had a marginal-to-deficient retinol status and avidly retained the oral dose of retinol. There is the need to repeat the observations in retinol-replete individuals. We have been unable to substantiate the claim that ascariasis causes malabsorption of vitamin A.

This work was supported by Nestlé Nutrition Research Grant Programme and the Geoffrey Taylor Memorial Fund.

Ahmed, F., Ellis, J., Murphy, J., Wootton, S. & Jackson, A. A. (1990). *Archives of Disease in Childhood* **65**, 589–593.

Effect of vitamin A status on serum and tear immunoglobulins in children. By S. M. FILTEAU[1], R. A. ABBOTT[1], P. ARTHUR[2], F. DINKA[2], J. GYAPONG[2], D. ROSS[3], M. STEWARD[3], R. BAILEY[3], B. KIRKWOOD[3] and A. M. TOMKINS[1], [1]*Centre for International Child Health, Institute of Child Health, 30 Guilford Street, London WC1N 1EH*, [2]*Ghana VAST Child Health Project* and [3]*London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

Vitamin A supplements have been reported to decrease mortality of children in developing countries (Keusch, 1990). To study possible mechanisms for this effect, serum retinol and serum and tear immunoglobulins were measured in a subgroup of Ghanaian children (6–60 months old) participating in a randomized, double-blind study of the effect on morbidity and mortality of 60 mg retinol (200 000 i.u.) as palmitate every 4 months. Before supplementation, 16% of the children had serum retinol levels in the deficient range (<0·35 μM), 52% in the marginal range (0·351–0·7 μM), and 32% in the adequate range (>0·701 μM).

Age-adjusted means (with their standard deviations (SD)) of immunoglobulin levels measured by ELISA using antibodies against the individual class heavy chains in serum (mg/ml) and tears (μg/mg protein) at baseline and 4 months after treatment with vitamin A or placebo are shown below for children in each range of serum retinol level.

		<0·35		0·351–0·7		>0·701	
Serum retinol level (μM)		Mean	SD	Mean	SD	Mean	SD
Baseline							
Serum	IgG (mg/ml)	8·8	2·0	7·7	2·0	6·8	2·0
	IgA (mg/ml)	0·75	0·31	0·68	0·33	0·40	0·33
	IgM (mg/ml)	0·74	0·27	0·63	0·29	0·56	0·29
n		10		13		13	
4 months							
Serum	IgG (mg/ml)	9·2	3·9	10·1	3·6	9·3	3·7
	IgA (mg/ml)	0·81	0·47	0·52	0·44	0·51	0·44
	IgM (mg/ml)	0·51	0·26	0·46	0·25	0·52	0·25
Tear	IgG (μg/mg)	11	30	51	28	44	28
	IgA (μg/mg)	13	25	32	23	36	23
n		7		25		16	

Serum IgG and IgA tended to be higher in children with lower serum concentrations in both groups although age-adjusted correlations were significant only for baseline samples (IgG: $r-0{\cdot}46$, $P=0{\cdot}018$; IgA: $r-0{\cdot}46$, $P=0{\cdot}021$). The opposite trend was seen for tear IgG and IgA with statistical significance only in the case of IgA (r 0·25, $P=0{\cdot}041$). These preliminary results suggest that vitamin A deficiency tends either to decrease membrane transport of immunoglobulins or increase their degradation at the conjunctival mucosa. The high serum IgG and IgA in the children with low serum retinol may result from infections subsequent to impaired mucosal defences.

The support of the Ghana VAST Study Health Team and the financial support of the Medical Research Council of Canada and the Overseas Development Administration, UK is gratefully acknowledged.

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Plasma vitamin A and E levels in healthy young women. By P. HALFORD and A. A. JACKSON, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

Few metabolic studies are carried out in women and frequently the results obtained in men are extrapolated uncritically to apply to women. Few workers have looked at the effect of the menstrual cycle or the contraceptive pill on the metabolism of nutrients.

Six healthy women aged between 23–28 years, three of whom were and three of whom were not taking an oral contraceptive pill (a low dosage combination pill of 30 μg oestrogen and 0·15 progesterone), were studied over the period of a menstrual cycle. Fasted, venous blood was taken on every 3rd day of the menstrual cycle and plasma vitamin A and E was measured by high performance liquid chromatography. Total plasma cholesterol was measured enzymically at 500 nm on samples from days 4, 10, 16 and 25, and on days 28 and 34 from subjects who had a menstrual cycle of greater than 28 d. Each individual's habitual intake of vitamins A and E and cholesterol was measured from a weighed food intake for the duration of the menstrual cycle.

The mean plasma vitamin A and E levels of the group (μmol/l) were 1·95 (SD 0·61) and 23·31 (SD 6·25) respectively. When the group was divided into pill and non-pill takers it was found that pill takers consistently had a significantly higher plasma vitamin A ($P<0·0001$) and vitamin E level ($P<0·002$) compared to non-pill takers. The vitamin E levels were not significantly different when expressed as μmol vitamin E/l:mmol cholesterol/l.

Mean plasma levels of vitamins A and E and cholesterol and vitamin E:cholesterol ratio

	Pill takers		Non-pill takers		
	Mean	SD	Mean	SD	*P*
Vitamin A (μmol/l)	2·35	0·45	1·75	0·49	<0·0001
Vitamin E (μmol/l)	25·54	6·97	20·90	4·64	<0·002
Cholesterol (mmol/l)	4·55	1·21	3·97	0·50	NS
Vitamin E:cholesterol (μmol/mmol)	5·48	0·49	5·38	0·29	NS

NS, not significant.

The difference in plasma vitamin A and E levels could not be explained by differences in dietary intake. The mean vitamin E intakes of the pill and non-pill takers were 6·78 (SD 2·79) and 8·01 (SD 4·25) mg/d respectively. This difference was not significant. Despite their lower plasma vitamin A, the non-pill takers were ingesting significantly more vitamin A compared with the pill takers ($P<0·05$). The mean vitamin A intake of the non-pill takers was 1246·0 (SD 579·0) μg/d compared with 809·3 (SD 536·7) for the pill takers; one pill taker was a non-meat eater.

The effect of the menstrual cycle on plasma levels of vitamins A and E was also assessed. There was no significant difference in the plasma level of vitamin E during the menstrual cycle for either pill or non-pill takers. There was, however, a significant rise ($P<0·05$) in the plasma level of vitamin A for the group from week 3 to week 4 of the menstrual cycle. This was shown to be significant ($P<0·01$) for the pill takers but not the non-pill takers.

It would appear that oral contraceptive pills influence the metabolic handling of vitamin A. Given the important role played by retinoids in cellular function, this difference should be explored in greater detail.

Unusual behaviour of a vitamin B_{12}-binder, Transcobalamin 1, in cattle plasma. By J. PRICE, S. G. BARRIE and S. UENO, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The concentration of vitamin B_{12} in plasma or serum is a reliable indicator of vitamin B_{12} status in man and the sheep, but growth-response trials have failed to establish critical diagnostic values for cattle. The reasons for this are uncertain but it has been suggested (Carlos *et al.* 1987) that microbiological and radioisotope dilution assays, which function satisfactorily with human and ovine samples, are incapable of measuring the total vitamin B_{12} present in bovine plasma. Since denaturation of plasma vitamin B_{12}-binders (Transcobalamins, Tc), with release of the free vitamin, is a primary requirement in all current vitamin B_{12} assays, we have separated the binders in cattle and sheep plasma by gel filtration and identified them by comparison of their properties with those of binders (reviewed Allen, 1975) which have been fully characterized in man.

Unsaturated binders in cattle, sheep and human plasmas were labelled directly by equilibration with ^{57}Co-cyanocobalamin and separated by chromatography on Sephadex G150; their elution characteristics (V_e/V_0) and apparent molecular weights (MW, $\times 10^3$ Daltons) are shown in the Table.

	Human		Cattle		Sheep	
Binder	V_e/V_0	MW	V_e/V_0	MW	V_e/V_0	MW
Tc0	1·00	>200	1·00	>200	not detected	
Tc1	1·28	103	1·30	96	not detected	
Tc2	1·65	37	1·62	40	1·58	44

Since failure to detect Tc0 and Tc1 in sheep plasma may be due to an inability to label these binders directly if already fully saturated, plasma samples were dialysed against 7·5 M guanidinium chloride to remove native cobalamin and its analogues, then against 0·2 M phosphate buffer (pH 7·4) before labelling. Despite a marked increase in label bound to Tc0, 1 and 2 in human and cattle plasma, Tc2 remained the only binder detected in sheep.

Prior to microbiological or competitive binding assay, displacement of vitamin B_{12} from binders in human plasma is normally carried out by heating at 100–120° for 15–30 min in the presence of cyanide (20 μM). The effectiveness of this treatment was verified by gel filtration, labelled vitamin being displaced from all binders in human and sheep plasma. Although the vitamin was also displaced from cattle Tc2, a substantial proportion of the label, equal to the sum that bound to Tc0 and Tc1, chromatographed as a single, unidentified high molecular weight complex.

The abnormal behaviour of the cattle Tc1–B_{12} complex under conditions resulting in complete release of the vitamin from binders in human and sheep plasma, suggests that vitamin B_{12} concentrations in cattle plasma are likely to be underestimated by current assay procedures if Tc1 is quantitatively important as a vitamin B_{12}-binder in the bovine.

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The effect of pyridoxine deficiency on copper status in male and female rats. By J. C. W. BROWN and J. J. STRAIN, *Human Nutrition Research Group, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB* and P. YOUNG, *Veterinary Services Division, Stormont, Belfast BT4 3SD*

Dietary supplementations with methionine (Strain & Lynch, 1990) or homocysteine (Hcy) (Brown & Strain, 1990) have been shown to lower copper status in the rat. This study investigated the effect of a deficiency of pyridoxine, a co-factor in sulphur amino acid metabolism, on Cu status.

One group (*n* 6) of male and one group (*n* 6) of female weanling Sprague-Dawley rats were provided with deionized water and fed a pyridoxine-deficient diet *ad lib.* A further two groups (*n* 6) of male or female rats were pair-fed a control diet (Brown & Strain, 1990) against the respective deficient groups. Diets contained 6·6 mg Cu/kg diet.

After 8 weeks the rats were killed and the activities of the Cu-dependent enzymes, cytochrome *c* oxidase (*EC* 1.9.3.1, CCO) and superoxide dismutase (*EC* 1.15.1.1, CuZnSOD) were measured in the liver. Cu content and CCO activity were also measured in the heart and kidney whilst erythrocyte SOD and plasma caeruloplasmin (*EC* 1.16.3.1, Cp) provided further indices of Cu status. Results were analysed by two-way analysis of variance testing for the main effects of pyridoxine deficiency and sex.

	Control				Pyridoxine-deficient					
	Male		Female		Male		Female		Main effects	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Pyridoxine	Sex
Body-wt	238	10·9	182	7·8	219	10·3	177	8·1	NS	***
Liver wt (% body-wt)	3·35	0·13	3·31	0·13	4·15	0·10	4·05	0·15	***	NS
Kidney wt(% body-wt)	0·71	0·02	0·81	0·02	0·86	0·03	0·84	0·03	**	NS
Liver										
Cu (μg/g dry wt)	16·7	0·57	18·8	0·40	13·7	0·59	14·1	0·85	***	NS
CCO (U/mg protein)	3·23	0·37	3·12	0·21	3·45	0·29	3·32	0·29	NS	NS
CuZnSOD (U/mg protein)	120·7	5·8	122·8	4·6	120·0	5·9	123·5	5·3		
Heart										
Cu (μg/g dry wt)	24·2	2·20	26·7	1·87	26·7	2·57	28·8	2·12	NS	NS
CCO (U/mg protein)	4·21	0·35	3·93	0·29	3·46	0·43	3·35	0·26	NS	NS
Kidney										
Cu (μg/g dry wt)	26·0	1·72	22·7	1·66	22·6	1·34	23·3	2·17	NS	NS
CCO (U/mg protein)	2·56	0·38	1·86	0·22	2·25	0·10	1·90	0·19	NS	*
Plasma										
Cp (U/l)	254	7·6	308	22·9	193	13·9	255	32·5	*	*
Hcy (μM)	3·7	0·51	2·7	0·31	19·0	0·68	11·0	0·88	***	***
Erythrocyte										
SOD (U/mg protein)	1·07	0·19	0·92	0·11	1·09	0·15	1·29	0·20	NS	NS

NS, not significant; *$P<0·05$; **$P<0·01$; ***$P<0·001$.

Although pyridoxine deficiency lowered Cu stores in the liver and plasma Cp activity, the Cu content of other tissues or other Cu-dependent enzymes were not affected over the experimental period.

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No effect of dietary boron on urinary oestrogen excretion in rats. By J. H. BEATTIE, *Division of Biochemical Sciences, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

A recent study with postmenopausal women volunteers suggests that changing dietary intake of boron from 0·25 to 3·25 mg boron/d under conditions of Mg deficiency significantly increases plasma oestradiol levels (Nielsen *et al.* 1987). The current study was designed to test in vivo, the in vitro finding, that borates inhibit catechol oestrogen methylation (J. H. Beattie and E. Weersink, unpublished results), and the proposal that this perturbation in oestrogen catabolism could influence the circulating level and metabolic fate of the major oestrogens.

Ten adult female rats which had been fed a diet low in boron (<0·1 mg B/kg) for 7 d and then either the same diet or a diet supplemented with boron (40 mg B/kg) for a further 5 d were ovariectomized to remove endogenous sources of oestradiol and were implanted with slow-release devices containing [^{3}H]oestradiol. After recovery from the operation the animals were maintained on the same diet and 24-h urine collections, which were preserved in an acetate–ascorbic acid buffer, were made over a period of 7 d. Urinary oestrogens were separated into conjugated, free and catechol forms using chemical extraction and enzymic techniques. Most radioactivity was detected in the conjugated oestrogen fraction and the dietary intake of boron had no significant influence on the distribution of tritium between conjugated, free and catechol oestrogen fractions.

Proportion (mean % with SD, n 5) of radioactivity in fractions from urine of rats continuously dosed with [^{3}H]oestradiol and fed a low- or high-boron diet

Boron intake (mg/kg)	Conjugated oestrogens		Free oestrogens			
			Non-catechol		Catechol	
	Mean	SD	Mean	SD	Mean	SD
<0·1	79·8	3·3	10·8	1·9	9·4	2·3
40	80·1	1·1	10·9	1·7	9·0	1·9

Under these study conditions, it is clear that a boron intake of 40 mg/kg diet has no significant effect on oestrogen excretion in ovariectomized rats. This conclusion supports evidence from separate studies that boron does not influence plasma sex steroid levels in rats (J. H. Beattie and A. Macdonald, unpublished results), although there is a marginal effect on bone growth which may be hormonally related (Beattie & Macdonald, 1991).

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Smoking, and correlations between dietary fat and adipose tissue linoleate (18:2*n*-6) using a food frequency questionnaire (FFQ). By C. BOLTON-SMITH, M. WOODWARD and H. TUNSTALL-PEDOE, *Cardiovascular Epidemiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY*

Low tissue 18:2*n*-6 levels may be objective markers for coronary heart disease (CHD) risk (Wood *et al.* 1987). Thus assessing the relationship between diet and tissue fatty acid composition is pertinent. Food frequency questionnaires (FFQ) have not been used to look at 18:2*n*-6 intake, probably because of the suspect adequacy of the fatty acid compositional data and the lack of fat biopsy and dietary data in the same population to provide validation.

As part of a cross-sectional study of risk factors for CHD in Scottish men and women (40–59 years) FFQ data were collected (Bolton-Smith *et al.* 1991) and approximately 4000 outer upper-arm fat biopsies were taken using a 3 mm syringe. Both adipose and dietary 18:2*n*-6 levels varied by smoking group (Table 1), and correlations between adipose and dietary fat variables were poorer in current smokers (Table 2). This suggests that the influence of smoking on the relationship between diet and adipose tissue fatty acid composition needs more detailed investigation. The dietary data from the FFQ would appear adequate to detect the previously-reported correlations between diet and tissue fatty acids, and confirms the usefulness of the FFQ as an epidemiological tool.

Table 1. *Mean dietary and adipose tissue linoleate (18:2*n-6*) values by smoking group*

	Men						Women					
Smoking group . . .	Current		Ex-		Never		Current		Ex-		Never	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dietary 18:2*n*-6 % energy	3·1	1·5	3·7	1·9	3·8	2·1***	3·4	1·9	4·0	2·2	3·9	2·2***
Adipose tissue 18:2*n*-6 % (w/w)	7·9	2·3	9·3	2·8	9·7	3·0***	8·4	2·1	9·9	2·6	9·8	2·5***
n	870		569		502		765		432		876	

***$P<0·001$ significant difference between smoking groups by analysis of variance on the (arcsine $\sqrt{}$) transformed data.

Table 2. *Pearson correlation coefficients*[a] *for dietary fat*[b] *v. adipose tissue linoleate % (w/w)*

	Men					Women				
Dietary . . .	% Fat	% SFA	% MUFA	% 18:2*n*-6	P:S	% Fat	% SFA	%MUFA	% 18:2*n*-6	P:S
Current	NS	–0·13	–0·11	0·31	0·30	–0·11	–0·21	–0·20	0·30	0·32
Ex-	NS	–0·22	–0·20	0·37	0·39	–0·20	–0·27	–0·27	0·29	0·32
Never	NS	–0·20	–0·20	0·37	0·39	–0·12	–0·25	–0·26	0·34	0·36

[a] All significant $P<0·001$ except NS for the (arcsine $\sqrt{}$) transformed variables.
[b] Percentage of energy including alcohol.
SFA, saturated fat; MUFA, mono-unsaturated fat; P:S, polyunsaturated:saturated fat ratio.

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The effect of dietary oil composition on lipid composition of pig meat. By C. A. MORGAN, *The Scottish Agricultural College, West Mains Road, Edinburgh EH9 3JG* and R. C. NOBLE, M. COCCHI and R. MCCARTNEY, *The Scottish Agricultural College, Auchincruive, Ayr KA6 5HW*

The polyunsaturated:saturated (P:S) ratio of fatty acids in the British diet should be increased (COMA, 1984). Also certain fatty acids (gammalinolenic (GLA) 18:3*n*-6, eicosapentaenoic (EPA) 20:5*n*-3 and docosahexaenoic 22:6*n*-3) have beneficial effects on health and increased dietary supply could be advantageous (Sanders, 1988). This experiment attempted to manipulate the composition of pig meat lipid by dietary means. Forty pigs (ten per treatment) were offered, from 25 kg live weight, one of four diets based on barley and soya-bean meal and containing per kg (1) 50 g tallow, (2) 50 g soya-bean oil, (3) 50 g soya-bean oil + 7·5 g GLAoil (Sturge Biochemicals, containing 170 g 18:3*n*-6/kg fatty acids) or (4) 50 g soya-bean oil + 9·5 g EPAnoil (Seven Seas Ltd, containing 212 g 20:5*n*-3 and 145 g 22:6*n*-3/kg fatty acids). Five pigs were slaughtered from each group at 70 and 100 kg live weight and samples were taken from the longissimus dorsi and semitendinosus muscles. The fatty acid composition of the lipids extracted from the semitendinosus muscle at 70 kg live weight is shown in the Table.

	P:S ratio and fatty acid composition (g/kg total) of the semitendinosus muscle							
Diet	P:S ratio	18:0	18:1	18:2*n*-6	18:3*n*-6 +*n*-3	20:4*n*-6	22:5*n*-3	22:6*n*-3
1	0·56	112	441	139	15·1	17·9	5·4	4·5
2	1·08	106	342	259	26·4	29·6	6·4	3·1
3	0·99	110	344	238	24·5	30·6	5·4	3·2
4	1·00	103	351	247	21·4	15·6	9·6	8·0
SD	0·116	11·3	23·7	22·1	4·68	6·31	1·66	1·12

Results were similar for the longissimus dorsi muscle and for pigs at 100 kg live weight. The P:S ratio was increased markedly by feeding soya-bean oil. Importantly, the considerable increase in polyunsaturates was achieved without obvious detriment to the physical or eating quality of the meat. The effects of the GLAoil on content of 18:3*n*-6+*n*-3 and 20:4*n*-6 were not consistent and showed no improvement over soya-bean oil alone. The EPAnoil resulted in 8·3 (SD 0·88) g 20:5*n*-3/kg total fatty acid in the semitendinosus muscle but none was detected with the other three treatments. The contents of 22:5*n*-3 and 22:6*n*-3 were increased by feeding EPAnoil. It is concluded that with respect to both the standard C18 acids and the less abundant C20 and C22 acids the health image of pig meat can be improved by manipulation of the muscle fat by dietary means.

This experiment was funded by the Beretta Company of Italy.

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Serum lipids and apolipoproteins (Apo-A1, A2 and B) in the Chinese from mainland and Singapore. By N. SAHA and J. S. H. TAY, *Department of Paediatrics, National University of Singapore, 5 Lower Kent Ridge Road, Singapore 0511*

The incidence of, and mortality from, coronary artery disease is very low in the Chinese and Japanese compared to that in the Caucasians and Asiatic Indians (Saha, 1987). Lower levels of serum total cholesterol have been reported in the Japanese, Chinese and Koreans as well as lower Apo B levels (Kesteloot *et al.* 1985; Saha, 1987). However, serum total cholesterol level in the Chinese of Singapore has not been found to be significantly different from that in Asiatic Indians and Caucasians. The present generation of the Singapore population are more affluent and 'Westernized' in their dietary habits. It was therefore thought that a comparative study of serum lipid and apolipoproteins in the Chinese of Singapore and a group of Chinese foreign workers from mainland China would provide some insight whether these differences in serum lipids in the Oriental could be of genetic or environmental origin.

Chinese males (*n* 167) recruited as foreign workers formed the sample from mainland China. They were studied on their arrival during the mandatory medical examination for employment as foreign workers. Singapore Chinese males (*n* 166) of comparable socio-economic background were selected at random from the same source. Serum lipids and apolipoproteins were estimated following the methods of Saha (1987), and LDL-cholesterol from total cholesterol–(HDL-cholesterol+(triacylglycerol/5)).

Anthropometry, serum lipids and apolipoproteins in the Chinese of Singapore and mainland China

	Mainland (*n* 167)		Singapore (*n* 166)		*P* (adjusted for age)
	Mean	SE	Mean	SE	
Age	21·6	0·15	26·1	0·42	<0·001
Height (cm)	171·2	0·41	169·7	1·85	NS
Weight (kg)	58·0	0·50	63·2	1·20	<0·001
Body mass index (BMI) (kg/m^2)	19·8	0·15	22·4	0·62	<0·001
Total cholesterol (mmol/l)	3·55	0·06	4·84	0·07	<0·001
HDL-cholesterol (mmol/l)	0·94	0·02	1·15	0·03	<0·001
Triacylglycerol (mmol/l)	1·26	0·05	1·05	0·04	<0·01
LDL-cholesterol (mmol/l)	2·05	0·06	3·20	0·07	<0·001
Apolipoprotein A1 (mg%)	131·0	4·04	135·8	4·36	NS
Apolipoprotein A2 (mg%)	44·6	1·22	44·1	1·03	NS
Apolipoprotein B (mg%)	71·2	1·89	70·7	1·57	NS

The higher serum triacylglycerol in the mainland Chinese may be due to their higher intake of dietary carbohydrates. There were no differences in serum apolipoprotein levels. Multiple regression analyses show higher age, and age–BMI-related rises in serum total cholesterol, LDL-cholesterol and triacylglycerol levels in the Chinese of Singapore, suggesting that the differences are due to nutritional differences.

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Which foods best discriminate between individuals with low-fat and high-fat diets? By S. A. GIBSON, D. A. ROSE and R. C. COTTRELL, *Leatherhead Food Research Association, Randalls Road, Leatherhead, Surrey KT22 7RY*

Dietary advice designed to reduce fat intake to under 35% of food energy normally focuses on foods such as meat, fats and dairy products, which contribute most fat to the national 'average' diet. Advice better targeted to those with high-fat diets would be more efficient, but is hampered by the lack of simple-but-reliable means of identifying such individuals and the dietary habits associated with their high-fat energy intake.

In this project the weighed 7-d diet records from the survey of British schoolchildren (Department of Health, 1989) were used to evaluate several statistical methodologies for the interpretation of multidimensional dietary data. The aim of the project was to identify whether individuals with high-fat diets (e.g. over 40% fat energy) have dietary habits which allow them to be characterized and differentiated from individuals with low-fat diets (<35% fat energy). Principal components analysis, used to identify dietary behaviours in other recent studies (Barker *et al.* 1990; Gregory *et al.* 1990), identified a number of patterns in each dataset. Only one component among the schoolchildren, associated with avoidance of breakfast cereals, was positively correlated with fat energy. Canonical discriminant analysis, to our knowledge not used previously for these types of dietary data, produced a classification into three fat energy groups from intake data on ten foods out of the thirty-eight in the study, the ranking of which differed slightly between the populations.

Top ten foods in the discriminant analysis (in decreasing order of importance)

Schoolgirls		Schoolboys	
+	−	+	−
Butter		Butter	
	Bread		Bread
Margarine		Margarine	
	Sugar		Sugar
	Soft drinks	Meat	
	Breakfast cereals	Eggs	
Meat			
			Breakfast cereals
Eggs			Soft drinks
Crisps		Crisps	
Cheese		Cheese	

We conclude that; 1, canonical discriminant analysis is a valid tool for identifying fat energy intake above 40% or below 35% with approximately 65% accuracy and less than 5% misclassification of extremes; 2, negative predictors are as important as positive predictor foods in assessing fat energy; 3, foods such as milk and chips, which contribute significantly to the (child) population intake of fat, do not necessarily discriminate well between high- and low-fat consumers.

The support of MAFF in funding the project is gratefully acknowledged.

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Nutritional status of subjects with middle-aged onset Parkinson's disease. By R. A. ABBOTT[1], H. MARCUS[2], M. COX[1], M. HODKINSON[3] and A. TOMKINS[1], [1]*Nutrition Research Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1,* [2]*Department of Neurology, Middlesex Hospital, London W1* and [3]*Department of Geriatric Medicine, St Pancras Hospital, 4 St Pancras Way, London NW1*

Parkinson's disease (PD) is a disabling neurological disorder that is characterized by tremor, bradykinesia, rigidity and disturbances of posture and balance. Although it is often noted that patients with PD tend to be thin, only two studies have reported this in the literature (Vardi *et al.* 1976; Yapa *et al.* 1989). It has, however, been shown that some elderly-onset patients with PD have increased energy expenditure at rest (Levi *et al.* 1990). This could contribute to the thinness observed. The present study investigated dietary intake and nutritional status in a group of subjects with middle-aged onset PD. Particular attention was given to assessment of protein intake as many patients are advised to reduce the protein content of the diet in order to maximize the efficacy of their drug treatment.

Forty-five patients (nineteen female, twenty-six male) ranging from 44–83 (mean 62) years were nutritionally assessed using anthropometry and dietary questionnaires. Weight loss occurring since the onset of PD was seen in 58% of the patients and of these, 38% had a self-reported loss >12·7 kg (2 stone). A body mass index (BMI) of <20 was seen in 29% of the patients. The range of both weight loss and BMI are shown in the Table.

Weight loss (kg)	None	0–6·4	6·5–12·7	12·8–19·1	>19·1
% of patients	42	15·5	20	15·5	7
BMI (weight/height^2)		<18·0	18·0–20·0	20·1–25·0	≥25·1
% of patients		9	20	51	20

Various factors affecting dietary intake were assessed. Of the patients who were previously able to shop for food, 50% were prevented from doing so as a result of their PD and, of those who could previously cook, 53% were no longer able to. Advice to follow a protein-restricted diet was given to 40% of the patients but compensatory dietary advice was not given. In fact only 44% of those who followed the advice found the diet to be of any benefit to their neurological state. Other factors influencing dietary intake were: difficulty in swallowing (44%), loss of taste or smell, or both, (38%), chewing difficulties (36%), loss of appetite (24%) and nausea (22%). Vitamin or mineral supplements without any specific dietary instruction were taken by 31% of the patients and 13% (all of whom had a BMI <20) took daily high-energy–protein drinks.

This study shows that PD patients are subject to considerable weight loss. The combination of increased energy expenditure, decreased ability to purchase and prepare food, advice to restrict protein intake and difficulty in eating is a serious problem for these patients. It is suggested that more careful, specific attention to these aspects of nutrition should be incorporated into the management of patients with PD.

We acknowledge the support of the Parkinson's Disease Society and the assistance of the medical staff at the Neurology Department of the Middlesex Hospital.

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The incidence and cause of weight loss in Parkinson's disease. An anthropometric study of eighty-five patients. By H. S. MARKUS[1], A. TOMKINS[2] and G. M. STERN[1], [1]*Department of Neurology, Middlesex Hospital, Mortimer Street, London W1N 8AA* and [2]*Clinical Nutrition Unit, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

Weight loss appears to be a common feature of Parkinson's disease but its incidence and cause have been little studied. Contributing factors might include a reduced energy intake due to social isolation, chewing or swallowing difficulties or impaired gastrointestinal function, or an increased energy expenditure due to involuntary movements (tremor and dyskinesias) or increased muscle tone during 'off' periods. Anthropometric indices (weight, height, triceps and biceps skinfold thickness, and mid-arm circumference (MAC)) were recorded in eighty-five individuals (fifty-one male, thirty-four female) with L-Dopa-responsive Parkinson's disease. Body mass index (BMI), mean skinfold thickness (MSFT) and MAC were correlated with the following clinical features: duration of disease, clinical disease stage (Hoehn and Yahr, 1967), living alone, chewing and swallowing difficulties, tremor grade (1–4) and dyskinesia grade (1–4), and presence of prolonged 'freezing'.

Mean values (SD) in all patients, and those with early (<5 years) and late (>15 years) disease are documentated in the Table.

	All	Early	Late
Male	*n* 51	*n* 18	*n* 11
BMI (kg/m^2)	23·0 (3·3)	25·1 (2·9)	21·3 (2·7)
MSFT (cm)	8·1 (2·6)	9·8 (2·0)	8·0 (2·1)
MAC (cm)	27·9 (2·5)	29·1 (2·7)	23·4 (2·1)
Female	*n* 34	*n* 16	*n* 8
BMI (kg/m^2)	22·5 (4·6)	24·4 (5·1)	19·2 (2·7)
MSFT (cm)	12·4 (5·2)	15·1 (5·2)	8·4 (3·4)
MAC (cm)	26·2 (3·6)	28·1 (3·5)	22·9 (2·8)

Within the male group a significant ($P<0·01$) negative correlation was found between all three anthropometric indices and dyskinesia grade. BMI and MSFT were significantly related to Hoehn and Yahr stage, duration of disease, episodes of prolonged freezing, and chewing difficulties. In contrast, no relationship was found between derived arm muscle circumference and these clinical variables, suggesting that the weight loss was predominantly body fat rather than lean body mass. No correlation was found with living alone, swallowing difficulties and tremor grade. Dyskinesia grade, Hoehn and Yahr stage and disease duration were interrelated variables but the negative correlation between anthropometric variables and dyskinesia grade was strongest. Multiple regression analysis revealed that BMI and MSFT were only independently related to dyskinesia grade ($P<0·05$). Within the female group similar correlations were found except that no correlation was found with episodes of prolonged freezing.

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Acid-base status of pre-menopausal vegetarian and omnivorous women. By D. BALL, J. D. ROBERTSON and R. J. MAUGHAN, *Environmental and Occupational Medicine, University Medical School, Aberdeen AB9 2ZD*

Short-term extreme variations in diet composition can induce changes in blood acid-base status (Greenhaff *et al.* 1987). We have examined two populations with different chronic dietary habits to examine whether a difference in their acid-base status exists. Healthy females (*n* 33) volunteered for the present experiment which was approved by the local ethics committee. Twenty of these subjects ate meat on a regular basis; the remaining thirteen subjects were designated as vegetarians, although two occasionally ate fish. All subjects completed a 7-d weighed food intake which was analysed using a computer-based system of food composition tables (Paul & Southgate, 1978). On two separate occasions during this week, subjects reported to the laboratory following an overnight fast, and arterialized–venous blood samples were obtained for the determination of acid-base status. A 24 h urine collection was completed by each subject and later analysed for pH and total acid. Each subject's height, weight and % body fat were measured. Statistical analysis was by Student's *t* test for paired and unpaired data; significance was declared at the $P<0{\cdot}05$ level.

		Dietary intake				Blood acid-base			Urinary acid-base	
		Energy (MJ)	Protein (g)	Fat (g)	CHO (g)	pH	HCO_3^- (mM)	BE (mM)	pH	Total acid (meq/d)
Omnivore	Mean	9·0*	69·9*	88·3*	278·8*	7·41*	19·7	−3·8	6·26	48·9*
	SD	1·5	8·8	18·0	69·1	0·02	1·3	1·2	0·50	20·3
Vegetarian	Mean	7·1	55·1	65·4	227·6	7·40	19·9	−3·8	6·45	35·3
	SD	1·1	10·0	22·2	26·1	0·01	1·3	1·1	0·56	23·3

Daily dietary intake and acid-base status of the two groups: * denotes a significant difference between the means.

Although there was no difference in body-weight and % body fat between the two groups, significant differences did exist in dietary intake. Blood acid-base status was not substantially different. The greater dietary protein intake of the omnivores resulted in an increased excretion of inorganic acids, as reflected in the increased total urinary acid output.

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Evaluating dietary surveys against energy requirements. A. E. BLACK, G. R. GOLDBERG, S. A. JEBB and A. M. PRENTICE, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

Dietary surveys may underestimate 'habitual' food intake. This conclusion rests on principles of energy physiology. FAO/WHO/UNU (1985) expressed energy requirements as multiples of basal metabolic rate (BMR). That for a sedentary lifestyle is given as 1·55 × BMR. Since most dietary surveys also measure height and weight, mean BMR can be calculated from equations (Schofield, 1985) and related to the reported mean energy intake (EI/BMR).

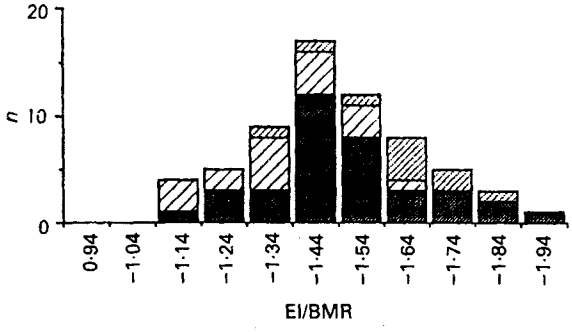

Fig. ▨, Diet history; ▨, diet recall; ▩, diet records. EI/BMR in published diet surveys of adults.

The distribution of EI/BMR in sixty-eight subgroups from thirty-seven studies of adults in nine countries of Europe and America, including sixteen major UK studies since 1979, fourteen European studies and six of the best known US epidemiological studies (Black *et al.* 1992), ranging from *n* 10 to *n* 10 000 and including different survey methods, is shown. In only seventeen groups does EI/BMR reach or exceed 1·55. It is improbable that all intakes below 1·55 × BMR are valid measures of 'habitual' intake, even for very sedentary lifestyles. Intakes above 1·55 × BMR may be underestimates of intakes of physically-active groups.

Dietary records, weighed or in household measures, give varied values for EI/BMR; recall method tends to give lower and diet history higher values. EI/BMR is higher for men (mean 1·49 (SD 0·17)) than women (mean 1·32 (SD 0·17)) indicating either better compliance or higher activity levels.

Evidence to indicate whether bias to underestimation is an observer effect operating equally on all subjects, or from identifiable subgroups, or to a continuum of good-to-bad records is scanty. At present data have to be taken at face value while maintaining a critical awareness of possible misinterpretations.

Practical steps for evaluating future studies could include: estimation of individual EI/BMR and study of means for groups and subgroups; questions on occupational and leisure activity; questions to identify weight conscious individuals; and inclusion of external validation procedures in protocol when submitting applications for funds (Black *et al.* 1992; Goldberg *et al.* 1992).

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The effects of potassium deficiency on energy balance in the growing rat. By R. HAILWOOD, S. A. WOOTTON and A. A. JACKSON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

The exclusion of potassium from an otherwise adequate diet inhibits growth in young rats (Dorup & Clausen, 1989) and results in abnormalities of protein metabolism (Gustafson *et al.* 1973). It has been suggested that diets deficient in minerals may result in increased energy expenditure and decreased efficiency of utilization of energy (Kleiber, 1945). The effect of a K-deficient diet on energy utilization has been studied in rats. Male Wistar rats, 80–100 g body-weight, were randomly assigned to four groups and housed individually. Time 0 rats were killed at the start of the study as a reference. K-adequate rats (K-AD) were given a prepared diet containing potassium chloride, 62·7 mmol K/kg diet. Deficient animals (K-DEF) were given the same diet *ad lib.*, with K excluded from the mineral mixture. A group of rats were pair-fed (K-AD-PF) the adequate diet to the intake of the K-deficient animals. For 14 d, body-weight and food intake were measured daily. The energy content of the diet and the dried carcass was determined by ballistic bomb calorimetry. Energy expenditure was derived as the difference between gross energy intake and carcass energy gain.

Group	Weight (g)	T-E-I (MJ)	Carc-E-gain (MJ)	TEE (MJ)	EE (MJ/100 g)	E density (kJ/g)
K-AD	186[a]	4·45[a]	0·52[a]	3·93[a]	2·12[a,b]	7·4[a]
SE	5	0·07	0·04	0·05	0·04	0·5
K-DEF	131[b]	3·13[b]	0·20[b]	2·94[b]	2·24[a]	10·3[a]
SE	4	0·10	0·03	0·07	0·03	1·3
K-AD-PF	145[c]	3·20[b]	0·37[c]	2·83[b]	1·95[b]	11·4[a]
SE	4	0·12	0·05	0·12	0·05	1·5

T-E-I, total energy intake; Carc-E-gain, carcass energy gain; TEE, total energy expenditure; EE, energy expenditure; E density, energy density.

ANOVA values with the same superscript are not different, $P<0·01$.

The rats on the K-DEF diet had a reduction in food intake and gained significantly less weight than the K-AD group. However, the changes in body-weight could not simply be accounted for by the reduced intake, as the K-AD-PF group gained weight at a significantly greater rate than the K-DEF animals. The energy expenditure per 100 g body-weight was not different between the K-AD and K-AD-PF groups, but was significantly increased in the K-DEF group, by about 15%. There was no significant difference in the carcass energy retained per g of weight gain, and therefore the composition of the tissue gained was similar amongst the different groups. Specific nutrient deficiencies can exert an important effect upon energy requirements.

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Effect of porcine somatotropin and insulin on nutrient uptake by the hindlimb of growing pigs. By DIANE WRAY-CAHEN, ALAN W. BELL, R. DEAN BOYD and DALE E. BAUMAN, *Department of Animal Science, Cornell University, Ithaca, NY, USA*

Exogenous porcine somatotropin (pST) greatly reduces the fat accretion and increases protein accretion rates in pigs. This shift in nutrient partitioning is accompanied by a decrease in insulin sensitivity for whole-body glucose uptake (Wray-Cahen *et al.* 1990); the relative contribution of metabolic changes in various tissues is unknown. Our objective was to quantify net uptake of glucose, lactate, non-esterified fatty acids (NEFA) and oxygen by the hindlimb under normal and hyperinsulinaemic (euglycaemic) conditions and to examine the impact of pST on these variables.

Eight pigs (55 (SD 6) kg) received either recombinant pST (120 μg/kg) or excipient (control) for 7 d. Blood flow (via dye dilution) and arterio–venous concentration differences of metabolites were measured immediately before initiation of treatment (day 0) and on day 7 of treatment. The catheterized external iliac arteries and veins served a tissue bed that was approximately 66% muscle and 8% fat. Net uptakes were measured under basal conditions and during jugular insulin infusions at low (physiological, 14 ng/kg per min) and high (maximal, 360 ng/kg per min) rates. During insulin infusion, arterial blood glucose was monitored every 5 min and jugular glucose infusion rate (GIR) was adjusted to maintain euglycaemia. GIR provides an estimate of whole-body glucose uptake in response to exogenous insulin.

Day 7 of treatment	Insulin (ng/ml)		GIR (mg/kg per min)		Hindlimb glucose:O_2 quotient		Net hindlimb uptake: Glucose (μmol/min)		Net hindlimb uptake: NEFA (μmol/min)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Basal										
Control	0·48	0·12	–	–	1·10	0·20	155	35	5·97	0·44
pST	1·34*	0·24	–	–	0·45*	0·18	98	51	−2·15†	4·66
Low insulin										
Control	0·93	0·09	7·1	0·9	1·76	0·16	290	50	6·66	1·25
pST	1·22*	0·06	1·5*	0·1	0·58*	0·31	120*	70	−2·07*	3·02
High insulin										
Control	15·56	2·34	24·1	1·7	3·31	0·24	590	68	5·33	0·88
pST	16·39	1·06	21·5	2·0	3·82	0·37	737	118	5·27	1·03

Significantly different from control: †$P<0·10$, *$P<0·05$.

The pST-induced alterations in hindlimb and whole-body uptake of nutrients were only evident at physiological levels of insulin (basal and low insulin). The decrease in hindlimb glucose uptake was entirely accounted for by the estimated reduction in glucose utilization by adipose tissue, calculated from the in vivo data of Dunshea *et al.* (1989) and present values for hindlimb tissue composition. This is consistent with the theory that pST directs nutrients away from adipose and towards lean tissue growth, by altering the response of tissues to homeostatic signals.

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5-Oxo-L-proline and orotic acid excretion during normal pregnancy. By C. PERSAUD and A. A. JACKSON, *Department of Human Nutrition* and G. WERKMEISTER, *Department of Human Reproduction, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

In the past we have used an increased urinary excretion of 5-oxo-L-proline as an index of glycine insufficiency. We have found 5-oxo-L-prolinuria in situations where the metabolic demand for glycine is high, viz during pregnancy (Persaud *et al.* 1987). Van der Werf *et al.* (1975) have shown in mice that L-dihydro-orotic acid can competitively inhibit the enzyme 5-oxo-L-prolinase. On this basis, Ribes *et al.* (1987) have speculated that accumulated orotic acid or its precursor, L-dihydro-orotic acid, could be the reason for a mild 5-oxo-L-prolinuria. Increased urinary excretion of orotic acid occurs in pregnancy and we have looked for an association between 5-oxo-L-proline and orotic acid excretion in pregnant women to see whether the increased excretion of orotic acid could account for the 5-oxo-L-prolinuria.

Urine samples (24 h) were collected from seventy-five pregnant women attending the antenatal clinic and compared with a control group of twelve non-pregnant women. 5-Oxo-L-proline was isolated by column chromatography and measured enzymically after acid hydrolysis to glutamic acid. Orotic acid was measured colorimetrically after interfering substances had been removed with a cation-exchange resin.

	Orotic acid (μmol/d)		5-Oxoproline (μmol/d)	
	Median	Range	Median	Range
Non-pregnant	4·3	2–4·9	137	29–477
Pregnant	6·1	1–26	910	40–2312
Wilcoxon rank	NS		$P<0·0001$	

NS, not significant.

There was no increase in the excretion of 5-oxo-L-proline or orotic acid as pregnancy progressed. However, there was increased urinary orotic acid in forty-six of seventy-five pregnant women and an increase in urinary 5-oxo-L-proline in fifty-nine of seventy-five (beyond the normal range). Regression of urinary 5-oxo-L-proline on orotic acid for all the pregnant women did not show a significant relationship (r 0·18, P value 0·124), nor when only the women with raised orotic acid were considered (r 0·24, P value 0·112). Therefore, at most, about 5% of the variability in 5-oxo-L-proline excretion could be accounted for by changes in orotic acid excretion.

Changes in renal function during pregnancy result in a generalized amino aciduria. The increased excretion of 5-oxo-L-proline is greater than can be accounted for by this mechanism (Persaud *et al.* 1987). Although increased orotic acid excretion may contribute to 5-oxo-L-prolinuria, an increased orotic acid level of itself is insufficient to account for the magnitude of the 5-oxoprolinuria seen in normal pregnant women.

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Oestrogen production in human adipose tissue in vivo. By K. N. FRAYN, D. U. HUDSON, K. L. BOULTON and S. W. COPPACK, *Sheikh Rashid Diabetes Unit, Radcliffe Infirmary, Oxford OX2 6HE*

Obesity and anorexia are both associated with disturbances of steroid sex hormone concentrations. Obese men, for instance, have elevated oestrogen:androgen ratios. This may reflect the fact that adipose tissue is an important site for the peripheral interconversion of steroid hormones. Both the cytochrome-P450-dependent aromatase (which converts androgens to oestrogens) and the 17-β hydroxysteroid dehydrogenase (*EC* 1.1.1.51) (which interconverts androstenedione and testosterone, or oestrone and oestradiol) are present in adipose tissue. Information on the occurrence of these processes in vivo is sparse. We have therefore measured arterio–venous differences for some steroid sex hormones across subcutaneous abdominal adipose tissue.

Seven men and eight women were studied, in various nutritional states and at various times of the menstrual cycle. The groups were heterogeneous and included two post-menopausal females and one with polycystic ovary syndrome, and one subject with insulin-dependent diabetes mellitus. Blood samples were withdrawn from a vein draining the subcutaneous abdominal adipose tissue, and from a vein draining a warmed hand ('arterialized') or from a radial artery. The cannulation techniques have been described previously (Frayn *et al.* 1989). Total plasma concentrations of testosterone, androstenedione, oestrone and oestradiol were measured by radioimmunoassay after extraction with diethyl ether. Within-batch coefficients of variation were: testosterone, males 3·3%, females 5·7%; androstenedione, males+females 4·7%; oestrone, males+females 3·2%; oestradial, males+females 6·0%.

Despite the heterogeneity of the groups, some very consistent findings emerged. Almost all subjects showed increases in oestrone and oestradiol concentrations across the adipose tissue (mean (SEM) arterio–venous differences −8 (2) and −22 (7) pmol/l respectively; $P<0·01$ for each hormone by paired t test). In all males but one, testosterone concentration decreased during passage through adipose tissue (arterio–venous difference +0·8 (SEM 0·2) nmol/l, $P<0·02$); the arterio–venous difference for testosterone was positively related to the arterial concentration (r 0·70, P0·05). In all females but one (in whom there was no change) the testosterone concentration increased (arterio–venous difference −0·4 (SEM 0·1) nmol/l, $P<0·05$). The exchange of androstenedione was less consistent. The arterialized plasma oestrone concentration was positively correlated with body mass index amongst the males (r 0·85, P0·01), despite a relatively narrow range of adiposity (19·3–26·9 kg/m^2).

We conclude that subcutaneous adipose tissue is an important site for interconversion of steroid hormones, in particular the production of oestrogens. Measurements of arterio–venous differences (together with blood flow) will provide a useful technique for investigation of disorders of sex hormone concentrations in obesity and other clinical states.

We thank Colin Selby of the City Hospital, Nottingham, for help in setting up the radioimmunoassays.

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Development of bio-markers for bone turnover: the excretion of pyridinium cross-links in pre-school children. By F. BRANCA[1], A. DUNCAN[2], C. CASEY[3], A. FERRO-LUZZI[1] and S. P. ROBINS[2], [1]*Unità di Nutrizione Umana, Istituto Nazionale della Nutrizione*, [2]*Skeletal Metabolism Research Unit, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and [3]*Department of Medicine and Therapeutics, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD*

We are currently investigating the urinary excretion of pyridinium cross-links, that are primarily derived from the degradation of mature collagen in bone and cartilage, as an indicator of bone turnover. Excretion is greater in children and in patients affected by metabolic bone diseases than in normal adults (Robins *et al.* 1991). A pilot study was designed to quantify different components of within- and between-subject variability. Urine collections were obtained from twelve normal healthy children aged 3–5 years, to analyse diurnal and weekly rhythms of excretion, and to assess the rate of excretion at different ages. Pyridinoline (PYD) and deoxypyridinoline (DPD) were assayed by high performance liquid chromatography (Black *et al.* 1988). The mean rates of excretion of PYD and DPD calculated from 24 h timed collections were, respectively, 0·22 (SD 0·03) and 0·06 (SD 0·02) nmol/min. Excretion rates were lower in the afternoon (PYD, 0·14–0·19 nmol/min; DPD, 0·04–0·05 nmol/min) than in the morning and night (PYD, 0·22–0·24 nmol/min; DPD, 0·06–0·08 nmol/min). However, only for DPD were the differences statistically significant. Within-subject variation was 20% on within-day and 30% on between-day spot samples. Between-day variation was reduced to 20% where timed overnight collections were used. In spite of the large intra-individual variance and the small number of subjects, an age-related significant difference in excretion of PYD could be revealed (Table).

Urinary excretion of pyridinium cross-links at different ages

	PYD (nmol/mmol creatinine)		DPD (nmol/mmol creatinine)	
Age	Mean	SD	Mean	SD
3 years (*n* 4)	168	8	49	8
4 years (*n* 4)	144	23	42	5
5 years (*n* 4)	128	13	40	8
	$P<0·05$		NS	

NS, not significant.

These preliminary results indicate that, given this high variability, in order to point out the correlation between cross-link excretion and growth rates, three repeated collections would be needed to reduce the error term of the correlation coefficient to below 10% (Liu *et al.* 1978). Careful age stratification should also be carried out.

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Total body water measurement in very low birth weight infants using bioelectrical impedance. By D. C. WILSON[1], T. BAIRD[2], C. M. SCRIMGEOUR[2], G. MCCLURE[1], H. L. HALLIDAY[1], M. MCC. REID[1] and M. J. RENNIE[2], [1]*Neonatal Unit, Royal Maternity Hospital and Department of Child Health, The Queen's University of Belfast, Grosvenor Road, Belfast BT12 6BB* and [2]*Department of Anatomy and Physiology, The University of Dundee, Dundee DD1 4HN*

Analysis of body composition in very low birth weight (VLBW; <1·5 kg) infants is extremely difficult. Methods such as skinfold thickness measurement are unreliable, whereas methods such as densitometry, total body electrical conductivity or magnetic resonance imaging are not practical. Bioelectrical impedance has recently been shown to be a valid, quick, safe and non-invasive means of body composition analysis in children and adolescents (Davies *et al.* 1988). The use of bioelectrical impedance in infancy has not been reported.

We have undertaken a study of the value of bioelectrical impedance to predict total body water (TBW) in VLBW infants, using dilution of the stable isotope $H_2{}^{18}O$ as a standard. TBW was calculated by the method of Schoeller *et al.* (1980). Results from seventeen studies in eight babies have been obtained. Babies were born at a mean (SD) gestational age of 24·9 (1·2) weeks, with a mean birth weight of 760 (64) g. Median (range) age at study was 44 (18–76) d, and median (range) weight at study was 1092 (661–2116) g. Bioelectrical impedance measurements were made using the Holtain body composition analyser (Holtain Ltd). Electrodes were placed on the hand and foot, and voltage drop was measured on a further set of electrodes placed 3 cm proximally on the forearm and lower leg. TBW was derived from impedance (I) using the association of impedance with length (L^2/I). TBW measured by isotope dilution was highly correlated (r 0·96; $P<0·0001$) with L^2/I. The regression equation of the relationship was TBW = 0·55 $L^2/I+0·094$. The possibility was studied that TBW measured by back extrapolation of the regression line of ^{18}O concentration against time gives a truer result than the plateau method, and that impedance pathway rather than length may be a better measurement in the prediction equation. The best agreement was given by the plateau method and length respectively.

Our results indicate that bioelectrical impedance is a valid, inexpensive, portable, rapid and non-invasive means of body composition analysis in VLBW infants. Furthermore, it can be used in VLBW infants requiring mechanical ventilation for respiratory disorders.

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Ultrafiltered cow's milk as a milk substitute for rat pups. By J. L. SMART, R. F. MASSEY and A. C. MCMAHON, *Department of Child Health, University of Manchester Medical School, Manchester M13 9PT* and D. G. DALGLEISH, *Hannah Research Institute, Ayr KA6 5HL*

Finding a satisfactory milk substitute (RMS) on which to rear rat pups artificially has proved troublesome and the best available RMS is complicated to produce (Auestad *et al.* 1989). The method outlined below is simple and produces a RMS similar to rat's milk in composition and which gives acceptable survival and growth.

Pasteurized cow's milk is concentrated by ultrafiltration: this can be achieved on a scale of several litres by using a hollow fibre membrane cartridge (UFP-500-D-6, AG Technology, Needham, MA, USA), or on a laboratory scale using an Amicon TCF10A cell and PM30 membrane. The casein, fat and colloidal material are concentrated by a factor of 3 by removing 0·67 volumes of permeate from 1 volume of milk, although some serum proteins are lost. Water-soluble vitamins are added to the retentate: 308 mg/l Solvito (Kabivitrum Ltd., Uxbridge, UK). The calculated composition of the resulting RMS is (g/l): casein 74·4, whey protein 18·6, total protein 93·0, lactose 42·9, fat 144·0, which compares favourably with rat's milk (Auestad *et al.* 1989). The RMS has excellent physical properties for delivery down the fine tubes used in artificial rearing, being fluid and non-viscous. An advantage of this material is that the casein micelles and fat globules of the original milk remain intact, so that the formation of a normal coagulum during digestion is likely to be facilitated. Other RMSs contain particles whose structures have been grossly altered, and which will form abnormal coagula, which may affect digestion deleteriously.

A variation of this method is to ultrafilter skimmed cow's milk and to add a mixture of oils designed to produce a similar fatty acid profile to rat's milk (Auestad *et al.* 1989) plus water- and oil-soluble vitamins, and to homogenize the milk at high pressure to prevent separation of the oil. Such preparations were acceptable, but the need for high-pressure homogenization may limit their applicability.

Rat pups have been artificially reared from 5–20 d on RMSs produced by ultrafiltration of whole or skimmed cow's milk (with additions, as above). Survival was as good as, or better than, for other milk substitutes that we have used (Smart *et al.* 1984). Growth in body-weight compared with mother-reared pups was satisfactory. After a slight initial lag (often encountered in artificial rearing), growth to 20 d was good such that by then there was no significant difference in body-weight between artificially-reared (AR) and mother-reared (MR) rats (mean (SD): AR, 40·25 (3·23) and 43·21 (2·93); MR, 38·74 (3·23) and 42·12 (3·42) g, in two experiments with a total of twenty AR and twenty-three MR rats). There was no significant difference in brain weight at 20 d: AR 1·295 (0·055) and MR 1·323 (0·046) g. The timing of eye-opening was normal.

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Energy cost of egg formation in quail. By S. WARD, *Department of Molecular and Biological Sciences, University of Stirling, Stirling FK9 4LA* and M. G. MACLEOD, *AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS*

Little information is available on the additional energy requirement for egg production in birds. In this study the daily energy expenditure (DEE) of 7-week-old female Japanese quail was measured using indirect calorimetry (MacLeod *et al.* 1985). At this age individuals showed a range of egg production from zero to daily laying.

Birds were placed singly in calorimetric chambers for 1 d of training followed by 3 d on experiment. Food and water were provided *ad lib.* The diameter of each ovarian follicle was marked at the start of each experimental day by feeding a gelatin capsule of lipophilic dye (Sudan B) which stained a distinct band in follicles undergoing the rapid growth phase (Gilbert, 1972). The yolks of those eggs laid and the follicles still within the ovary were examined at the end of the experiment. The increase in volume of each yolk was used to calculate the total energy content of yolk formed. The energy content of any albumen formed was added to that of the yolk, to calculate mean daily energy content of egg formed.

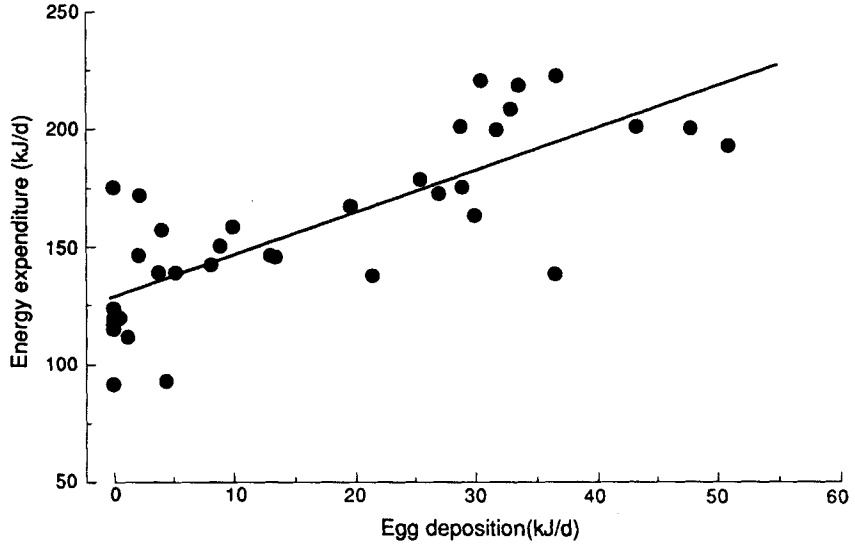

Fig. 1. Increase in daily energy expenditure of quail with daily energy content of egg deposited ($y=1{\cdot}75x + 129$, r^2 0·59, $P<0{\cdot}0001$, n 35 birds).

These measurements showed that females which laid daily had 49% greater DEE than birds which had not begun to lay, and that each unit of energy deposited in the eggs required 1·75 additional units of DEE. The birds in this experiment deposited only 36% of their additional energy output (additional DEE+energy deposited in the egg) as egg, about half of the efficiency usually quoted for the domestic fowl.

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Urea kinetics in free-living adults measured with a single-dose method. By I. MCCLELLAND, M. DANIELSEN and A. A. JACKSON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

The measurement of urea kinetics may be used to characterize the adequacy of dietary protein, in terms of quality and quantity. However, the methods available for measuring urea kinetics require a steady metabolic state and are not suitable for application to free-living subjects. Therefore, we have developed a simple approach based upon single-dose methodology suitable for application in the field.

Six normal men in whom urea kinetics had been measured with a prime and intermittent oral doses of $^{15}N^{15}N$-urea whilst taking 70 g protein/d (Danielsen & Jackson, 1992), repeated the study. A single dose of $^{15}N^{15}N$-urea, 100 mg, was taken in the morning and all urine was collected for the next 48 h. The amount of $^{15}N^{15}N$-urea in urine was measured and used to calculate the rate of urea production and salvaging. The same method was used in six women to measure urea kinetics whilst they were on their habitual intake of protein on sixteen separate occasions.

	mg N/kg per d				Production / Intake %	Excretion / Production %
	Intake	Production	Excretion	Salvage		
Men						
Inter dose	165	199	128	71	121	65
SD		20	18	20		
Single dose	165	186	146	40	113	79
SD		37	37	18		
Women						
Single dose	215	238	147	92	126	70
SD	65	67	36	46	23	13

These data demonstrate that the single-dose method for measuring urea kinetics gives similar results to those obtained with the prime and intermittent doses under controlled conditions in men. When the single-dose method is used to derive urea kinetics in women taking their habitual diet under free-living circumstances, the relative pattern of changes is similar to that found under controlled conditions. We conclude that the single-dose method can be used for measuring urea kinetics non-invasively in free-living individuals.

Danielsen, M. & Jackson, A. A. (1992). *Proceedings of the Nutrition Society* (In the Press).

Limits of adaptation to a low-protein diet in normal adults: urea kinetics. By M. DANIELSEN and A. A. JACKSON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

We have shown that normal adults can maintain nitrogen balance on a diet containing 35 g protein/d through the enhanced salvaging of urea-N in the lower bowel (Langran *et al.* 1990). In the present study the response to an intake of 30 g protein/d has been measured.

Six healthy young men were studied on two occasions while receiving a mixed diet which provided about 150 kJ/kg per d and either 70 or 30 g protein/d. The diets were given for 5 d and during the last 24 h urea kinetics were measured using a prime and oral intermittent doses of $^{15}N,^{15}N$-urea (Jackson *et al.* 1984). On 70 g protein/d urinary urea was 8·46 (SD 2·2) mg N/d on the first day and 7·89 (SD 0·82) mg N/d on the final day of the study, compared with 9·88 (SD 2·8) and 4·47 (SD 0·81) mg N/d respectively on 30 g protein/d. On 30 g protein/d the subjects had stable weight, but were not in N balance during the measurement of urea kinetics.

	mg N/kg per d								
		Production		Excretion		Salvaged		Production / Intake %	Excretion / Production %
	Intake	Mean	SD	Mean	SD	Mean	SD		
Study 1	165	190	20	128	18	71	20	121	65
Study 2	68	123	30	67	8	56	24	181	56
ANOVA, *P*		<0·001		<0·001		NS		<0·01	NS

NS, not significant.

There was a statistically-significant decrease in the rate of urea production and excretion on 30 g protein/d, with little change in the salvage of urea-N between the two diets. Relative to the dietary intake, urea production was enhanced on the 30 g protein/d diet, but not to the same extent as had been seen on 35 g protein/d.

These data show that whereas on 35 g protein/d N balance can be maintained by the enhanced salvaging of urea-N, when the intake is reduced to 30 g/d the system is no longer able to accommodate to maintain N balance. On 35 g protein/d the urea-N salvaged was 132 mg N/kg per d with 66 mg N/kg per d going to excretion (Langran *et al.* 1990). On 30 g protein/d the urea salvaging was reduced to almost one-third with urinary excretion being similar.

We conclude that the salvaging of urea-N is critical to the mechanism whereby the body accommodates to a low protein intake, and this system fails at an intake of 30 g protein/d. The fact that N balance on 35 g protein/d requires the effective salvaging of urea-N raises questions of fundamental importance about our perceptions of minimal requirements for protein in man.

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Whole-body protein turnover in healthy young women at different stages of the menstrual cycle. By G. GROVE and A. A. JACKSON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

As few metabolic studies are carried out in women, frequently the results obtained in men are extrapolated uncritically to apply to women. From the studies which have been carried out it is clear that during the reproductive years there are important metabolic changes associated with the normal hormonal cycle. Changes in energy expenditure and balance (Bisdee *et al.* 1988) can be associated with changes in overall nitrogen status (Calloway & Kurzur, 1982). We were interested to know whether it was possible to define changes in protein turnover at different stages of the cycle. In a preliminary study the results had suggested that, around the time of ovulation, protein turnover might be increased in association with increased circulating levels of progesterone, oestradiol, luteinizing hormone and follicle-stimulating hormone, relative to earlier in the cycle. Here we report the preliminary results of measurements of protein turnover in a group of five women carried out 7 and 14 d after the onset of menstruation.

Five healthy women, aged 21–27 years, who were not taking an oral contraceptive pill, were studied on two occasions 3–5 weeks apart. An assessment of each individual's habitual intake was made from a diet history. For the duration of the study the subjects were provided with their habitual intake of energy and about 1·2 g protein/kg per d as sandwiches at 3 h intervals from 06.00 h. Protein turnover was measured by the single-dose-end-product method using ^{15}N-glycine (Fern *et al.* 1984). The bladder was emptied at 09.00 h and a single dose of ^{15}N-glycine (200 mg) was taken orally. Urine was collected for 9 h. The enrichment in urinary ammonia was measured by mass spectrometry. Differences were sought using the paired *t* test.

	Nitrogen flux (mg N/kg per 9 h)		Protein synthesis (mg N/kg per 9 h)		Protein degradation (mg N/kg per 9 h)	
	Mean	CV%	Mean	CV%	Mean	CV%
Day 7	245	9	185	15	137	17
Day 14	317	25	237	35	210	38
P value	0·138		0·097		0·104	

For all measurements there may be greater variability in the results at day 14 than at day 7. There was a trend towards increased values for flux, synthesis and degradation at day 14 compared with day 7, but with relatively large interindividual variation this failed to reach conventional statistical significance.

For the second measurement at day 14, no attempt was made to standardize the timing of the study to coincide with ovulation, and so different subjects might have been at slightly different stages of the cycle. This could account for the wider interindividual variation at this time. The data do show that there is wider intra-individual variation in measures of protein turnover in women in relation to the timing of the menstrual cycle than has been found for repeated studies in men. Clearly, therefore, it is not satisfactory simply to extrapolate findings in men to women. There is the need to define with much greater care the metabolic and nutritional status of women throughout the menstrual cycle.

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Effect of manipulation of the efficiency of nitrogen retention by diet on plasma insulin-like growth factor-1 (IGF-1) concentration. By G. V. KRIEL and M. A. LOMAX, *Department of Biochemistry and Physiology* and M. J. BRYANT, *Department of Agriculture, University of Reading, Whiteknights, Reading RG6 2AJ*

It has been proposed that plasma concentrations of IGF-1 mediate the growth responses to diet (Pell & Bates, 1990). This experiment was performed to establish whether manipulation of the efficiency of dietary nitrogen retention by feeding growing sheep diets differing in fibre content and energy source (MacRea *et al.* 1985), would also alter plasma IGF-1 levels and their response to growth hormone administration.

Thirty-two female crossbred lambs (Suffolk cross Mule, average body-weight 26·4 (SD 2·1) kg) were allocated to four dietary groups with the following composition (% natural basis) and metabolizable energy intakes: diet 1, 80% hay and 20% barley-based concentrate (5·96 MJ/d); diet 2, 70% hay and 30% barley-based concentrate (9·64 MJ/d); diet 3, 95% barley-based concentrate and 5% hay (9·40 MJ/d); diet 4, 95% molassed sugarbeet-based concentrate and 5% hay (10·27 MJ/d). Diets 2, 3 and 4 were fed at a similar N intake (17·6 g/d). After a 3-week period of dietary adaptation blood samples were taken via jugular vein catheters over a 14 h period at hourly intervals. Animals were then given an intravenous injection of pituitary oGH (0·1 mg/kg) and 11 h after the GH injection sampling was resumed at hourly intervals for a further 25 h. Plasma was prepared for the analysis of glucose, insulin, growth hormone and IGF-1. N balances were then performed on all animals.

Treatment . . .	Diet 1		Diet 2		Diet 3		Diet 4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
N retained (g/d per $W^{0·75}$)	0·46[a]	0·07	0·80[b,c]	0·09	0·91[c]	0·05	0·60[a,b]	0·09
IGF-1 (ng/ml)								
Before oGH	198[c]	16·7	247[d]	24·0	295[e]	14·6	246[d]	32·4
After oGH	181[c]	15·7	292[e]	24·5	394	14·2	300[e]	28·4

Numbers in the same line with different superscripts differ significantly by Student's *t* test ($P<0·05$).

The Table shows that N retention was lowest for animals fed near-to-maintenance energy requirements (diet 1), and was highest for animals fed the 95% barley concentrate (diet 3) with diets 2 and 4 intermediate between these extremes. Plasma IGF-1 concentrations, both before and after oGH administration, followed a similar pattern to N retention and are significantly correlated to N retention [IGF-1 = 170·88 + 9·23 × N retention; r 0·72, n 31, $P<0·001$].

It is concluded that the effects of the different diets on N retention are associated with changes in plasma IGF-1 concentration and support the concept that growth responses to nutrition may be linked to the endocrine action of IGF-1.

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Interaction between endotoxaemia and low protein intake on rat skeletal muscle contractile characteristics. By L. B. LEVY and S. A. WOOTTON, *Department of Human Nutrition, Southampton University, Bassett Crescent East, Southampton SO9 3TU*

Malnutrition is often associated with disease and infection, yet the implications that this may have for the functional capacity of skeletal muscle has not been studied. Endotoxin alone does not result in impaired rat skeletal muscle function (Levy, 1991). Consumption of low-protein diets, however, resulted in prolongation of twitch time in slow twitch muscles, raised force-generating capacity, alterations in force–frequency relationship and increased fatigue susceptibility of both fast and slow twitch muscles (Levy & Wootton, 1991). The aim of the present study was to examine the influence of endotoxin administration on the contractile characteristics of rat skeletal muscle following consumption of low-protein diets. Muscles were selected to reflect the different muscle fibre types: soleus (type I), extensor digitorum longus (EDL, type IIB) and tibialis anterior (TA, type IIA/B).

Male Wistar rats (initial weight 97 g (SEM 1·9 g)) were fed on a 15% protein diet (FED) or a 0·5% protein diet (LP) *ad lib*. After 21 d animals received either *Escherichia coli* endotoxin (0·5 mg/kg body-weight) or sodium chloride solution 9 g/l intraperitoneally. Skeletal muscle function was assessed 8 h later *in situ* under Sagatal anaesthesia, as previously described (Levy & Wootton, 1989).

The contractile characteristics of the muscles of animals in the FED + endotoxin group were not different to the FED–control animals, except a lower force-generating capacity in the TA muscle (49% twitch, 77% maximal, $P<0·01$). This was associated with a lower TA muscle weight in the FED + endotoxin animals. Twitch characteristics of the LP + endotoxin animals were not different for the EDL and TA, but the soleus muscle exhibited a 37% and 32% faster contraction ($P<0·05$) and relaxation ($P>0·05$) respectively. Maximal force-generating capacity was lower in the LP + endotoxin group, attaining significance for the EDL muscle (58%, $P<0·05$). All three muscles exhibited greater fatigue, although some muscles of LP + endotoxin animals exhibited normal fatigue indices. No difference in the soleus and EDL force–frequency relationship was discerned, but the TA muscle exhibited a shift to the left at 20 and 50 Hz compared to non-endotoxin-treated animals ($P<0·05$).

The results suggest that endotoxin may compound the deficits in skeletal muscle function resulting from the consumption of low-protein diets. The relative force generation in the LP + endotoxin group were of the same magnitude as the well-nourished animals, indicative of a reversal of the previously-described adaptive response (Levy & Wootton, 1991). We might therefore hypothesize that malnourished infected individuals would perform work-related tasks less satisfactorily than well-nourished or malnourished individuals.

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MRI estimates of changes in adipose tissue area with weight loss. By J. LOVE[1], M. A. FOSTER[2], G. MCNEILL[1] and V. ANTFANG[2], [1]*Human Nutrition Unit, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and [2]*Department of Biomedical Physics, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD*

This study assessed the usefulness of magnetic resonance imaging (MRI) in detecting the changes in area and distribution of adipose tissue in obese women after a period of sustained weight loss. Estimates of adipose tissue area in nine transaxial planes were made in eight healthy obese women by MRI proton imaging before and on completion of a 3·35 MJ (800 kcal)/d residential weight-reducing regime lasting 3 weeks. The women were aged 30–45 years with a mean starting weight of 87·73 (range 78·45–94·45) kg, and mean body mass index 31·9 (range 29·2–35·2) kg/m^2. Weight loss was on average 4·48 (range 3·90–4·80) kg.

Nine images were taken at positions distributed between the sternal notch and the mid calf. The sites of these images were first determined in each subject from anatomical reference points. The MRI proton imager operated at 3·4 MHz, using pulse sequences which produce an inverse recovery image giving strong contrast between adipose tissue and other tissues (Foster *et al.* 1984). Four repeated measurements of adipose tissue area in one subject had coefficients of variation ranging from 2·2% to 9·6% at these sites.

The sum of the adipose tissue area (ATA) at the nine sites at the beginning of the regime correlated significantly with total fat mass (kg) as estimated from densitometry (r 0·74, $P<0·05$). The Table shows the initial percentage of ATA (% ATA) at each site, and ATA as a percentage of total area lost (% TAL) at each site. Sixty-two per cent of the adipose tissue area measured was distributed between four sections. These four sections also accounted for 67% TAL from these sites. There was a significant relationship between ATA and TAL (r 0·86, $P<0·01$). The variability in TAL at each site reflects not only differences in the site of loss between subjects, but also differences in the positioning of soft tissue relative to anatomical landmarks. The usefulness of MRI for detecting changes in adipose tissue at different sites therefore depends on careful consideration of the sites imaged.

Section . . .	1	2	3	4	5	6	7	8	9
% ATA	9·1	12·3	11·1	14·6	19·3	15·9	10·0	4·3	3·3
SD	1·1	2·1	1·8	1·6	0·9	1·1	2·0	1·4	0·7
% TAL	8·4	18·8	3·0	20·1	19·1	23·8	6·5	−1·7	2·0
SD	8·0	10·8	20·1	9·8	14·1	17·3	11·6	11·7	3·4

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The effect of smoking cessation on energy expenditure and body-weight. By MARY CURSITER, *Department of Dietetics and Nutrition* and M. HOLMES, *Department of Management and Social Sciences, Queen Margaret College, Edinburgh EH12 8TS* and SHEILA JENNETT, *Institute of Physiology, University of Glasgow, Glasgow G12 8QQ*

Weight gain after cessation of smoking has been attributed to a combination of changes in energy intake and energy expenditure (EE). Smoking increases 24 h EE (Hofstetter *et al.* 1986); Perkins *et al.* (1989) showed that nicotine has a thermogenic effect on EE during light exercise. The aim of the present study is to investigate energy balance and body-weight in women smokers, before and after cessation of smoking.

This preliminary report presents data on EE and body-weight in forty subjects: twenty-seven successful ex-smokers (mean (SD) age 34·5 (6·0) years and body mass index 22·6 (2·2)) and fifteen who relapsed (35·9 (6·4) years and 22·0 (2·4)). Subjects were assessed as smokers (baseline) and 1 month after an agreed cessation date (return). Smoking status was monitored at each visit by measuring end-expired carbon monoxide. Resting metabolic rate (RMR) and EE during light exercise (EEX) were measured by open-circuit indirect calorimetry using a Douglas bag. The exercise test used a cycle ergometer, set at a level (determined at a practice visit) to produce light exercise (approximately 50% of maximum heart rate, EE 15–20 kJ/min) for each subject. Exercise (5 min warm-up, 6 min measurement) was repeated after a 15 min break, during which subjects either smoked (baseline) or rested (return). The thermogenic effect of smoking on EEX was calculated by subtracting the change in EEX after rest from the change in EEX after smoking. Data were analysed by *t* tests; complete data were not obtained for all subjects.

	Success group					Relapsed group				
		Baseline		Return			Baseline		Return	
	n	Mean	SD	Mean	SD	*n*	Mean	SD	Mean	SD
Weight (kg)	27	62·0	5·8	64·1**	6·3	15	61·7	7·0	62·4	7·1
RMR (kJ/d)	25	5835	723	5876	755	13	5762	650	5853	765
RMR (kJ/kg per d)	25	94·6	15·3	91·7	11·8	13	94·7	8·4	95·0	8·5
EEX (kJ/min)	23	15·3	2·5	15·0	2·5	15	16·2	2·7	16·3	2·8
EEX (kJ/kg per h)	23	14·9	2·5	14·1*	2·5	15	15·8	2·2	15·8	2·6

*$P<0·05$, **$P<0·001$. Significance tested by paired *t* test between baseline and return.

All successful ex-smokers gained weight (mean gain 2·11 kg, range 0·30–4·95 kg) and their EEX (kJ/kg per h) decreased significantly, representing a potential energy saving of 384 kJ/d for a 60 kg woman (assuming 8 h light activity); RMR (kJ/kg per d) decreased slightly, though not significantly; these changes were not found in the relapsed group. A thermogenic effect was not found: there was no significant difference between the pre-smoking EEX (15·5 (2·4) kJ/kg per h) and the net post-smoking EEX (15·8 (2·5) kJ/kg per h).

These results show that stopping smoking leads to a reduction in the energy cost of light exercise; this response, together with other minor decreases in EE, may contribute to weight gain after cessation of smoking.

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Energy intake and expenditure in divers during a 450 m working dive. By PAUL HAGGARTY, GERALDINE MCNEILL, BRIAN A. MCGAW, SUSANNAH L. CHRISTIE, DEBORAH C. MORRISON, ERIC MILNE and GARY DUNCAN, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB*

Divers often undergo substantial weight loss during saturation diving with the severity of the weight loss generally being proportional to the depth achieved. At least part of this weight loss is the result of negative water balance but it has been suggested that saturation diving could also impose additional energetic stresses which may not be met by food intake (Webb, 1976). With the advent of the heavy water technique (Lifson & McClintock, 1966) it has become possible to quantify the energy expenditure of subjects without restriction, and in hostile environments remote from calorimetric equipment. The results of a heavy water study carried out during a 450 m dive are presented here.

A 31 d onshore 450 m simulated dive was undertaken by six divers at the National Hyperbaric Centre in Aberdeen. During the 10 d bottom phase the energy intake of the divers was assessed by monitoring the food entering and leaving the chamber airlock and CO_2 production was determined by analysing the isotopic composition of daily urine samples after administration of triply-labelled water (TLW) (Haggarty *et al.* 1988). Energy expenditure was calculated using respiratory quotients derived from food quotients and the change in body energy. During this time the divers undertook a pipeline repair requiring significant physical work and manual dexterity.

The five divers studied all lost weight during the bottom phase; a mean of 266 (SEM 36) g/d, representing a loss of 3·5% of normal body-weight over the 10 d active period of the dive. Over this same period the mean energy intake was 9·69 (SEM 0·33) MJ/d and energy expenditure was 12·55 (SEM 0·98) MJ/d. The resulting energy deficit of 2·70 MJ/d could account for around a third of the measured weight loss. The remaining two-thirds of the weight loss was assumed to be water. This conclusion was supported by haematocrit values which indicated that the divers became dehydrated as the dive progressed.

Using the FAO/WHO/UNU (1985) prediction equations it can be calculated from the body-weight of these divers that their mean basal metabolic rate (BMR) would be 7·39 MJ/d. On the basis of the FAO/WHO/UNU classifications the energy expenditure of the divers (1·68 × BMR) was not excessive, falling below the moderate activity classification, whereas the intake (1·33 × BMR) would not be sufficient to support even light activity on the surface. Thus, there is indeed an energy imbalance during deep saturation diving and this can explain some of the weight loss found in divers. However, the measured expenditure could easily be sustained on the surface therefore it must be concluded that the energy imbalance is due to a fall in food intake under hyperbaric conditions. Since the divers had access to a wide variety of palatable foods the reduced intake must have been due to loss of appetite.

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Feeding pattern in humans. 1. The relationship between feeding pattern and body-weight.

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Feeding pattern describes the energy intake over a 24 h day as judged by the time of consumption and size of all feeding occasions. Increased feeding frequency and a shift in daily energy intake towards the beginning of the waking day have been associated with lower body-weights compared to controls (Adams & Morgan, 1981). Feeding pattern is thought to affect body-weight by altering the total daily thermic effect of food (TEF) (Fabry, 1969).

Feeding pattern and body mass index (BMI) were studied in 231 free-living humans, separated for analysis into five age-groups. Total daily energy intake (TDI) was assessed using 7-d weighed dietary intakes. TDI was divided into six feeding periods; breakfast, mid-morning snacks, lunch, afternoon snacks, dinner and evening snacks (Summerbell & Moody, 1992). The variance between the mean energy intakes over the six consecutive feeding periods, as a per cent of TDI, was used as an assessment of feeding pattern and called the Feeding Pattern Index (FPI).

			TDI (kJ)						
	n		Males		Females		BMI		Regression
Group	Male	Female	Mean	SD	Mean	SD	Mean	SD	slope†
Adolescent	12	21	10 802[a]	2041	7174	1785	21·0[c]	3·4	0·3105*
Student	0	11	—	—	7543	1012	20·6	1·7	0·1141
Young adult	29	30	10 332[a]	2285	8618[a]	2537	22·4	2·1	−0·0970
Middle-aged	6	34	9929	2247	6829[b]	1512	25·1[a]	4·3	0·1191
Elderly	24	64	8652[b]	1817	6917[d]	1420	23·6	5·9	−0·0245

† Regression coefficient (slope) showing the change in BMI associated with a unit increase in FPI; *$P<0·05$.
Differences between age groups were assessed using ANOVA and Scheffe's multiple comparison test, and are represented by the superscript letters a–d where: a *v.* b = $P<0·05$; a *v.* c = $P<0·01$; a *v.* d = $P<0·001$.

A positive linear relationship was found between FPI and BMI in the adolescent group, i.e. a more even distribution of energy intake over a day (a snacking pattern) was associated with lower BMI. Also, the amount of energy consumed at breakfast was inversely related to BMI ($P<0·05$) in the adolescent group. Feeding pattern was not related to BMI within the other four age groups.

Overall, these results show no significant relationship between feeding pattern and BMI in the population as a whole. The lack of a clear or consistent definition of a feeding occasion in earlier studies makes it difficult to draw relevant comparisons.

Although weak, the relationship between feeding pattern and body-weight in the adolescent group did not support the work of Fabry (1969), unlike those from the other age-groups. Two possible explanations for this result are suggested. Firstly, it has been shown that TEF decreases with age (Golay *et al.* 1983); secondly, the relative timing of physical activity in relation to food intake has been shown to affect TEF (Miller *et al.* 1967) and it is conceivable that the adolescent group expended greater levels of energy after feeding occasions as compared with the other age-groups.

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Feeding patterns in humans. 2. Feeding patterns and sources of energy intake. By C. D. SUMMERBELL, *St Stephen's Clinic, 369 Fulham Road, Chelsea, London SW10 9TH* and R. C. MOODY, *School of Food and Consumer Studies, The Robert Gordon's Institute of Technology, Queen's Road, Aberdeen AB9 2PG*

Feeding pattern was studied in 231 free-living humans. These subjects were separated for analysis into five age-groups. Total daily energy intake (TDI) was assessed using 7 d weighed dietary intakes. TDI was divided into six feeding periods: 1, breakfast; 2, mid-morning snacks; 3, lunch; 4, afternoon snacks; 5, dinner and 6, evening snacks.

Mean energy intake (SD) as a percentage of total daily energy intake

Group	*n*	Median age	Feeding periods 1	2	3	4	5	6
Adolescent	33	13	13·3[c]	7·0[a]	31·5[c]	9·5[a]	29·7	9·1[c]
			(8·5)	(5·0)	(6·7)	(7·3)	(6·9)	(9·1)
Student	11	18	15·3	4·0	21·9[df]	8·7	29·9	20·3[a]
			(6·4)	(3·5)	(5·7)	(3·8)	(5·7)	(9·8)
Young adult	59	31	14·4[d]	4·2[b]	29·8[de]	5·0[ce]	36·7[a]	10·0[c]
			(7·2)	(3·1)	(6·5)	(3·4)	(6·9)	(6·3)
Middle-aged	40	48	15·2[b]	4·7[e]	29·1[c]	4·0[d]	33·7[e]	13·4
			(8·4)	(5·7)	(8·2)	(8·0)	(9·2)	(7·2)
Elderly	88	77	20·2[a]	2·4[df]	35·3[a]	2·9[df]	27·0[dh]	12·2[b]
			(8·2)	(3·1)	(8·5)	(4·6)	(7·7)	(7·6)

Significant differences between age-groups are represented by the superscript letters a–h where: a *v.* b and e *v.* f = $P<0·05$; a *v.* c and e *v.* g = $P<0·01$; a *v.* d and e *v.* f = $P<0·001$.

These results suggest that the changes in feeding pattern of the British population observed in the early 1980s compared to the 1950s (BNF, 1985) appear to be continuing in the same direction. The population is consuming relatively more food and drink during the latter part of the day and adolescents are placing less emphasis for energy intake on the traditional meal. These changes are strongly related to social changes in the British society.

Typical snack food such as chocolate and sweet confectionery, fizzy drinks and squashes contributed to the relatively high proportion of energy intake at mid-morning and afternoon in the adolescent group, whereas alcohol accounted for the relatively high proportion of energy intake during the evening in the student group.

The degree of snacking (Summerbell & Moody, 1992) was related to sugar consumption ($P<0·05$) due to the fact that food commodities consumed as snacks were relatively higher in sugar ($P<0·001$) than those commodities consumed as meals. These results add to the concern (BNF, 1987) about high sugar intakes, particularly between meals, leading to dental decay, especially in adolescents.

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Carbohydrate balance and day-to-day food intake in man. By R. J. STUBBS, P. R. MURGATROYD, G. R. GOLDBERG and A. M. PRENTICE, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

Experiments in mice have demonstrated that energy intake is negatively correlated with carbohydrate (CHO) balance on the preceding day (Flatt, 1987), suggesting that a drive to maintain CHO balance is a major factor regulating energy balance.

This hypothesis was tested by whole-body calorimetry of nine men (body-weight 72 (SD 6) kg, body mass index 23 (SD 6) kg/m^2) studied twice on the following protocol (% fat:CHO:protein by energy): Day 1, maintenance (40:47:13); Day 2, manipulation (carbohydrate depletion (85:3:12) or control (40:47:13) diets fed to an identical, slightly positive energy balance); Day 3, outcome. Nutrient composition was calculated using the values from Paul & Southgate (1978). Three-hourly urine samples were analysed for nitrogen (Kjeldahl) to determine protein oxidation rates. Fat and CHO oxidation rates were calculated from non-protein gaseous exchange, using the coefficients of Elia & Livesey (1988). Carbohydrate fluxes on the manipulation day were: depletion intake 23 g, net oxidation 70 g, balance −47 g; control intake 309 g, net oxidation 208 g, balance 101 g. Thus, hepatic glycogen was severely depleted on the depletion diet (evidenced by low net CHO oxidation and negative balance) and was replete on the control diet. On the outcome day subjects had *ad lib.* access to a normal diet (40:47:13) and were encouraged to eat to satisfaction. Outcome variables are summarized in the Table.

	Depletion				Control			
	Energy (kJ)	Protein (g)	Fat (g)	CHO (g)	Energy (kJ)	Protein (g)	Fat (g)	CHO (g)
Intake	12 729	96	134	384	12 717	97	134	381
SD	224	17	24	68	201	15	21	62
Oxidation	9193	87	120	174	9212	95	89	256
SD	797	20	11	41	753	35	12	39
Balance	3536	9	14	210	3505	2	46	125
SD	2140	22	26	66	2045	39	30	46

We interpret the marked positive energy balance as a failure to down-regulate habitual intake to match the low activity in the calorimeter. The primary mechanism for re-establishing CHO balance on the outcome day was a suppression of CHO oxidation (paired t value 4·782, $P<0·001$) on the depletion protocol. Under these experimental conditions food intake was not influenced by the previous day's hepatic glycogen status (paired t value 0·016, $P = 0·987$).

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Effect of dietary fat content and choice on intake and performance of growing pigs. By RHONA URQUHART[1] and K. J. MCCRACKEN[1,2], [1]*The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX* and *Food and Agricultural Chemistry Department, Department of Agriculture, Northern Ireland*

It has been established that energy intake may be limiting lean growth rate in pigs of high genetic potential (Rao & McCracken, 1991). The role of dietary fat in promoting energy intake and growth is controversial (Cole & Chadd, 1989). This may be partly due to variability of the quality of fat sources used. A blended fat designed to be close to the composition of pig adipose tissue was prepared and the apparent digestible energy content was found to be 34·1 MJ/kg. This was incorporated into a wheat–barley–fish–soya-bean diet to produce four diets (1–4 respectively), containing 0, 50, 100, 150 of supplemental fat/kg feed. Four replicates of five littermate pigs were obtained from pedigree breeders at around 25 kg. They were allocated within litters to one of the four diets or to a fifth treatment offering free choice of diets 1 and 4. All diets were offered *ad lib.* from 33–90 kg.

Feed weighbacks were determined daily and pigs were weighed twice a week to find live weight gain (LWG). The digestible energy (DE) and metabolizable energy (ME) contents of the diets were measured by 6 d balance at around 60 kg live weight.

Diet	1	2	3	4	5	SEM
ME intake (MJ/d)	30·4	30·5	30·5	30·8	31·6	0·98
LWG (kg/d)	1·01	1·04	1·05	1·01	1·01	0·053
ME:LWG	30·1	29·5	29·4	30·5	31·5	0·92

The DE contents were 15·7, 16·9, 17·7 and 19·1 MJ/kg dry matter for diets 1–4 respectively. There were no significant effects of dietary treatment on ME intake, daily gain or conversion of ME to gain. There were large differences between litters in ME intake (MJ/d) (27·2, 31·3, 32·7 and 31·9 (SEM 0·88); P=0·04) and in feed efficiency (MJ/kg gain) (28·9, 32·6, 30·8 and 28·5 (SEM 0·82); P=0·014). There were also marked differences in the proportion of energy from the basal diet selected by the four pigs given a choice (0·23, 0·50, 0·58, 0·67).

The results confirm the report of Cole & Chadd (1989) that boars regulate energy intake very closely over a wide range of energy density, and suggest that regulation of energy intake is also maintained when a choice of energy density is offered despite differences in the level of dietary fat selected by individual pigs.

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The effect of adding 10% maize oil to the diet on body fatness and food intake in broiler chickens. By A. Y. YALDA and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Results of studies on the effect of adding fat to the diet on growth and carcass composition of chickens are conflicting. Griffiths *et al.* (1977) observed no differences in the composition of the carcass when various levels of fat were fed, while Donaldson (1985) indicated that body fat was increased by feeding a fat-rich diet. Adding maize oil to the diet will not only increase its energy content, but may also decrease food intake to maintain energy intake.

To study further the effect of adding maize oil to the diet on voluntary food intake and body fatness in broiler chickens, twenty-four male broiler chickens were housed individually at 4 weeks of age. Twelve were fed normal grower diet until 9 weeks of age, while the other twelve were fed the same food with the addition of 10% maize oil until 7 weeks of age and thereafter fed the grower diet with no added maize oil. Body-weights were obtained weekly, while food intake was measured daily. For carcass analysis, birds were killed from each treatment at 7 and 9 weeks.

Weeks . . .	7			9		
	Control	Oil	SED	Control	Oil	SED
Body-wt (g)	1563[a]	1663[a]	85·3	2070[a]	2053[a]	131·9
Food intake (g/bird per d)	130[a]	106[b]	7·66	147[a]	135[a]	14·1
ME intake (kcal/bird per d)	420[a]	394[a]	25·3	379[a]	372[a]	45·5
Abdominal fat (g/bird)	21[b]	57[a]	4·7	45[b]	65[a]	6·1
Total carcass lipid (g/bird)	113[b]	179[a]	16·2	159[b]	192[a]	11·7
Total carcass protein (g/bird)	200[a]	208[a]	10·8	267[a]	235[a]	24·3
Total carcass ash (g/bird)	42[a]	44[a]	11·2	86[a]	67[a]	15·5

[a,b] Means in the same row for the same age with different superscript letters were significantly different ($P<0·05$).

SED, standard error of difference.

Addition of maize oil decreased food intake significantly and, despite a similar caloric intake, the weight of carcass lipid and abdominal fat were increased by 58 and 170% respectively at the end of the oil-feeding period.

This increase in body fatness did not prevent the birds subsequently eating almost as much normal food as controls so that, at the end of the experiment, they were still significantly fatter. Therefore, food intake in modern male broiler chickens is not affected to a great extent by body-fat manipulation.

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Griffiths, L., Lesson, S. & Summers, J. D. (1977). *Poultry Science* **56**, 1018–1026.

Effect of dietary supplemental ascorbic acid on performance and choice feeding of broiler chicks under heat stress. By H. R. KUTLU and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Heat stress caused by a high ambient temperature leads to increased mortality, reduced feed intake and growth rate in broilers. Ascorbic acid (AA) can alleviate the effect of heat stress on broiler performance, but the success is variable. Our preliminary results showed that dietary supplemental AA improved body growth and feed intake under heat stress. The objective of this study was to determine whether chicks, when receiving both AA-supplemented diet and normal diet simultaneously, would prefer the supplemented one when each feed was of a different colour. Unsupplemented red (R^-), 0·2 g/kg AA-supplemented red (R^+), unsupplemented green (G^-) and 0·2 g/kg AA-supplemented green (G^+) coloured standard starter diets were prepared. Sixty-four, 1-week-old female broiler chicks were divided up into eight groups. Following an 8 d training period, the chicks were given a choice between either G^- and R^- or G^+ and R^- or G^- and R^+ or G^+ and R^+ for 14 d under either heated (14 h 26°: 10 h 38°) or non-heated (constant 26°) conditions.

		Feed combinations				
		G^- and R^-	G^+ and R^-	G^- and R^+	G^+ and R^+	SED
Feed intake (g/bird) over testing period	Heated	816 + 438 (65%) (35%)	785 + 543 (60%) (40%)	432 + 877 (33%) (67%)	678 + 752 (47%) (53%)	—
	Non-heated	656 + 846 (44%) (56%)	356 + 995 (26%) (74%)	942 + 593 (61%) (39%)	758 + 612 (55%) (45%)	—
Body-wt (g/bird) at 29 d of age	Heated	872[d]	966[b,c,d]	912[c,d]	984[a–d]	
	Non-heated	1059[a,b]	980[a–d]	1080[a]	1009[a,b]	16·4

SED, standard error of the difference between means.
[a–d] Means with different superscript letters are significantly different: $P<0·05$.

Heating reduced feed intake and body growth. AA supplementation increased body growth and feed intake under heat stress. AA supplementation also affected choice feeding: under heat stress, the groups that were given a choice between AA-supplemented diet and unsupplemented diet preferred the AA-supplemented diet to the unsupplemented diet, while under the non-heated conditions, the choice-fed groups consumed more unsupplemented diet than AA-supplemented diet. Although the differences between intakes of AA-supplemented diets and unsupplemented diets were not statistically significant ($P>0·05$), the results tended to support the proposition that the birds could associate AA supplementation and colour and they preferred AA-supplemented diet to unsupplemented diet under high environmental temperature.

The effect of force-feeding various levels of protein on diet selection and growth of broiler chickens. By F. SHARIATMADARI and J. M. FORBES, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Chickens are able to regulate their intake when offered high- and low-protein feeds (Shariatmadari & Forbes, 1990*a*). The composition of recently-digested food is a factor in determining short-term regulation (Shariatmadari & Forbes, 1990*b*).

In order to assess the ability of broilers to control their protein intake over a longer period of time, four groups of ten male broiler chickens, 3 weeks of age, were force-fed 40 g per d of isocaloric diets with protein (CP, nitrogen 6·25) contents of 60, 135, 215 or 295 (T1, T2, T3 and T4 respectively) g CP/kg fresh food for 10 d with *ad lib.* access to a choice of feeds with 60 (LP) and 295 (HP) g CP/kg. Half of the broilers were killed for analysis of carcass protein while the rest were given free access to the LP and HP feeds for a further 35 d and then killed for carcass analysis.

	Diet				
	T1	T2	T3	T4	SED
Force-feeding period					
AFI	48[a]	50[a,b]	53[a,b]	60[b]	3·2
API	13·5	13·2	13·0	12·6	0·90
CW	315[d]	350[c]	401[b]	432[a]	20·3
PCC	600[b]	604[b]	616[b]	643[a]	20·6
Post-treatment period					
AFI	145[a]	144[a]	159[b]	162[b]	4·6
API	27	28	28	28	1·04
CW	1670[a]	1650[a]	1700[a]	1680[a]	75[b]
PCC	600[a]	625[a]	530[b]	533[b]	24·7

[a–d] Values in the same row with different superscript letters are significantly different: $P>0·05$.

AFI, average voluntary food intake (g/d); API, average voluntary protein intake (g/d); CW, carcass weight (g); PCC, protein composition of carcass (g/kg dry matter).

The force-feeding of a higher protein level resulted in a significantly higher food intake ($P>0·05$) but lower proportion of HP so that API was unaffected. As a result of higher total protein intake, carcass weight and carcass protein content were increased at the end of a 10 d force-feeding period. During the post-treatment period feed intake was higher in T3 and T4 birds while protein intake was unaffected. This lower protein:energy ratio resulted in reduced carcass protein deposition and showed that these birds had not readjusted their protein intake after the end of force-feeding with high protein feeds.

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Effects of meat species and particle size on postprandial satiety. By JENNIFER A. FRENCH, CLARE J. WAINWRIGHT and DAVID A. BOOTH, *School of Psychology, University of Birmingham, Edgbaston, Birmingham B15 2TT* and JAN HAMILTON, *School of Home Economics, Texas Technological University*

Toughness of connective tissue and particle size in a meat soup were predicted to slow passage from the stomach and hence to prolong satiety as measured by subsequent food consumption.

Subjects were twelve undergraduate students in normal health who were not at the time restricting their diet in any way. Each subject completed test sessions with pre-loads of two meat species (beef and chicken) and two sizes of meat particles (2 and 4 mm) in a meat-flavoured clear soup base: for men, 200 g meat, 20 g pasta, 285 ml stock; for women, 140 g meat, 14 g pasta, 200 ml stock.

These were served at the subject's normal lunch time on days when the dietary record showed normal consumption from the previous evening. In the first four sessions subjects had free access to a selection of pre-packed and weighed foods as a test meal given 30 min after each pre-load; a further four sessions presented the test meal 3 h later.

Table 1. *Composition of pre-loads*

Food item	Content (g/kg)			MJ/kg
	Protein	Fat	Carbohydrate	
Pre-load				
Chicken	210	30	0	4·85
Beef	290	40	0	6·52
Vermicelli	90	10	780	14·84

Intake of food energy was reduced after consumption of the large particle size relative to the small at both time delays ($P<0·01$), and after beef relative to the chicken at 30 min ($P<0·001$), but not at 3 h.

The test meal intakes (MJ) for the different combinations of meat species, particle size and time since pre-load are given in Table 2.

Table 2

Size	Delay by 30 min				Delay by 3 h			
	Beef		Chicken		Beef		Chicken	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2 mm	2·27	1·04	3·20	0·95	3·14	1·26	3·76	1·04
4 mm	1·85	0·73	2·47	0·74	2·59	1·26	2·84	0·48

The present results show that differences in satiation can arise from physical differences in the type and form of food, presumably altering the rate of breakdown of particles and hence of satiety signals at the intestine wall or in the circulation. Thus what has been described as a protein effect could be a meat effect arising from differences in connective tissue. The satiating effects of different foods cannot be understood without manipulating macronutrient contents and other properties of the food independently in ways that distinguish between mechanisms that could be operative during the test for satiety.

Gene expression and the metabolic role of zinc. By J. K. CHESTERS, R. BOYNE and LINDA PETRIE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Both in animals and in cell cultures, low availability of zinc restricts growth and specifically inhibits DNA synthesis. In 3T3 cells, the timing of the requirement for Zn coincided with the period of induction of the enzymes involved in DNA synthesis (Chesters *et al.* 1989) and lack of Zn inhibited the corresponding increase in thymidine kinase (*EC* 2.7.1.21, TK) activity. The latter was associated with a reduced concentration of TK mRNA (Chesters *et al.* 1990) consistent with the hypothesis that Zn is necessary for certain changes in gene expression (Chesters, 1978).

Since this hypothesis was largely based on effects of Zn deficiency on replicating cells, the generality of this requirement has now been investigated by examining the impact of low Zn availability induced by addition of a chelator, diethylenetriaminepentaacetic acid (DTPA, 600 μM), on the differentiation of myoblasts in culture. During differentiation, the cells lose the ability to produce DNA, leave the replication cycle and begin to synthesize muscle-specific proteins such as creatine kinase (*EC* 2.7.3.2, CK). Although inhibition of DNA synthesis by lack of Zn might have been expected to encourage differentiation, the latter was actually impaired and CK activity was reduced by DTPA and these effects were specifically reversed by Zn (400 μM). Similar additions of calcium, cobalt and copper did not alter the inhibition by DTPA while iron, manganese and nickel produced only partial reactivations probably associated with displacement of endogenous Zn from the chelator.

Treatment	Creatine kinase activity (IU/mg protein)	Creatine kinase mRNA CK/18S*	Creatine kinase mRNA CK/S6†
Control	2·41	1·62	1·40
DTPA alone (−Zn)	0·27	0·09	0·15
DTPA+Zn (+Zn)	2·33	1·13	1.20
DTPA+Fe	0·73		
DTPA+Mn	1·33		
DTPA+Ni	0·81		

* 18S ribosomal protein RNA.
† S6 ribosomal protein MRNA.

Further investigations using Northern hybridization to quantify specific RNAs revealed that, as with thymidine kinase, the reduction in creatine kinase activity was accompanied by a corresponding decrease in the concentration of CK mRNA. This did not result from a generalized inhibition of mRNA synthesis since the decrease was equally apparent whether CK mRNA concentrations were expressed per unit of 18S ribosomal RNA or relative to the concentration of the constitutively expressed mRNA for ribosomal protein S6. It appears that adequate Zn is a prerequisite for induction of normal concentrations of mRNA not only for certain enzymes involved in DNA synthesis but also for others induced in its absence.

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Stable isotope studies of short-term plasma zinc kinetics in normal human subjects. By N. M. LOWE, J. M. RHODES, A. GREEN and M. J. JACKSON, *Department of Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX*

Studies in experimental animals have indicated that analysis of the kinetics of isotopic zinc turnover in plasma may be of use in the assessment of body Zn status and provide information on the size and kinetics of a rapidly-exchangeable, intracellular 'pool' of Zn primarily located within the liver (Lowe *et al.* 1991). Previous data on the immediate fate of injected radioactive Zn in man have indicated a similar pattern of distribution of the isotope to that seen in experimental animals (Wastney *et al.* 1986) and we have therefore attempted to examine plasma Zn turnover in normal human subjects using enriched stable ^{70}Zn.

Six normal subjects (four male, two female, aged 25–38 years) were given a bolus intravenous injection of 0·5 mg 98%-enriched ^{70}Zn as zinc chloride in isotonic saline (9 g sodium chloride/l). Blood samples were removed at frequent intervals for periods of up to 2 h, the plasma was separated and analysed for ^{70}Zn content by inductively-coupled plasma-mass spectrometry following extraction of the Zn by ion-exchange chromatography. The decay curves of ^{70}Zn enrichment were analysed using the techniques previously used to study ^{65}Zn kinetics in rat plasma (Lowe *et al.* 1991), as originally described by Shipley & Clarke (1972).

Over the time course of the study (120 min) plasma ^{70}Zn enrichment closely followed two compartment kinetics and was analysed appropriately to derive the sizes of the two exchangeable pools (Q_a and Q_b), their fractional turnover rates and the fluxes between these pools. It was assumed that on injection the ^{70}Zn rapidly equilibrated with all of the Zn in plasma. The initial exchangeable pool was calculated to have a size of 0·047 (SEM 0·006) mg/kg body-weight, which was approximately one-quarter that of the second pool (Q_b: 0·235 (SEM 0·061)). The initial pool was also found to have a very rapid turnover (fractional turnover rate: mean (SEM) 0·099 (0·004)/min) compared to that of Q_b (0·019 (0·002)).

Results therefore indicate that similar plasma Zn kinetics occur in man to those previously described in the rat (Lowe *et al.* 1991) and the pig (Chesters & Will, 1981). Mathematical analysis of these decay data appears to provide access to information on the sizes and turnover of Zn pools in man which is likely to be of relevance in various situations, such as the assessment of Zn status and the determination of optimal requirement of dietary Zn.

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In vitro studies on transepithelial phosphate transport in the small intestines of goats and sheep. By G. BREVES, H. KAEPPNER and B. SCHROEDER, *Department of Veterinary Physiology, University of Giessen, D 6300 Giessen, Germany*

It is well documented that the upper small intestine is the major site for net absorption of inorganic phosphate (P_i) in simple-stomached animals as well as in ruminants (Harrison & Harrison, 1961; Pfeffer *et al.* 1970). Whereas the localization, the mechanisms involved and their regulation have been intensively studied in simple-stomached species, little is known about P_i transport characteristics in ruminants.

It was the aim of the present study to measure the unidirectional transepithelial P_i fluxes in the proximal duodenum and mid-jejunum of goats and sheep. Stripped epithelial tissues from the duodenum and the jejunum were mounted between the two halves of Ussing-type chambers (surface area 1 cm^2). The tissues were incubated in the presence of 0·01 mM-indomethacin in Krebs–Henseleit bicarbonate buffer (pH 7·4; 38°) containing 3·0 mM-phosphate and 10 mM-glucose (serosal) or 10 mM-mannitol (mucosal). Indomethacin was added to minimize epithelial prostaglandin effects. For measurement of unidirectional P_i fluxes corresponding tissues were paired. In paired tissues the transepithelial conductance (G_t) differed by less than 25%, and 6–8 μCi carrier-free ^{32}P-orthophosphate were added either to the mucosal or to the serosal side of the tissue. Flux measurements were initiated 20 min after the addition of ^{32}P and consisted of at least two 15 min flux periods. The tissues were short-circuited during the flux measurement and chemical gradients were eliminated, thus allowing measurement of active P_i transport.

In both segments of the small intestine, P_i fluxes from mucosal to serosal side (J_{ms}) were significantly higher than in the opposite direction (J_{sm}) resulting in a significant net flux (J_{net}). This clearly indicates the presence of a process for active P_i absorption in the duodenum and jejunum of ruminants (Table). In both species jejunal J_{net} was significantly higher compared with the duodenum.

P_i flux rates (nmol/cm² per h), G_t (mS/cm²) and short circuit currents I_{sc} (μeq/cm² per h) of duodenal and jejunal tissue preparations obtained from goats and sheep (means (SEM) for N animals)

			J_{ms}		J_{sm}		J_{net}		G_t		I_{sc}	
Species	Tissue	*n*	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Goats	Duodenum	7	82·2[a]	12·7	33·0[a]	5·2	49·3[a]	9·4	14·7[a]	1·2	+0·35[a]	0·04
	Jejunum	6	272·8[b]	33·8	88·3[b]	21·8	173·3[b]	47·9	27·5[b]	2·4	−1·29[b]	0·57
Sheep	Duodenum	6	130·3[a]	19·1	81·4[b]	11·1	49·0[a]	9·9	29·0[b]	3·0	+0·38[b]	0·04
	Jejunum	13	197·8[b]	22·1	81·3[b]	14·0	116·4[b]	21·6	30·8[b]	2·8	+0·37[b]	0·15

[a,b] Mean values in a vertical column with different superscript letters are significantly different ($P<0·05$; unpaired *t* test).

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Stimulation of trace element absorption by major metals in vitro. By J. B. ADEKALU and F. W. HEATON, *Division of Biological Sciences, Lancaster University, Lancaster LA1 4YQ*

A simple intestinal sac technique was used to screen for interactions that might be of importance during trace element absorption from the small intestine.

Everted duodenal sacs were prepared from the intestines of adult male Wistar rats, filled with 0·15 M-Tris-Krebs buffer, pH 7·3, and incubated for 30 min at 37° as described previously (Seal & Heaton, 1983). Control sacs were incubated in the same buffer containing 2·0 mM concentrations of copper, zinc, manganese or iron salts. Previous studies had shown this concentration to be in the linear range of the concentration–uptake relationships for all four metals. Calcium, magnesium, sodium or potassium chlorides were added to the incubation buffer of test sacs at concentrations giving the same ratios to the trace metal under study as are found in normal human diets. This resulted in NaCl concentrations as high as 2·5 M. The trace metals present in the sac contents after incubation were determined by atomic absorption flame photometry.

	Metal uptake (μmol/g dry tissue per 30 min)				
	Additive				
Trace element	None	Ca	Mg	Na	K
Cu Mean	0·40	0·35	0·56	0·17	0·24
SEM	0·08	0·06	0·16	0·03	0·03
Zn Mean	1·54	2·01	3·49**	1·23	4·25**
SEM	0·20	0·19	0·51	0·20	0·60
Mn Mean	4·35	7·54**	11·60**	4·99	7·35*
SEM	0·41	0·81	1·23	1·21	0·81
Fe Mean	1·40	2·57**	3·32**	1·19	2·44**
SEM	0·10	0·32	0·52	0·09	0·18

n 11 for controls without additive, *n* 6 when salts of major metals were present.
Values significantly different from control; *$P<0·01$, **$P<0·001$.

Mg and K both markedly increased the uptake of Zn, Mn and Fe into the sacs. Ca also stimulated the uptake of Mn and Fe, and the results suggested a similar effect on Zn although it was not statistically significant. However, Cu uptake was not affected by any of the major metals tested and the presence of Na did not alter any of the four trace elements studied.

The similarities between the effects of Ca, Mg and K on the absorption of Zn, Mn and Fe suggest the existence of some unrecognized general mechanism of action that is subject to further investigation.

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Effect of ascorbic acid and lemon juice on tea-inhibited iron absorption in suckling rats.

By A. FLYNN and M. REDDY, *Department of Nutrition, University College, Cork, Republic of Ireland*

Absorption of non-haem iron (NHI) in a meal is dependent on its relative content of inhibitors and enhancers of absorption. We have shown that the suckling rat is a useful model for investigation of dietary inhibitors of NHI absorption, such as tea polyphenols (Reddy *et al.* 1991) and phytate (Cashman *et al.* 1991). This report describes how this model may be used to investigate the interactions of enhancers (ascorbic acid (AA) and lemon juice (LJ)), and inhibitors (tea polyphenols) of NHI absorption.

Tea infusion was prepared as previously described (Reddy *et al.* 1991). Fe content was 0·1 mg/l. Lemon juice (LJ) containing AA (290 mg/l) and citrate (46 g/l) was purchased locally. Tea infusion, mixed with water (9:1), LJ (9:1) or 100 mM-AA (9:1) and a control (water) were labelled with $^{59}FeCl_3$ to give a final concentration of 6 mg Fe/l and 1 μCi^{59}Fe/ml, and 0·3 ml was given by gavage to 17-d-old rats, previously fasted for 18 h. Animals were killed 6 h later and stomach, small intestine (SI), caecum–colon and liver removed. SI was perfused with 6 ml 0·15 M-sodium chloride. ^{59}Fe was determined in a well gamma counter.

Uptake (% dose) of ^{59}Fe from water, tea, tea+AA and tea+LJ in suckling rats

	Water (*n* 5)		Tea (*n* 6)		Tea+AA (*n* 6)		Tea+LJ (*n* 6)	
Organ/tissue	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Stomach	0·9	0·1	0·5	0·1	0·9	0·1	0·7	0·1
SI	5·3	0·5	7·1	0·5	7·2	0·5	6·0*†	0·6
SI perfusate	0·2	0·1	2·8	1·1	2·0	0·8	2·1	0·9
Caecum–colon	2·4**	0·2	52·7	2·9	32·6**	4·6	22·9**†	1·7
Liver	10·1**	0·9	3·6	0·6	7·0*	1·1	9·7**††	1·4
Absorbed‡	96·5**	0·2	44·1	2·6	64·7**	4·9	74·3**†	1·9
Carcass§	91·1**	0·5	37·0	2·6	57·5*	5·4	68·3**†	2·5

Significantly different from tea: *P<0·05, **P<0·01.
Significantly different from tea+AA: †P<0·05, ††P<0·01.
‡ Absorbed = 100 − (stomach + SI perfusate + caecum–colon) (%).
§ Carcass = absorbed − SI.

Absorption of ^{59}Fe from tea was much lower than from water. Inclusion of AA (final concentration, 10 mM) or LJ (final concentration of AA, 0·16 mM; citrate, 23·9 mM) in tea increased ^{59}Fe absorption, and the increase with LJ was significantly greater than with AA. Since the dietary intake of citrate is much higher than AA (Hazell & Johnson, 1987) these results suggest that citrate may be more significant than AA as an enhancer of NHI absorption in human diets. The study shows that the suckling rat is a useful model for investigation of the interactions of enhancers and inhibitors of NHI absorption.

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Progressive effects of selenium deficiency on the acute cold-induced stimulation of type II iodothyronine deiodinase activity in rat brown adipose tissue. By J. R. ARTHUR[1], G. J. BECKETT[2], F. NICOL[1], Y. GUO[1] and P. TRAYHURN[1], [1]*Division of Biochemical Sciences, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and [2]*University Department of Clinical Chemistry, The Royal Infirmary, Edinburgh EH3 9YW*

The trace element selenium is now recognized to be essential for normal thyroid hormone and iodine metabolism (Arthur *et al.* 1990*b*). Recent work has shown that Se is a key component of the type I iodothyronine deiodinase enzyme in liver (Arthur *et al.* 1990*a*), which converts thyroxine to the biologically-active thyroid hormone, 3′,3:5-triiodothyronine (T_3). Brown adipose tissue (BAT) has a high activity of the type II iodothyronine deiodinase (ID-II), and the activity is greatly increased in response to cold exposure (see Arthur *et al.* 1991). Severe Se deficiency appears to lead to an impairment of ID-II activity in BAT, and there is also an inhibition of the acute cold-induced stimulation of the enzyme (Arthur *et al.* 1991). In the present study we have examined the progressive effects of Se deficiency on the stimulation by cold of ID-II in BAT.

Male, weanling, Hooded Lister rats were offered either a Se-deficient (<0·005 mg Se/kg; −Se) or a Se-supplemented (0·1 mg Se/kg; +Se) diet for periods of up to 6 weeks (Arthur *et al.* 1990*a*). Interscapular BAT was removed from groups of twelve rats after 2, 4 and 6 weeks; half the animals in each group were exposed to 4° for 18 h before sampling, the other animals being kept at 21°.

Increase in ID-II activity (fmol/min per mg protein) in BAT of rats exposed to 4° for 18 h

Weeks on diet	+Selenium (*n* 6)	−Selenium (*n* 6)
2	2·84	2·52
4	1·89	0·25
6	2·51	0·13

The basal ID-II activity in BAT of +Se rats declined progressively between 2–6 weeks after weaning. A decline also occurred in −Se animals, and at 4 and 6 weeks the basal activity was signficantly lower in the −Se than the +Se animals. Cold exposure caused an increase in ID-II activity of 1·89–2·84 fmol/min per mg protein (thirteen–thirtyfold) in +Se rats at each time-point of the study (see Table). The cold-induced increase in activity was lower in −Se than in +Se rats at 4 and 6 weeks, and declined from 2·52 fmol/min per mg protein (fifteenfold) at 2 weeks to a mere 0·13 units (fourfold) by 6 weeks.

These results confirm that Se deficiency leads to an impairment in the cold-induced activation of ID-II in BAT. The results also show that the defect is evident by 4 weeks after the introduction of the Se-deficient diet. In view of the importance of T_3 to thermogenesis in BAT, it is likely that the response to cold will be impaired in Se deficiency; this may be of particular relevance to neonatal animals born to dams with a very low Se status.

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Food consumption and nutritional status of adolescents in Birmingham. By FATI RABIEE, J. MUNN and J. LUTZ, *South Birmingham Health Education Department, Highgate Street, Birmingham B12 0YA*

As part of a larger study to develop programmes for preventing coronary heart disease (CHD), anthropometric, health knowledge, food frequency and 24 h food recall (over 2 d; a weekday and a weekend day) data were collected on 625 pupils aged 14–15 years. Schools chosen were socio-economically and ethnically diverse. Fifty-eight per cent of pupils were Asians. Selected nutrients were compared with recommended daily intake (RDI) (Department of Health and Social Security, 1979).

Mean (SD) weights and heights for girls and boys were 51 (5) kg, 157 (6) cm and 61 (9) kg, 168 (10) cm respectively. Six per cent of the population studied were thin (<90% weight for height), 8% overweight (>110% weight for height), and 3% were short (<95% height for age) (National Centre for Health Statistics, 1976; Bray, 1975–76). Eleven per cent of the girls and 2% of the boys said they were currently dieting.

Mean daily energy intake was low both for girls and boys (86% and 83% of the RDI respectively). Protein and ascorbic acid were more than adequate, iron was adequate in boys (108% of RDI) and marginally adequate in girls (94% of RDI), but calcium was inadequate in both (87% of RDI). Fat contributed 34% of the total energy in girls and 37% in boys. Carbohydrate provided 53% of the energy in girls and 50% in boys. Sugar contributed 21% of the energy in girls and 15% in boys. Mean daily fibre intake was 16 g for girls and 17 g for boys.

The diet is therefore higher in fat and sugary food and lower in fibre than the NACNE recommendation on healthy eating (NACNE, 1983).

Analysis of food frequency data suggests that 74% of the pupils drink full-fat milk as compared to only 22% who drink semi-skimmed and skimmed milk. Cheddar cheese is still the most popular cheese (69%) and only 10% of the pupils eat lower-fat varieties. Thirty-nine per cent of the pupils eat at least one packet of crisps and 25% have one helping of chips every day. Fifty-one per cent of the pupils take fizzy drinks, Ribena and Lucozade, and 48% have at least one helping of chocolate, sweets, biscuits and cake every day. Fifty-six per cent of pupils consume white bread and 52% cornflakes or rice crispies for breakfast. This pattern of dietary practice echoes the findings of Balding's study (Balding, 1987); however, in girls there is lower fat and higher carbohydrate consumption than in Wenlock's survey (Wenlock *et al.* 1986).

In conclusion, two-thirds of pupils knew about the importance of healthy eating in preventing CHD. However, dietary practices did not reflect their knowledge. Health Education programmes in schools should aim to give practical guidance on healthy eating and also to develop skills to implement knowledge.

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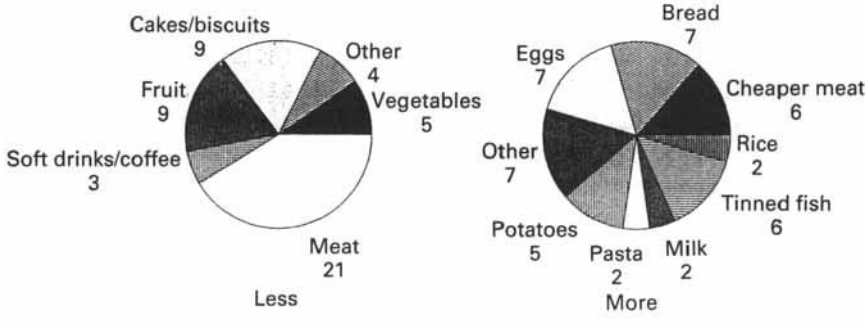
A pilot study to investigate nutritional deprivation among low-income households in London. By PENNY ALLEN and ELIZABETH DOWLER, *Centre for Human Nutrition, London School of Hygiene and Tropical Medicine, 2 Taviton Street, London WC1H 0BT*

Reform of the UK means-tested benefit system in 1988 was designed to target resources to those most in need. Concern has been expressed that the low levels of benefit, and the stringent access rules, mitigate against many low-income households being able to afford a reasonably healthy diet, or even an adequate one. In 1989, a 3-month pilot study was carried out during preparation of a proposal to investigate the nature of nutritional deprivation in such households. The pilot study had two objectives: to assess a semi-structured interview questionnaire to explore food poverty and deprivation among low-income households; and to test the proposed method for contacting low-income households.

The interview questionnaire included five broad areas: household details, budgeting priorities, the food budget, shopping patterns, and eating patterns. Many useful observations about the design and management of the interview schedule were made. Low-income households were contacted through the network of Citizens' Advice Bureaux (CAB), and interviewed by a nutritionist.

Fifty householders volunteered to remain for interview after seeing their CAB officer during July and August, 1989 in two London CAB, and ten householders were interviewed in Homeless Families' Centres. Response rates in the CAB were poor (approximately 16% of clients per day, and averaging 4·2 respondents/half day) because of operational difficulties in using CAB offices. As a result, the main study was redesigned.

The average expenditure on food per week was 37% of income: £33 per household and £14 per person. These figures were said to be very variable, especially in the low-income households. Nearly 60% interviewed said they cut back on food purchases when they were short of money, although they mostly cut out other items first. Nearly 40% said they sometimes ran out of money for food; five households said they did so fortnightly or more often. Most of the latter were 'unemployed childless' or single parents on Social Security benefits. Homeless families and basic State pensioners also had problems buying the quantity and quality of food they wanted. The main items respondents wanted to eat more often were fresh meat, fresh fruit and fresh vegetables. These were also the items most regularly omitted when money was tight (see Figure).



Foods bought less or more when cutting back.

Are practice nurses an effective means of delivering dietary advice as part of health promotion in primary health care? By ALISON KYLE, *Department of Public Health Medicine, Somerset Health Authority, Taunton, Somerset TA2 7PQ*

The introduction of the new GP contract in April 1990 has given the primary health care team (PHCT) greater incentive to carry out health promotion activities. One area of health promotion which many practice nurses (PN) have undertaken is giving dietary advice to patients both in a preventative role and in disease management.

The present study looks at nutrition training of PN within Somerset Family Health Services Authority (FHSA). The aim of the study was to evaluate the efficacy of the dietary training provided and the quality of information being imparted to clients in terms of: skills at imparting simple healthy-eating messages accurately and that are easily understood; giving different dietary advice e.g. weight-reducing or simple diabetic advice; assessing skills at tailoring practical dietary advice to suit individuals; and ability to give positive *v.* negative advice e.g. what they can eat as well as what they cannot.

The different methods used were: 1, basic questionnaire to assess how useful PN found the nutrition training and how applicable it was in their role as health educators (*n* 83); from these eighty-three PN three smaller groups were randomly selected to carry out: 2, a case-study questionnaire to assess knowledge of NACNE/COMA recommendations and understanding of different issues covered in the healthy-eating messages (*n* 10); 3, tape recorded interviews (*n* 10); and 4, observation interviews by a community dietitian (*n* 10).

There was a 78% response rate to the initial questionnaire. Of PN, 74% rated their previous nutritional knowledge as 'average' whereas after training 72% perceived their knowledge to be 'good'. When discussing dietary issues with patients, after training, 33% of PN described their confidence to be 'average', 64% 'good' and 3% 'excellent'. Only one PN felt the information in the training to be too detailed. Input on additional topics and the opportunity to sit in with a clinical dietitian was requested by 31% of PN.

Six case-study questionnaires were returned. In questions requiring a specific answer on healthy-eating issues, 50% of PN gave the correct response and in most cases this was greater than 80%. However, when asked what advice they would give a hypothetical patient there was still a tendency to offer 'blanket' healthy-eating advice rather than tailoring it to the patient.

Tape-recorded interviews proved to be unacceptable to the PN and so these nurses agreed to observations. Fourteen PN were visited who saw a total of eighteen patients and three groups. Using an assessment checklist each PN was given a score of 0–5 where 0= 'of very little value to understanding' and 5= 'very comprehensive advice'. Scores obtained by the PN were 2 (*n* 2), 3 (*n* 12), 4 (*n* 4) and 5 (*n* 3).

Results obtained from this small study would suggest that given adequate training, practice nurses can increase their knowledge and skills to enable them to deliver basic healthy-eating advice. It was highlighted by the PN, however, that this initial training should be followed up by regular study days to ensure PN are kept up to date and have access to expert nutrition advice when needed. This will ensure that nutrition information is based on sound scientific and dietetic principles. In conclusion, PN can be a resource to help promote healthy eating.

Nutritional knowledge and attitudes towards nutrition education in medical students at Southampton University Medical School. By P. HEYWOOD and S. A. WOOTTON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

Whilst several workers have examined the nutritional knowledge and attitudes towards nutrition education in medical schools in the United States (Phillips, 1971; Dugdale *et al.* 1979; Weinsier *et al.* 1988), little attention has been directed towards evaluating nutritional education in UK medical schools. This study was conducted in order to assess the nutritional knowledge of medical students graduating from Southampton University during Oct–Nov, 1989. In particular we were interested to determine how the nutritional knowledge of medical students and their attitudes towards nutrition and nutrition education altered throughout their medical school education.

A questionnaire consisting of twenty-seven statements and thirteen multiple choice questions covering eight major topic areas considered to be essential to the education of medical students (Weinsier *et al.* 1989) was completed by medical students in all 5 years of training (year 1, *n* 94; 2, *n* 96; 3, *n* 87; 4, *n* 50; 5, *n* 51). The respondent was required to indicate whether the statement was true or false, or if they could not confidently identify the correct answer to indicate 'not sure'. Nutritional knowledge was expressed as both 'perceived' and 'actual' knowledge, as described by Dugdale *et al.* (1979). Eight further questions examined attitudes towards the teaching of nutrition. In addition, the questionnaire was also completed by Humanity students (*n* 25), State Registered Dietitians (*n* 61), Junior Medical Staff (*n* 44) and General Practitioners (*n* 52).

The mean nutritional knowledge of Humanity students and Dietitians was 18% (range 0–33%) and 71% (range 49–87%) respectively. The nutritional knowledge of medical students on admission to medical school was greater than that of Humanity students (mean 28%, range 8–59%; $P<0{\cdot}01$) and increased over the initial 3 years of training (Year 3, mean 44%, range 21–77%; $P<0{\cdot}05$). There were no significant differences between the nutritional knowledge of 3rd year medical students and that of final year students, GPs or Junior Medical Staff. Over 70% of medical students and qualified medical staff self-rated their knowledge of nutrition to be 'poor' or 'very poor' and believed that the teaching of nutrition was inadequate; 90% of respondents felt that there should be more taught on the practical application of nutrition support.

From this cross-sectional comparison of nutritional knowledge, it would appear that the nutritional training of the graduating medical student or qualified doctor is inadequate. These findings are similar to those reported in an earlier UK study (Brett *et al.* 1986) and by other workers in the United States (Phillips, 1971; Dugdale *et al.* 1979; Weinsier *et al.* 1988) and suggest that further attention is directed towards the teaching of nutrition within the medical curriculae.

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Attitudes to food allergy: families in Dublin. By S. SUGRUE and N. P. KENNEDY, *Division of Nutritional Sciences, Department of Clinical Medicine, Trinity College, University of Dublin, TCD Medical School Building, St James's Hospital, Dublin 8, Republic of Ireland*

Increased media interest reflects the public concern about food allergy in recent years. That the general public in the UK considers food intolerance to be widespread is indicated by prevalence estimates varying between 15% and 30% (Bender & Matthews, 1981; Burr & Merrett, 1983; Young *et al.* 1987), although these cannot reliably distinguish food allergy from intolerance. This degree of public concern raises the possibility that inappropriate food avoidance practices may be common. This study was designed to estimate the perceived prevalence of 'food allergy' in families in Dublin, to determine the foods most commonly implicated and to explore the extent to which foods are avoided.

Self-administered questionnaires were distributed to 300 families through a sample of primary schoolchildren selected randomly from the 6th class of thirty schools which had been selected by stratified random sampling. The overall response rate was 90% (264 families). Response rates to attitude questions ranged between 71% and 100%. Of respondents, 24% felt that 'food allergy' was very common (affecting 50% of people or more) and 49% felt that it was common (affecting 10–50% of people). 'Food allergy' was attributed to milk by 28% and to food additives by 26%. Less frequently blamed food ingredients were artificial colours (11%), wheat (7%) and E numbers (6%). One or more family members were reported to suffer from 'food allergy' in 44% (106 families, 139 individuals). This represents a perceived percentage of 8% (139 individuals of the 1734 reached by the survey).

Of the 139 individuals suffering from 'food allergy', 39% (54) were self-diagnosed or diagnosed by a parent. Among the wide range of foods avoided by this group were milk in 34% (48), chocolate in 27% (38), artificial colours in 15% (33), sugar in 14% (31), food additives in 14% (31), E numbers in 14% (31), eggs in 10% (22) and wheat in 8% (17). In contrast with other studies, cutaneous symptoms were much more commonly reported than gastrointestinal symptoms. The diagnosis of food allergy was most commonly made (57%) by food exclusion without challenge. Unorthodox methods of diagnosis, such as hair analysis and vega testing (Katelaris *et al.* 1990), were rarely reported.

From this survey, we conclude that the general public of Dublin feel that food allergy is common. One or more individuals in a large proportion of families avoid foods because of food allergy, of whom a substantial number avoid staple food items. The concern remains that many of these individuals may be avoiding foods inappropriately.

S.S. is the recipient of a postgraduate scholarship from the National Dairy Council (Ireland).

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Basal metabolic rate and body composition in Mexican males of different body mass index.

By M. E. VALENCIA and S. Y. MOYA, *Centro de Investigación en Alimentación y Desarollo, Hermosillo, Sonora, Mexico* and G. MCNEILL and P. HAGGARTY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Thirty-two Mexican male adults from diverse social backgrounds in northern Mexico, divided into four body mass index (BMI) groups (<20; 20–25; 26–30 and 31–40: age range 18–40 years), were selected for basal metabolic rate (BMR) and body composition determinations. Weight range was 51·75–118 kg and height was 1·61–1·89 m.

BMR was determined in the subjects after an overnight stay at the unit in which an evening meal was provided at one-third of their estimated energy requirement based on body-weight (FAO/WHO/UNU, 1985). The measurement was performed by means of a ventilated hood system using a Deltatrac metabolic monitor. The system was regularly checked by analysis of recovered gases from propane gas burns: mean (SD) of O_2 and CO_2 recoveries were 99·7 (1·8) and 98·7 (1·6) % respectively. Body composition was determined by ^2H_2O dilution assuming that the ^2H_2O dilution space is 1·04 × body water and that fat-free mass (FFM) consists of 73·2% water. The isotope dilution protocol required a blood sample to be taken after an overnight fast for the determination of background enrichment. Each subject was then given sufficient isotope to raise their ^2H enrichment by 300 ppm. A second blood sample was taken 4 h after the dose, during which time the subject did not eat or drink.

BMR was analysed with body-weight by regression and compared to the values predicted using the FAO/WHO/UNU equations (1985). These equations overestimated measured BMR by 6·71, 8·44, 5·68 and 4·39% in the four BMI groups respectively. The overall difference of 6·3% in all groups was statistically significant when analysed by paired *t* test ($P<0·001$). The BMR prediction equation based on weight obtained for twenty-eight of the thirty-two subjects in the 18–30 age range, when compared to the FAO/WHO/UNU equations for the same age, showed the difference between intercepts to be statistically significant ($P<0·001$) by covariance analysis. BMR between the different BMI groups was not significant ($P=0·82$) when expressed per kg FFM as determined by ^2H_2O dilution.

BMI (kg/m^2)		Weight (kg)		Fat (%)		BMR (kJ/d)		BMR (kJ/kg BW)		BMR (kJ/kg FFM)	
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
18·2	1·4[a]	58·5	6·5[a]	13·7	3·4[a]	6137	386[a]	105·5	6·5[a]	128·1	9·0[a]
23·0	1·4[b]	67·7	8·0[a]	21·7	3·9[b]	6590	869[a]	97·2	2·7[a,b]	125·5	9·3[a]
27·4	1·3[c]	86·1	6·7[b]	29·4	3·4[c]	7748	954[b]	90·0	8·3[b,c]	126·9	11·5[a]
33·9	2·4[d]	101·5	11·5[c]	36·3	5·4[d]	8698	834[b]	86·4	9·9[c]	130·4	11·5[a]

[a–d] Mean values in a vertical column with different supercript letters were significantly different; $P<0·001$.

The overestimation of BMR by the FAO/WHO/UNU equations in these subjects is similar to, if a little less than, those seen in different ethnic groups by Henry & Rees (1988). This may need to be taken into account when estimating BMR from FAO/WHO/UNU prediction equations.

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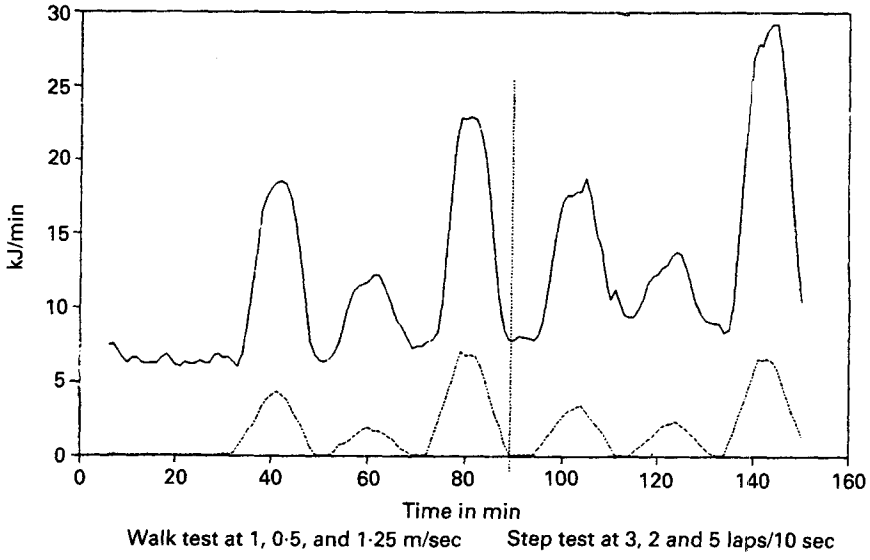
A new approach to the measurement activity and work efficiency in a whole-room indirect calorimeter. By T. J. HORTON, M. SUN and J. O. HILL, *Clinical Nutrition Research Unit, Vanderbilt University, Nashville, Tennessee, USA*

Refinements in the technique of indirect whole-room calorimetry have enabled an expansion of the information relating to energy balance in man. However, one limitation with this technique has been the inability to measure accurately the quantity of physical activity performed by subjects occupying a metabolic chamber. To address this methodological limitation one of our group (MS) designed a force floor which has been incorporated into our whole-room indirect calorimeter. This system enables the accurate measurement of activity whilst simultaneously measuring energy expenditure.

The floor (2·5 × 2·5 m) rests on four force transducers positioned at each of its corners. Both the vertical and horizontal movements of an individual can be measured and the external work performed can be calculated. The energy cost of the external work is computed from the associated incremental change in total energy expenditure measured by the indirect calorimeter. Calibration of the floor, using a mechanical device capable of delivering a known amount of work to the system, has shown it to be highly accurate and reproducible. Combining the floor with the indirect calorimeter enables us accurately to break down daily energy expenditure into its component parts, that is, sleeping metabolic rate, resting metabolic rate, the energy cost of activity and thermogenesis.

A further application of the system is to the measurement of work efficiency. This is done by comparing the mechanical work of an exercise to its energy cost measured simultaneously. The graph below illustrates the results from a preliminary study measuring the energy expenditure (EE) and mechanical work (MW) during different walking and step tasks. These initial results have shown that the work efficiency of walking (31%) is greater than that of stepping (26%). This method can be used to investigate further the work efficiency of different exercises and to measure the efficiency of different groups and changes following various interventions.



Work efficiency of walking and stepping. Energy expenditure, ———; mechanical work, ...

Validation of the doubly-labelled water (DLW) method in growing pigs. By PAUL HAGGARTY, MALCOLM F. FULLER, SUSANNAH L. CHRISTIE, BRIAN A. MCGAW, ERIC MILNE, GARY DUNCAN and JOHN S. SMITH, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The DLW technique is based on a simple principle (Lifson & McClintock, 1966), but there are a number of complicating processes in mammals which have the potential to affect the accuracy of the technique. Having quantified three potentially important sources of error: ^2H incorporation into body fat during growth, ^2H losses in methane, and ^2H losses in the exchangeable groups of faecal solids (Haggarty, 1991), we set out to assess the accuracy of the DLW method before and after correction for these processes.

The CO_2 production (r_{co_2}) of eight Large White cross (Large White × Landrace) pigs was determined over 21 d using the DLW method and, simultaneously, by continuous collection of CO_2 in closed-circuit respiration chambers. Four animals were allowed free access to food, achieving a moderate growth rate (group M) of 491 (SEM 101) g/d and four were fed sufficient food to produce a low growth rate (group L) of 103 (SEM 14) g/d. Fat synthesis was determined from ^2H incorporation into body fat, methane production from the change in composition of the chamber air and faecal dry matter content from complete collection of faeces in the chamber. The values for these variables are given in the Table together with their consequences for the calculation of r_{co_2}.

	Group L		Group M	
	Mean	SEM	Mean	SEM
Start wt (kg)	33·80	3·058	41·65	3·920
End wt (kg)	35·97	3·319	51·96	5·779
Chamber r_{co_2} (l/d)	282	21·6	757	79·4
DLW r_{co_2} (l/d)	264	9·4	636	53·2
Fat synthesis (g/d)	10	0·9	120	32·1
Effect on r_{co_2} (l/d)	−4	0·3	−46	12·4
Faecal dry matter (g/d)	83	3·0	320	47·2
Effect on r_{co_2} (l/d)	−6	0·2	−22	3·3
Methane production (l/d)	2	0·3	6	1·6
Effect on r_{co_2} (l/d)	−1	0·2	−4	1·0

DLW-derived r_{co_2} underestimated the true value by 6% in group M and 16% in group H. After correction for fat synthesis, faecal losses and methane production, the underestimates were 2% and 7% respectively. This validation highlights the need to make appropriate corrections for the known sources of error.

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Validations of dietary assessment using doubly-labelled water. By A. E. BLACK, G. R. GOLDBERG, S. A. JEBB, S. A. BINGHAM, M. B. E. LIVINGSTONE and A. M. PRENTICE, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL* and *Department of Biological and Biomedical Sciences, University of Ulster, Coleraine BT52 1SA*

Daily variation in food intake means that true intake is rarely measured at individual level, but random sampling should give valid measures of mean ('habitual') intake at group level. Most studies of diet and health make the tacit assumption that either a valid measure of 'habitual' food intake is obtained, or that bias operates equally across all subjects and thus within-study comparisons remain valid. Only independent external markers of intake can verify these assumptions.

In weight-stable populations energy intake (EI) must equal energy expenditure (EE). Free-living EE measured by the doubly-labelled water technique (DLW) can thus be used to validate EI. Results from studies measuring both EE and EI are presented.

Study	Method	Wt change (kg)	EE (MJ/d)	EI (MJ/d)	EI:EE Mean	EI:EE SD	EI:EE Range
Prentice *et al.* (1989)							
14 elderly women	7d Ob	N/A	6·10	6·30	1·05	0·17	0·80–1·41
Diaz *et al.* (1991)							
10 men	21d Ob	0·0	13·40	13·30	1·01	0·09	0·09–1·23
Black *et al.* (unpublished)							
7 student volunteers	10d WI(1)	0·95	10·50	10·70	1·02	0·13	0·84–1·18
	10d WI(2)		11·16	10·59	0·96	0·10	0·82–1·05
Prentice *et al.* (1986)							
15 lean women volunteers	7d WI	N/A	8·44	7·97	0·96	0·17	0·63–1·14
Goldberg *et al.* (1991)							
10 lean women volunteers	7d WI	N/A	9·7	9·42	0·98	0·17	0·69–1·30
Livingstone *et al.* (1990)							
16 men R	7d WI	N/A	14·23	11·21	0·81	0·22	0·46–1·40
15 women R	7d WI		9·93	8·00	0·82	0·21	0·45–1·18
Black *et al.* (1991)							
11 post-obese volunteers	10d WI	−0·78	9·73	6·66	0·74	0·17	0·47–0·95
	10d PT		9·73	7·22	0·79	0·22	0·46–1·14
	DH(A)		9·57	6·61	0·64	0·20	0·38–0·98
	DH(B)		9·57	7·66	0·84	0·21	0·55–1·15
Prentice *et al.* (1986)							
9 obese women volunteers	2 × 7d WI	−0·69	10·22	6·73	0·64	0·17	0·38–0·87

R, part of a random sample stratified by a previously-reported energy intake; WI, weighed records; PT, weighed records PETRA system; DH, diet history; Ob, observed.

Where IE was observed by investigators there was good agreement with EE. Lean volunteers on a demanding project showed good agreement between EI and EE; a randomly-selected group showed poorer agreement suggesting poorer motivation to comply; obese and post-obese showed poor agreement, confirming suspicions that these subjects misrepresent their intake. Further work is needed to quantify errors and identify poor compliers.

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Energy intake and basal metabolic rate of children with chronic asthma. By S. ZEITLIN[1], S. A. BOND[2], M. RADFORD[1] and S. A. WOOTTON[2], *Departments of* [1]*Child Health* and [2]*Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

The energy intake and basal metabolic rate (BMR) of pre-pubertal children (twenty-four male, nine female) with chronic asthma was compared with that of thirty-three healthy controls matched for sex and fat-free mass in an attempt to determine the extent to which poor growth in asthmatic children could be attributed to poor energy intake and increased metabolic demand. All asthmatic children received regular inhaled β-agonist therapy; twenty-three received regular inhaled steroid therapy. Energy intake was estimated from 7 d records of weighed food intake using computerized food composition tables, and BMR was determined by indirect calorimetry using a ventilated hood.

The BMR of the asthma group was significantly greater than controls when expressed in absolute units (5·11 (0·12) *v.* 4·79 (0·10) MJ/d; mean (SEM), $P<0·05$) or as a percentage of the BMR predicted (Schofield, 1985) from age, sex and weight (109 (2) *v.* 101 (2)%; mean (SEM), $P<0·001$). Despite the elevated BMR, the energy intakes of the asthmatic group were comparable with that of the control group whether expressed in absolute units (8·23 (0·34) *v.* 7·87 (0·22) MJ/d; mean (SEM), NS) or as energy intake:BMR ratio (1·62 (0·06) *v.* 1·65 (0·04); mean (SEM), NS). Further analysis revealed that these results were not influenced by accounting for differences in stature or steroid usage. These results suggest that whilst the asthmatic children receiving conventional inhaled therapy have a slightly greater metabolic demand at rest than size-matched controls in this study, this would be unlikely to contribute to an energy deficit sufficient to limit growth.

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Energy intake and basal metabolic rate in paediatric oncology patients receiving chemotherapy. By S. BOND[1], J. KOHLER[2] and S. A. WOOTTON[1], *Departments of [1]Human Nutrition and [2]Child Health, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

Previous studies have demonstrated that children with cancer are adequately nourished at diagnosis and that malnutrition in children with cancer may be largely iatrogenic (Smith *et al.* 1990). Attention has been principally directed towards studying the changes in energy intake and expenditure during the initial period of intensive treatment following diagnosis (Kien & Camitta, 1987). The extent to which the energy requirements are satisfied during the longer period of maintenance chemotherapy have not been studied. Energy intake and basal metabolic rate (BMR) were determined in twenty-six children aged 5–16 years receiving maintenance chemotherapy following attainment of complete continuous remission at least 6 months after diagnosis of acute lymphoblastic leukaemia (three female, thirteen male) or solid tumour (seven female, three male).

Energy intake was estimated from 7 d records of weighed food intake using computerized food composition tables, and BMR was determined by indirect calorimetry using a ventilated hood in the week prior to a chemotherapy block. Skinfold callipers were used to estimate fat-free mass (FFM). The results were compared against those of age-, sex- and FFM-matched healthy controls. The results are shown in the Table.

	BMR						Energy intake					
	kJ/d		kJ/kg FFM per d		% predicted		kJ/d		kJ/kg FFM per d		% RDA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Patients receiving maintenance												
Chemotherapy	4873	207	194	7	98	2	7705	459	304	14	85	4
Control	4987	183	197	9	101	3	7773	261	308	14	87	9

There were no statistically-significant differences between the two groups in BMR expressed in absolute units, per kg FFM or as a percentage of that predicted from age, sex and weight (Schofield, 1985), or in energy intake expressed in absolute units, per kg FFM or as a percentage of the current recommended daily allowance (RDA) (Department of Health and Social Security, 1979). Both children receiving chemotherapy and their controls had a mean energy intake: BMR ratio of 1·59. The ranges of 0·96–2·73 (chemotherapy) and 1·23–2·46 (control) indicate the variation within each group. These results suggest that neither food intake nor energy expenditure at rest is altered during the period between chemotherapy blocks in paediatric oncology patients undergoing long-term maintenance chemotherapy.

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Raised resting energy expenditure in subjects with middle-aged onset of Parkinson's disease. By M. COX[1], H. MARCUS[2], R. ABBOTT[1], M. HODKINSON[3] and A. M. TOMKINS[1], [1]*Nutrition Research Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT,* [2]*Department of Neurology, Middlesex Hospital, Mortimer Street, London W1N 8AA* and [3]*Department of Geriatric Medicine, 4 St Pancras Way, London NW1 2PE*

In a previous study we demonstrated that energy expenditure, measured by indirect calorimetry, is elevated among elderly patients who were hypertonic or who had involuntary movements as a result of temporary withdrawal of anti-Parkinson's drug therapy (Levi *et al.* 1990). In the present study we examined a group of middle-aged subjects (four females, eight males, aged 58·0 (SD 9·9) years) who were regularly attending the Department of Neurology at the Middlesex Hospital for control of their Parkinson's disease. Each subject had taken their normal breakfast and anti-Parkinson drugs before 09.00 hours. They did not take their anti-Parkinson drugs thereafter but at the time of measurement of energy expenditure their neuromuscular abnormality was controlled by injections or infusions of apomorphine. A group of healthy middle-aged subjects without Parkinson's disease were also measured (three females, five males, aged 55·0 (SD 5·7) years). Each subject lay under an acrylic hood and energy expenditure was measured using apparatus and calculations as described by Buchdahl *et al.* (1988). Measurements were taken for each Parkinson patient while they were in the untreated 'off' state, and while in the treated 'on' state after subcutaneous apomorphine. Muscle hypertonicity, present in some patients in the 'off' state, was scored (0–3 in each limb). Patients with Parkinson's disease weighed less (mean (SD) 63·0 (7·8) *v.* 73·6 (10·1) kg $P<0·01$), and were thinner (mean (SD) BMI 21·6 (3·16) *v.* 24·4 (2·29) $P<0·04$) than control subjects. The resting energy expenditure of the Parkinson's disease group while treated (mean (SD) 75·76 (12·29) J/kg body-weight per min) was considerably higher than in control subjects (mean (SD) 57·78 (4·24) J/kg body-weight per min) ($P<0·002$). Within the Parkinson's disease group resting energy expenditure was higher in the untreated state (results above; $P<0·05$), and comparing individual subjects the percentage increase in energy expenditure occurring in the untreated state, compared with the treated state, was related to the severity of muscle rigidity seen in the untreated state ($P<0·05$). These results show that energy expenditure is still elevated in patients with Parkinson's disease despite the use of treatment which was sufficient to eliminate any Parkinsonian tremor or involuntary movement. Furthermore, muscle rigidity is likely to be a factor in the additional increase in energy expenditure seen in the untreated compared with the treated state.

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The blood pressure of Gambian children – effects of maternal weight during pregnancy.

By B. M. MARGETTS[1], A. M. CRUDDAS[2], M. G. M. ROWLAND[3], F. A. FOORD[4], T. J. COLE[5] and D. J. P. B. BARKER[2], [1]*Department of Human Nutrition*, [2]*MRC Environmental Epidemiology Unit, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*, [3]*East Anglia Regional Health Authority, Cambridge*, [4]*MRC Dunn Nutrition Group, Keneba, The Gambia* and [5]*MRC Dunn Nutrition Unit, Cambridge CB4 1XJ*

There is increasing evidence that maternal nutrition and fetal growth have a major effect on blood pressure levels and the risk of cardiovascular disease in adult life (Barker *et al.* 1989). The objective of the present study was to relate children's blood pressure levels to their mother's weight in pregnancy. The blood pressures of 675 children aged 1–9 years living in three villages in rural Gambia were measured. In order to reduce observer variation, blood pressure was determined using a DINAMAP (Model 18465X); its relative validity has been determined in comparison with a Hawksley random zero sphygmomanometer (Silas *et al.* 1980). Blood pressure data were matched to antenatal clinic data which had been collected from all pregnant women in the three villages since 1980. Data on maternal weight were available for 351 pregnancies. Further details are published elsewhere (Margetts *et al.* 1991).

Among children under 8 years of age, those born in the dry season had the highest blood pressure and were heavier and their mothers gained more weight in the last trimester of pregnancy. Their blood pressures were positively related to body-weight and to mothers' weight at 6 months of pregnancy. These relationships were independent of mothers' age, parity, birth weight, gestational age and placental weight.

The Table shows mean systolic pressures with children divided into thirds of mother's weight gain in the last three months of pregnancy.

Wt gain (kg) . . .	<1·85			1·85–3·80			>3·80		
Age (years)	*n*	Mean	95% CI	*n*	Mean	95% CI	*n*	Mean	95% CI
1–4	53	93·3	91–96	64	93·4	91–96	59	95·0	92–98
5–7	54	98·0	95–101	34	99·6	95–104	40	97·7	96–100
8–9	11	104·5	99–110	18	104·1	99–108	18	99·3	100–105

The relationship between children's systolic pressure (adjusted for age, body-weight and time since last meal) at ages 8–9 years and mother's weight gain ($P<0·02$) was independent of weight at 7·5 months, mother's height and blood pressure.

An interpretation of these findings is that among young children differences in blood pressure are largely determined by rates of maturation. However, the long-term effects of adverse intra-uterine influences which elevate blood pressure become apparent in older children.

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Effect on birth weight of a community-based supplementation programme for pregnant Gambian women: first year results. By S. M. CEESAY, S. SAIDYKHAN, A. M. PRENTICE, T. J. COLE, K. C. DAY, M. G. M. ROWLAND, L. T. WEAVER and R. G. WHITEHEAD, *MRC Dunn Nutrition Unit, Keneba, The Gambia and Cambridge CB4 1XJ*

We have previously reported that the provision of a groundnut-based biscuit supplement to pregnant Gambian women achieved a highly-significant beneficial effect on birth weight during the annual wet (hungry) season when judged against retrospective controls (Prentice *et al.* 1987). Compliance with the supplement was intensively encouraged and monitored. We now report interim results from a controlled trial of supplementation performed under more realistic field conditions.

Commencing July 1989, twenty-seven villages in the West Kiang region of The Gambia (population 12 000) were randomized into supplement (S; 2 biscuits of 2·1 MJ (508 kcal) and 11 g protein each from the 5th month of pregnancy) or control (C; supplement for first 5 months of lactation but none in pregnancy). Biscuits were distributed in a primary health care setting by traditional birth attendants. Compliance (mean 152 biscuits) was assessed by token exchange. Maternal height, weight (monthly) and parity, and infant birth weight, length, head circumference and gestational age were measured by fieldworkers. After a 6-month start-up period, 1st year birth weight results (S 199 births, C 207 births) were analysed by multiple regression (df 398).

Variable	Coefficient (g)	SE (g)	*t*	*P*
Constant*	2886	569	10·5	<0·001
Female sex	–55	33	–1·7	NS
Parity >0	5	6	0·7	NS
Gestation (weeks)	205	14	14·8	<0·001
Maternal wt (kg)†	13	2	5·7	<0·001
Wet season	–102	49	–2·1	<0·05
Supplement	48	40	1·2	NS
Supplement × wet season	173	69	2·5	=0·013

* Represents; dry season, male sex, 40 weeks gestation, 50 kg maternal weight, first parity.
† Mean value for whole of gestation.

The results indicate a significant increase in birth weight during the nutritionally-stressful wet season. This confirms our earlier highly-supervised trial. Length, head circumference and gestation were not significantly affected. Infant growth and mortality are being monitored. The trial continues for another 2 years. Supplementation of pregnant women may be a realistic primary health care intervention.

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Effect of weaning foods on breast milk intake in Gambian infants. By R. M. DOWNES, A. M. PRENTICE, W. A. COWARD, R. G. WHITEHEAD and L. T. WEAVER, *MRC Dunn Nutrition Unit, Keneba, The Gambia* and *Cambridge CB4 1XJ*

The introduction of weaning foods may suppress breast milk intake by increasing satiety in the infant or by decreasing the suckling stimulus in the mother. At the Dunn's Gambian field station all infants receive an energy-dense weaning food (4·18 MJ/kg; 1000 kcal/kg) initially at 3–6 months. To measure its effect on breast milk intake, infants received the supplement (alternately in order of birth) from either 3–12 months (group E) or from 6–15 months (group L).

Eighty-eight infants were studied: 12 h breast milk intakes were measured monthly from 2–9 months by test weighing in sixty-seven infants; 24 h intakes were measured by D_2O dose-to-mother method at 10, 20 and 30 weeks in twenty-one infants.

There were no significant differences in maternal anthropometry, parity, infant birth weight or gestation length. Infant growth (mean weight-for-age, NCHS standards) was initially faster in group E, but ceased to be so when group L first received the supplement. Growth rates between 6 and 12 months were the same, although group E fell behind group L in the period 12–18 months, when the latter were still receiving supplement. There were no significant differences in other anthropometry between the two groups during the study. The Table shows mean 12 h milk intakes.

Time of supplement . . .	Early (3 months)			Late (6 months)		
Age (months)	*n*	Mean (g)	SEM	*n*	Mean (g)	SEM
2	30	346	12	28	355	19
3	34	356	13	30	372	17
4	31	292*	21	32	348	14
5	29	253**	13	30	374	16
6	28	249**	16	32	342	16
7	36	253	14	28	266	15
8	24	256	17	32	266	15
9	24	230	17	32	237	11

Significantly different from late group (*t* test): *P=0·026, **P<0·001.

There was a significant decrease in milk intake in both groups after introduction of supplement (P<0·01). At 5 months intakes differed by 121 g on 12 h measures (P<0·001) and by 228 g on 24 h measures (P<0·05). When change between 3–5 months was examined for mother–infant pairs, using 24 h data, mean differences were −99 g for group E and +209 g for group L (P<0·001). Greater milk intake at 5 months by group L was due to both increased feed frequency (P<0·03) and average volume (P<0·001). By 7 months there was no difference in milk intake between groups.

Supplementary feeds caused acute suppression of breast milk intake, whether introduced first at 3 or 6 months, but this is not long-lasting. When they are introduced late (at 6 months) infant growth rate is not affected, potentially-contaminated feeds are avoided and infants enjoy optimal intake of immuno-protective breast milk factors.

Effects of maternal tissue protein depletion at parturition on subsequent lactational performance in rats. By A. P. PINE and N. S. JESSOP, *Institute of Ecological and Resource Management, University of Edinburgh EH8 9YL* and J. D. OLDHAM, *Scottish Agricultural College, Edinburgh EH9 3JG*

Maternal protein reserves can be catabolized both during gestation and lactation in rats (Naismith *et al.* 1982). Mahan & Mangan (1975) have suggested that there may be interactions between maternal protein depletion at parturition and response to dietary protein level during lactation. The present study investigated the effect of a depletion in maternal protein at parturition on lactational performance when dietary protein was limiting.

Following mating, forty Sprague-Dawley multiparous rats were offered a high protein (H_G, 215 g crude protein (CP)/kg dry matter (DM) diet *ad lib.* until day 12 of gestation. Subsequently half were offered either the high or a low protein diet (L_1, 60 g CP/kg DM) *ad lib.* until parturition and were then allocated factorially to a high (H_L, 215 g CP/kg DM) or low (L_2, 90 g CP/kg DM) protein diet *ad lib.* until day 13 of lactation. Litters were standardized to 12 pups on day 1 of lactation. Groups of females (*n* 4) were sacrificed for body composition analysis at days 2 and 12 of gestation and 1 and 13 of lactation. All diets were isoenergetic with a constant carbohydrate:fat ratio (2·3:1 in DM).

Dietary sequence from day 12 of gestation–day 13 of lactation

	HH		L_1H		HL_2		L_1L_2	
Lactation effects	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Feed intake (g DM/12 d)	386·2	15·2[a]	390·7	18·9[a]	164·4	10·3[b]	129·6	27·3[b]
Maternal wt change (g/12 d)	−11·6	10·5[a]	10·1	10·1[a]	−109·1	4·4[b]	−85·7	6·2[c]
Maternal gains (g DM/12 d) of:								
Carcass CP*	−0·9	1·5[a]	2·3	1·3[a]	−9·8	1·1[b]	−5·5	1·1[c]
Carcass fat*	−15·3	3·7[a]	−16·9	0·9[a]	−20·2	1·5[a]	−19·3	0·8[a]
Litter wt gain (g/12 d)	264·9	9·3[a]	270·7	15·6[a]	87·5	3·7[b]	61·1	11·0[b]
Days 1–6	97·8	8·2[a]	104·3	7·3[a]	62·0	1·7[b]	34·3	3·7[c]
Days 7–13	167·1	5·3[a]	166·6	8·9[a]	25·5	3·9[b]	26·8	7·6[b]

[a,b,c] Means in the same row with different superscripts were significantly different: $P<0·05$.

* Maternal carcass composition changes adjusted for initial composition.

Carcass protein and fat masses (Mean (SE)) at parturition (g DM) were 44·7 (1·7), 27·5 (2·8) for H_G and 39·3 (1·73), 26·8 (1·8) for L_1. Dams offered L_2 ate significantly less food than those offered H_L, and lost significantly more weight. Those previously offered H_G also lost more weight and protein than those eating L_1. Fat loss during lactation appeared to be unaffected by dietary treatment. Litter weight gain reflected maternal intake patterns. Litters of HL_2 mothers showed a clear biphasic pattern of growth, being significantly greater than L_1L_2 litters during the first half of lactation.

The results suggest that when dietary protein supply is limiting during early lactation rats can mobilize tissue protein, but its influence on lactational performance is limited by the extent of the available reserve.

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Performance of dairy cows of different genetic index offered diets differing in forage: concentrate ratio over a full lactation. By J. D. OLDHAM, H. PARKINSON, P. PERSAUD and G. SIMM, *Scottish Agricultural College, West Mains Road, Edinburgh EH9 3JG*

The nutritional efficiency of milk production, measured as (milk energy):metabolizable energy (ME) intake is presumed to vary for a given diet only in proportion to variations in the partial contributions of maintenance, tissue change and the rate of milk secretion (ARC, 1980). There have been suggestions (Taylor *et al.* 1986) of between-breed differences in maintenance efficiency of lactating cattle and the possibility exists of there being variations in nutritional efficiency, both partial and gross, within breeds.

To study the consequences of selection for fat plus protein (kg) yield in dairy cattle, we are measuring food intake, milk output, weight and condition changes in dairy cows over their first three consecutive lactations with a view to establishing the extent to which there may be real variation in nutritional efficiency within a breed. Results of the first 2 years of this study are reported.

Cows are selected for high (selected) or average (control) weights of combined fat plus protein yield by breeding to bulls of proven merit using AI. Over 2 years, selected (n 59) and control (n 66) animals were offered, *ad lib.*, two complete mixed diets differing in forage:concentrate proportions over 38 weeks of lactation. Diets contained grass silage:draff:concentrates (DM basis) in the ratio either 50:5:45 (high concentrate, HC) or 75:5:20 (low concentrate, LC) and contained, on average, (per kg/DM) 11·7 and 11·3 MJ ME and 174 and 179 g crude protein (CP) respectively. Input and output data were regressed on predicted cow genetic index (CGI). Regressions on predicted CGI were significantly different from zero for milk ($P<0·001$), fat plus protein yield ($P<0·05$), protein % ($P<0·01$) and dry matter intake ($P<0·05$) for HC animals; that for efficiency approached significance. For LC animals, the regressions on predicted CGI were significantly different from zero for milk ($P<0·01$), fat plus protein yield ($P<0·001$) and efficiency ($P<0·001$). From these regressions, values for input and output traits are presented (Table) for cattle of average (500) and above average (700) predicted CGI in the two systems of feeding.

Forage concentrate ratio (DM basis) . . .	55:45		80:20	
Predicted CGI . . .	700	500	700	500
Milk yield (kg)	7032	6164	6030	5309
Fat (%)	4·25	4·48	4·62	4·51
Protein (%)	3·04	3·15	3·04	3·13
Fat plus protein (kg)	512	466	463	406
Dry matter (kg)	4645	4408	4065	4010
Efficiency (MJ/MJ)	0·388	0·366	0·414	0·367

In both feeding systems, high CGI cattle produced more milk, fat and protein and, in gross terms, were more nutritionally efficient. The differences in efficiency, for the LC group in particular, were greater than would be expected from simple dilution of maintenance, although these differences were not significant with the number of observations made thus far.

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Nutrition–endocrine interrelations and growth in suckled lambs. By J. M. BASSETT, *University of Oxford, The Growth and Development Unit, University Field Laboratory, Wytham, Oxford OX2 8QJ*

During natural suckling, the nutrition of the lamb and its growth depend vitally on the milk production of the ewe and are therefore primarily influenced by ewe nutrition and by the number of lambs suckled. However, hormones of the endocrine pancreas also play vital roles in regulating growth and in reordering metabolic priorities among tissues as nutrition varies.

To provide further information about the role of these hormones in modulating nutritional regulation of growth in sucking lambs, jugular blood samples were collected from eighty-two lambs once or twice weekly between birth and weaning at 7 weeks of age. Prefeeding blood samples were obtained from the ewes at the same time. The ewes (Swaledale × Blue Faced Leicester, mated with Suffolk rams) were kept in individual pens and offered water and hay *ad lib.*, with a concentrate ration twice daily. In the 1989 study, twenty-eight ewes (six suckling a single lamb, twenty with twins and two with triplets) received approximately 23 MJ metabolizable energy (ME) and 223 g crude protein (CP) daily, while in 1990, two groups of sixteen ewes suckling twins were offered either 28 MJ ME and 400 g CP, or 45 MJ ME and 730 g CP each day. No attempt was made to restrict access of the lambs to the food, or to the ewes before sampling, so their metabolite and hormone concentrations should be representative of the entire feeding cycle.

Plasma insulin concentrations in the lambs were influenced markedly both by maternal nutrition and by the number suckled. Geometric mean insulin concentrations of the six lambs reared as singles in the first study (274 pmol/l) were fourfold those of the thirty-eight lambs reared as twins (66 pmol/l) and sixfold those of the six triplets (42 pmol/l). In the second study, plasma insulin concentrations of the sixteen twin lambs suckled by ewes fed 45 MJ ME daily (275 pmol/l) were approximately twice those of lambs suckled by ewes offered half the amount of the same concentrate (145 pmol/l). These differences were closely associated with differences in live weight growth.

The rate of live weight gain by all eighty-two individual lambs over the entire suckling period was highly correlated with their mean plasma insulin concentration (r 0·81) and with glucose (r 0·73), but was negatively correlated with plasma free fatty acids (r − 0·65) and growth hormone (r − 0·43), even though GH concentrations in ram lambs were 50% higher than in ewe lambs. Enteroglucagon (r 0·38), pancreatic glucagon (r 0·18) and pancreatic polypeptide (r 0·36), showed little relation to the rate of lamb growth. Plasma insulin in the ewes, by contrast, showed strong negative associations with lamb live weight gain during the first month of lactation in each of the separate groups (1989, r − 0·73; 1990, r − 0·89 and r − 0·81).

Despite the range of maternal nutrition and numbers of lambs suckled, the pattern of changes with age in plasma and pancreatic hormone concentrations in the lambs during each of the studies was similar. Glucose and insulin concentrations were highest 1–2 weeks after birth and declined steadily until weaning, while pancreatic polypeptide and glucagon increased steadily from a nadir 1–2 weeks after birth. The rate of gain by the lambs also declined after the first 3 weeks as insulin concentrations declined.

These observations reinforce the significance of insulin secretion as a modulator of nutritional effects on growth during the suckling period.

Effect of rearing neonatal lambs in a cold or warm environment on thermogenesis during slow-wave sleep. By C. J. DARBY, L. CLARKE, M. A. LOMAX and M. E. SYMONDS, *Department of Biochemistry and Physiology, University of Reading, Whiteknights, Reading RG6 2AJ*

A failure to maintain normal thermoregulatory control mechanisms is a major cause of mortality in neonatal lambs. This may be associated with the transition from nonshivering (NST) to shivering (ST) thermogenesis in response to acute changes in ambient temperature (Ta) over the first 2 weeks of life in the lamb, as brown adipose tissue is replaced by white adipose tissue (Symonds *et al.* 1989). The following study investigates the extent to which metabolic rate and the ability to respond to warm and cold challenges is altered by rearing lambs in warm or cold environments.

Eight sets of twins (mean (SEM) birth weight 5·50 (0·21) kg), all born normally at term, were removed from the ewe on the morning after birth (Day 1) and one lamb was then reared in the warm (25°, WR) and its twin in the cold (15°, CR). They were fed daily a 2 litre volume of milk containing 200 g milk replacer (VOLAC LAMLAC, Royston, Herts), and attained a mean growth rate of 80 g/d over the study period. Oxygen consumption ($\dot{V}_{O_2}$) was then measured during slow-wave sleep after exposure to warm or cold Ta for at least 1 h on day 1 and days 7–8 of life.

	Oxygen consumption (ml/min per kg)							
	Day 1				Day 7			
	Warm (27·7°)		Cold (14·4°)		Warm (25·6°)		Cold (11·7°)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
WR	12·48	0·68	19·92	1·24	14·35	0·70	19·71	1.94
CR	16·37	1·34	21·09	1·15	16·83	1·02	20·36	1·04

CR lambs exhibited a significantly higher ($P<0·05$) $\dot{V}_{O_2}$ at a warm Ta than WR controls. Both groups had a similar $\dot{V}_{O_2}$ during cold exposure, but CR lambs showed a 50–60% ($P<0·05$) lower $\dot{V}_{O_2}$ response to cold exposure. At 1 d of age three WR and two CR lambs responded to cold via ST, but by 7 d, seven WR compared to three CR animals responded via ST.

It is concluded that rearing lambs in a warm environment does not alter their ability to increase metabolic rate in response to an acute cold challenge. At 7 d of age, however, WR animals adopted ST rather than NST, suggesting a more rapid loss of brown adipose tissue in this group. The level of GDP-binding to uncoupling protein in isolated mitochondrial preparations was 54% higher in CR lambs at 8 d of age (WR 123 (SD 21) and CR 189 (SD 31) pmol/mg protein; $P<0·01$).

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

Total energy expenditure of free-living Chinese infants and toddlers in Hong Kong from 6 months–2 years. By S. S. H. LUI, S. S. F. LEUNG and F. H. Y. YUEN, *Department of Paediatrics, The Chinese University of Hong Kong, Hong Kong* and W. A. COWARD, *MRC Dunn Nutrition Centre, Cambridge CB4 1XJ*

It was suggested in the 1985 WHO/FAO/UNU Expert Committee Report (FAO/WHO/UNU, 1985) that estimates of energy requirements should be based on measurements of energy expenditure. The availability of the doubly-labelled water (DLW) method provides the first non-invasive means of assessing total habitual energy expenditure in free-living subjects. In 1989, forty-two healthy, bottle-fed Hong Kong Chinese infants and toddlers were studied. ^{18}Oxygen (2·8 g/kg body-weight of 10% ^{18}O) and deuterium (0·11 g/kg body-weight of 99% $^{2}H_2O$) were administered orally after which urine samples were collected daily for the following 7–10 d. Baseline urine samples were collected prior to isotope administration. Total energy expenditure (TEE) was calculated according to Weir's equation (Weir, 1949). Food quotient was used instead of respiratory quotient. Ethical approval was obtained from The Chinese University of Hong Kong and MRC Dunn Nutrition Centre.

It was found that there was a gradual rise of energy expenditure (EE) from 0·34 MJ (82 kcal)/kg per d at 6 months to 0·46 MJ (110 kcal)/kg per d at 18 months (see Table) suggesting an increase in physiological need of energy for more physical activity.

Energy expenditure (EE) of study children in Hong Kong

		EE (MJ (kcal)/kg body-wt)		Weight (kg)		Length (cm)	
Age (months)	*n*	Mean	SD	Mean	SD	Mean	SD
6	8	0·34 (82)	0·11 (26)	7·18	0·72	65·66	2·2
9	6	0·38 (90)	0·07 (17)	8·08	0·90	69·12	3·1
12	4	0·45 (108)	0·11 (26)	8·91	1·04	72·70	2·9
15	7	0·44 (105)	0·14 (33)	9·29	0·23	75·90	1·5
18	5	0·46 (110)	0·07 (18)	9·69	0·66	77·64	1·9
21	8	0·42 (100)	0·06 (15)	10·28	0·88	81·64	2·3
24	4	0·39 (94)	0·08 (20)	10·45	0·49	84·05	1·1

Between 6–9 months, the EE of the study infants was lower than the current WHO recommendation for energy requirement (FAO/WHO/UNU, 1985), but comparable to those reported by Prentice *et al.* (1988) and Davies *et al.* (1989). Between 12–18 months the EE of the Hong Kong Chinese children was comparable to the 1985 FAO/WHO/UNU recommendation but higher than that reported by Prentice *et al.* (1988).

This study supports Prentice *et al.* (1988), that the recommendations for energy requirement by the FAO/WHO/UNU (1985) is over-generous before 9 months and after 21 months of age, but too low for the Hong Kong Chinese children between 12–18 months.

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The effect of morbidity on the growth of stunted and non-stunted children. By S. P. WALKER, C. A. POWELL, S. M. GRANTHAM-MCGREGOR and D. T. SIMEON, *Tropical Metabolism Research Unit, University of the West Indies, Kingston, Jamaica* and J. H. HIMES, *Division of Human Development and Nutrition, University of Minnesota, Minneapolis, USA*

The long-term effect of morbidity on growth remains controversial. Most studies have not addressed the effect of catch-up growth following illness or the confounding effect of socio-economic status.

We have measured the growth and morbidity of 129 stunted children and twenty-one non-stunted children for 2 years. Children aged 9–24 months were recruited by a survey of poor areas in Kingston, Jamaica. Stunted children were randomly assigned to receive nutritional supplementation or not. The supplement comprised 1 kg/week milk-based formula providing 3·1 MJ (750 kcal)/d if taken completely. Weight and length were measured every 2 months. A history of any symptoms of illness was taken weekly by paraprofessionals. The effect of morbidity on growth was analysed by multiple regression using 2-month child intervals (n 1759), with change in either weight or length as the dependent variables. The number of days ill with apathy, fever, anorexia, nasal discharge, cough, tachypnoea/dyspnoea, ear infections, vomiting or diarrhoea were entered in separate regressions controlling for sex and initial age. The mean effects of symptoms significantly related to growth are shown in the Table.

	Apathy	Fever	Anorexia	Tachypnoea/ dyspnoea	Diarrhoea
Wt (g/d ill)	−13·2**	−15·7***	−3·8*	−3·6*	−5·0*
Length (mm/d ill)	−0·25**	−0·007	−0·004	−0·004	−0·11*

*$P<0·05$, **$P<0·01$, ***$P<0·001$.

When all morbidity variables were offered stepwise, only fever was significant in the regression of weight gain, and apathy in the length regression. The unique effect of diarrhoea approached significance ($P<0·07$) for weight and length. Nutrition group (stunted or not), supplementation group, toilet and water facilities, and crowding did not contribute significantly to any of the regressions.

To determine whether there was a long-term effect of morbidity on growth, the regressions were repeated using weight and length change over 4 month intervals. Weight gain was reduced if apathy, fever and anorexia occurred in the second 2 months of the interval. Neither gain in weight nor length was affected by any morbidity in the first 2 months. Thus, the effects of morbidity on growth in this population were transient.

Brown fat-specific mitochondrial uncoupling protein in adipose tissues of neonatal goats.

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Although brown adipose tissue (BAT) is widely recognized to play a significant role in thermoregulation and the regulation of energy balance in small rodents, its presence and function in large, agricultural, species is much less well established. This is partly because the primary criterion for determining whether an adipose tissue is functionally 'brown' or 'white' is now considered to be biochemical rather than histological, BAT being identifiable by the presence of the tissue-specific mitochondrial uncoupling protein (UCP), M_r 32 000.

Recent studies have demonstrated that UCP, and hence BAT, is present in newborn lambs, cattle, red deer and reindeer (Casteilla *et al.* 1987; Soppela *et al.* 1991). Studies based on general histological appearance, together with the ion conductance of mitochondria, have suggested that BAT may also be present in the neonatal goat, at least in the perirenal region (Vatnick *et al.* 1987). We have now examined several adipose tissue depots of newborn goats for the presence of UCP, using immunoblotting procedures, to establish whether BAT is indeed present in this ruminant species.

Male goats, British Saanen breed, were obtained from the Rowett herd within 12 h of birth and at 7 d of age (four goats each). Adipose tissues were removed from five distinct sites: perirenal, pericardial, omental and subcutaneous (hind limb and neck regions). The tissues were frozen in liquid nitrogen and stored at –80°. Mitochondria were isolated and mitochondrial proteins separated according to molecular weight (Mwt.) by SDS-polyacrylamide gel electrophoresis. The proteins were blotted onto nitrocellulose membranes, and probed for the presence of UCP using a rabbit anti- (ground squirrel UCP) serum (Soppela *et al.* 1991). Antigen–antibody complexes were detected with a goat anti-(IgG) horseradish peroxidase-linked conjugate.

Examination of the immunoblots indicated that immunoreactivity at the 32 000 M_r position characteristic of UCP was present in mitochondria isolated from each of the adipose tissue depots, including the subcutaneous, of newborn goats. No immunoreactivity was evident at any other Mwt. Immunoreactivity consistent with UCP was also present in the adipose tissue depots at 7 d of age, but the amount of the protein was reduced relative to the newborn.

This study confirms, on the basis of the immunological identification of UCP, that BAT is present in the neonatal goat. It also indicates that even subcutaneous adipose tissues are functionally 'brown' in newborn goats, an observation consistent with recent reports identifying BAT in the subcutaneous region of other ruminants at birth (Casteilla *et al.* 1987; Soppela *et al.* 1991).

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Genetic variation in diet-induced thermogenesis in birds. By P. A. GERAERT and SOLANGE GUILLAUMIN, *Station de Recherches Avicoles INRA, 37380 Nouzilly, France* and A. BORDAS and P. MERAT, *Laboratoire de Génétique Factorielle INRA, 78350 Jouy-en-Josas, France* (Introduced by M. G. MACLEOD)

From a Rhode Island Red population, two experimental lines, R+ and R−, have been divergently selected since 1976 for high or low residual feed intake. The residual feed intake is determined as the individual variation from the regression of feed intake on body-weight and weight gain in adult males, plus egg mass in females. In 1989, observed feed intake differed by 40% for males for the same body-weight and 20% for females for the same egg production.

Energy metabolism of twelve adult cockerels of each line was investigated using an automated indirect calorimetry system with six respiratory chambers. Birds were fed *ad lib.* a standard complete diet containing 12·84 MJ metabolizable energy (ME)/kg and 126 g crude protein per kg. The results are presented in the Table.

	R−		R+		
	Mean	SE	Mean	SE	
Body-wt (W; kg)	3·281	0·071	3·338	0·044	NS
Food intake (g/d)	80	2	112	5	$P<0·001$
ME intake (kJ/kg $W^{0·75}$ per d)	425	17	596	24	$P<0·001$
TME/GE (kJ/kJ)	0·82	0·02	0·80	0·01	NS
Heat production (kJ/kg $W^{0·75}$ per d)					
Fasted	418	10	488	21	NS
Fed	507	15	652	26	$P<0·01$

ME, metabolizable energy; TME, true ME; GE, gross energy, NS, not significant.

Heat increment or diet-induced thermogenesis (DIT) was significantly enhanced in R+ cockerels; +84% when expressed as the difference between fed and fasted heat production and +31% when calculated as per cent of ME intake ($P<0·01$). Using a force-feeding technique to reduce physical activity associated with feed intake, similar results were obtained. Moreover, oral administration of a β-blocking agent (DL-propranolol, 5 mg/kg W) decreased fed heat production of force-fed R+ birds by 15% without any effect on the R− birds, suggesting the existence of a regulatory component of DIT in R+ birds as observed in mammals (Rothwell & Stock, 1981).

Surprisingly, while plasma thyroxine (T_4) level was not significantly different between genotypes, plasma triiodothyronine (T_3) level appeared lower in fasted R+ birds than in R− birds (2·19 *v.* 1·65 ng/ml, $P<0·01$) and equal in fed birds of both lines (2·22 *v.* 2·09 ng/ml, NS), whereas thermogenesis was always higher in the less efficient line (R+). These experimental R+ and R− lines appear to be an interesting model for investigating energy expenditure, and particularly diet-induced thermogenesis and its regulation in birds.

Rothwell, N. J. & Stock, M. J. (1981). *Annual Review of Nutrition* **1**, 235–256.

The influence of genotype, diet and stage of lactation on erythrocyte ATPase activity in dairy cattle. By A. P. PINE and N. S. JESSOP, *Institute of Ecological and Resource Management, University of Edinburgh, Edinburgh EH8 9YL* and J. D. OLDHAM, *Scottish Agricultural College, Edinburgh EH9 3JG*

Evidence suggests that dairy cattle of different milk yield potential may vary in the efficiency with which metabolizable energy is used for maintenance (Taylor *et al.* 1986). As ion transport is a major contributor to metabolic maintenance (Milligan & Summers, 1986), differences in maintenance efficiency may be manifested in different rates of ion transport activity. Using the activity of erythrocyte Na^+,K^+-ATPase (*EC* 3.6.1.3) as a measure of membrane transport we have made an exploratory study on the influence of genotype, diet and stage of lactation on this process. The activity of Mg^{2+}-ATPase (*EC* 3.6.1.4) was also measured.

First lactation heifers from the selected (*n* 18) and control (*n* 14) genetic lines within the Langhill dairy herd were used. Approximately half (*n* 17) received a high forage diet (0·8 kg forage dry matter (DM)/kg total DM) and the remainder a high concentrate diet (*n* 15, 0·55 kg forage DM/kg total DM). Jugular venous blood samples were taken at weeks 8, 19 and 36 post-partum. Erythrocyte membranes were prepared using a modification of the procedure developed by Wheeler & Whittam (1964) and the activities of Na^+,K^+-ATPase and Mg^{2+}-ATPase were measured (Gilbert & Wyllie, 1976).

Erythrocyte ATPase activities (μmol Pi/mg protein per h)

Dietary forage: concentrate ratio . . .	80:20				55:45				Pooled			
	Na^+,K^+-ATPase		Mg^+-ATPase		Na^+,K^+-ATPase		Mg^+-ATPase		Na^+,K^+-ATPase		Mg^+-ATPase	
Week	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
8	0·118	0·028[a]	0·689	0·108[a]	0·163	0·029[a]	0·702	0·139[a]	0·142	0·020[a]	0·697	0·089[a]
19	0·076	0·014[a]	0·334	0·045[b]	0·114	0·019[a]	0·374	0·058[b]	0·096	0·012[b]	0·356	0·038[b]
36	0·045	0·007[b]	0·293	0·025[b]	0·065	0·015[b]	0·241	0·037[b]	0·056	0·009[c]	0·264	0·023[c]

[a,b,c] Values within columns with different superscript letters were significantly different: $P<0·05$.

Both Na^+,K^+- and Mg^{2+}-ATPase activities decreased significantly with stage of lactation ($P<0·05$) suggesting that they reflect increased energy expenditure during early lactation. Diet had no effect on ATPase activities. There was no correlation between enzyme activity and predicted cow genetic index or elements of lactational performance including milk yield and DM intake.

The lack of association in heifers between enzyme activity and genotype suggests that Na^+,K^+-ATPase is not an indicator of lactational potential, although this may change in subsequent lactations.

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Effects of tumour necrosis factor-α (TNF-α) on lungs and liver in mice are modified by fats of varying saturated, monounsaturated and polyunsaturated fatty acid content. By S. BASHIR and R. F. GRIMBLE, *Department of Human Nutrition, Southampton University, Bassett Crescent East, Southampton SO9 3TU*

Activated macrophages produce cytokines among which is tumour necrosis factor-α (TNF-α). While cytokines are beneficial in combating infections, excessive exposure to some, particularly TNF-α, can be deleterious. The cytokine has been implicated in increased mortality following sepsis and in adult respiratory distress syndrome (ARDS) which accompanies that condition (Grimble, 1990). We have demonstrated that fats rich in *n*-3 polyunsaturated fatty acids (PUFAs) or poor in linoleic acid can modify the intensity of the acute-phase response to TNF-α in rats (Mulrooney & Grimble, 1991). In the present study we examine the effects of a range of fats on changes in lung and liver protein metabolism of mice in response to TNF-α. Nutritionally-adequate diets, similar in all respects except for the fat source (100 g/kg), were fed for 7 weeks to weanling MF1 mice. The fat in each diet was maize oil (high in linoleic acid), or coconut oil (CO, high in short-chain saturates and low in linoleic acid), or suet (SU, high in medium-chain saturates and monounsaturates, low in linoleic acid) or fish oil (FO (MaxEPA), high in *n*-3 PUFA and low in linoleic acid). The latter three diets contained 10 g maize oil/kg among the total fat to prevent essential fatty acid deficiency. Half of each dietary group received 30 μg recombinant human TNF-α (endotoxin content <2·9 pg/mg protein) intra-peritoneally, the other half received sterile non-pyrogenic saline (9 g sodium chloride/l). The latter groups were pair fed the intakes of the TNF-α groups.

Twenty-four h after injection, fractional rates of protein synthesis (FSR) and protein content of lungs and liver, zinc content of liver and serum caeruloplasmin (CP) were measured (Schosinsky *et al.* 1976; Mulrooney & Grimble, 1991).

Dietary fat . . .	Maize oil		Coconut oil		Suet		Fish oil	
Injection (*n* 6/group) . . .	Saline	TNF-α	Saline	TNF-α	Saline	TNF-α	Saline	TNF-α
Liver FSR (%/d)	82	99	117	99	95	78	91	81
Lung FSR (%/d)	16	35**	15	27*	22	20	24	11**
Liver protein (mg/g)	153	159	141	150	153	141	169	152
Liver Zn (μg/g)	35	42*	38	39	39	38	42	46
Lung protein (mg/g)	177	207**	190	199	200	204	231	190**
CP (units/l)	64	100*	64	149**	53	113**	70	97

Significantly different from saline group by ANOVA, *P<0·05, **P<0·01.

The response of liver and lung to TNF-α was modulated to different extents by the range of fats fed. While the increase in liver Zn was blocked by all fats with a low linoleic acid content, that of CP was only affected if the fat was rich in *n*-3 PUFA. The change in lung protein FSR was also reduced by all fats low in linoleic acid, but other fatty acid characteristics exerted a further influence leading to reduction in the case of CO, total suppression in the case of SU and reversal in direction in the case of FO.

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Oleic acid as a determinant of the difference between the suppressive effects of coconut oil and butter on responses to tumour necrosis factor α (TNFα) in rats. By HILDA MULROONEY and R. F. GRIMBLE, *Department of Human Nutrition, Southampton University, Bassett Cresceni East, Southampton SO9 3TU*

Fats low in linoleic acid suppress responses to TNFα (Mulrooney & Grimble, 1991). However, major differences in the degree of suppression were apparent between rats fed coconut oil and butter. Oleic acid (C18:1*n*-9) is the major monounsaturated fatty acid of butter (21·5% compared with 6·2% of coconut oil). This study examined whether oleic acid content is the major factor responsible for the differences in suppression of response.

Forty-eight male weanling Wistar rats (mean (SD) weight 63 (2) g), were fed *ad lib.* for 8 weeks on one of four 10% lipid synthetic diets, maize oil- coconut oil- oleic oil- or butter-based. The oleic acid diet contained coconut oil supplemented with oleic acid (21·5% of total lipid). Diets were isoenergetic and identical in all constituents; all contained 10 g maize oil/kg to prevent essential fatty acid deficiency. Half of each group received 100 μg TNFα/kg body-weight intraperitoneally; the other half received saline (9 g sodium chloride/l, pair-fed controls). After 24 h, protein synthetic rates were measured in the livers, lungs and kidneys using a modified flooding-dose method (Jepson *et al.* 1986). Protein and zinc content of the tissues were measured as previously described (Mulrooney & Grimble, 1990). Plasma albumin and complement (C3) were measured using rocket immunoelectrophoresis and radial immunodiffusion respectively. Caeruloplasmin was measured colourimetrically (Schosinsky *et al.* 1974).

Dietary oil . . .	Maize		Coconut		Oleic		Butter	
Injection . . .	Saline	TNFα	Saline	TNFα	Saline	TNFα	Saline	TNFα
Liver FSR (%/d)	56·8	106***	52	77·8*	62	73	65	51**
Liver Zn (μg/g)	35	43·4**	33	40·5***	27·4	29	33·4	36
Albumin (mg/ml)	13·5	10·3*	16·4	13·3	16·3	19·4*	17	16·3
C3 (mm)	18	23**	27·5	25	27	28·7	19·6	32***
Caeruloplasmin (U/l)	23	81·5**	38·6	94***	23	50·5***	30·5	57·2
Lung FSR (%/d)	41·1	54·3**	24·3	74·2***	35·3	41·3	25·9	27·1

Significantly different from own saline control (one-way ANOVA): *$P<0·05$, **$P<0·01$, ***$P<0·001$.

The response of the butter- and oleic acid-fed animals was similar. This suggests that oleic acid content is a major determinent of the differences in suppression by fats with a low linoleic acid content. In general, an oleic acid-supplemented diet diminishes the severity of response, and in cases where the production of TNF is inappropriately increased, this fatty acid may be a useful supplement to the diet.

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Changes in basal metabolic rate, fasting respiratory quotient and body fat following energy supplementation of chronically-undernourished individuals. By M. J. SOARES, R. N. KULKARNI, L. S. PIERS, M. VAZ and P. S. SHETTY, *Nutrition Research Center, Department of Physiology, St John's Medical College, Bangalore 560 034, India*

This study examined the changes in basal metabolic rate (BMR) and body composition following acute perturbations in energy intake, in order to assess their physiological importance to chronic undernutrition. Seven, apparently healthy, free-living, chronically-undernourished subjects were supplemented with 3·35 MJ/d (15 g protein, 34·5 g fat and 105 g carbohydrate) for 12 weeks. Measurements of BMR, body-weight and body fat (four skinfold measurements, Durnin & Womersley, 1974) were made before, and serially during the 12 weeks of supplementation. Five subjects were followed up to 12 weeks post cessation of supplementation. All results of serial measurements were analysed by analysis of variance of repeated measures.

	Baseline data		Week of supplementation								12th week post cessation (*n* 5)	
			3		6		9		12			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body-wt (kg)	43·5	2·18	44·8†	2·59	45·4†	2·62	45·3*	2·43	45·3	3·00	44·6[a]	2·78
Fat (kg)	4·80	0·70	5·40*	1·03	5·90**	1·03	5·90	1·27	5·80	1·55	5·7	1·27
Fat-free mass (FFM) (kg)	38·68	2·22	39·4**	2·25	39·5*	2·15	39·4	1·83	39·5	2·41	38·9†	2·49
Respiratory quotient	0·97	0·08	1·08	0·11	1·17*	0·15	1·13*	0·10	0·98*	0·10	0·88	0·04
BMR/kg FFM (kJ/kg per d)	124·3	6·20	132·3**	5·73	137·5†	7·63	143·4†	6·25	148·5*	13·8	129·8†	0·89

[a] $P = 0·06$, $*P<0·05$, $**P<0·01$, $†P<0·005$.

There were significant increases in body-weight up to week 6, due to increases in body fat and fat-free mass (FFM) in the ratio 58% to 42% respectively. During the 12 weeks of supplementation, BMR was higher than that accounted for by the increases in FFM. This would indicate an increase in the metabolic activity of FFM. An added cost of lipogenesis in the fasting state, denoted by a fasting respiratory quotient (RQ) > 1·00, may also have contributed to this increase in BMR (Schutz *et al.* 1982). When RQs rose above 1·00, a significant increase in body fat could be demonstrated and when subjects showed no further accretion of body fat (i.e. by week 9), the RQs declined to basal values.

Twelve weeks after cessation of supplementation, BMRs were lower even when expressed per kg FFM. This suggests a 'metabolic economy' during the negative energy balance phase, in these individuals. The study demonstrates that changes in the BMR of chronically-undernourished individuals are reversible and hence physiologically important in their adaptation to low energy intakes. The demonstration of a lower BMR when expressed per kg FFM, in chronically-undernourished subjects, probably denotes a metabolic response to an acute or chronic deficit in energy intake.

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The effect of changes in dietary carbohydrate *v.* fat intake on 24 h energy expenditure and nutrient oxidation post-menopausal in women. By G. MCNEILL, D. C. MORRISON, L. DAVIDSON and J. S. SMITH, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

To investigate the effect of altering energy intake in the form of carbohydrate (CHO) while maintaining fat and protein intake, five women age 65–71 years, weight 50·65–87·05 kg and per cent body fat (by 4 site skinfold thickness) 28·0–42·8 were studied on a 5-week residential protocol (McNeill *et al.* 1989). In weeks 1, 3 and 5 they received a diet containing 45% energy (% En) as CHO, 40% En as fat and 15% En as protein, in amounts designed to maintain energy balance for each woman. The mean (SD) metabolizable energy (ME) intake for these weeks was 8607 (874) kJ/d, with 247 g CHO (of which 97 g were sugars), 91 g fat and 75 g protein. In week 2, the energy intake was increased by 40% by the addition of CHO only, while in week 4, the energy intake was reduced by 40% by the removal of CHO only. Twenty-four h energy expenditure (EE) was measured on day 7 of each week by whole-body indirect calorimetry. Twenty-four h nutrient oxidation patterns were determined from O_2 consumption, CO_2 production and urinary nitrogen excretion on the 7th day. Body-weight was measured to the nearest 50 g after voiding but before breakfast, wearing standardized clothing, on the first morning of each week. The results were compared with results for the same subjects from a previous study in which fat intake was altered and CHO and protein intake held constant on an identical protocol.

The Table shows the changes in weight, 24 h EE/kg body-weight and nutrient oxidation patterns on day 7 for changes of CHO and fat intake.

	Changes on overfeeding				Changes on underfeeding			
	CHO		Fat		CHO		Fat	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Wt (kg)	+1·35	0·24	+0·84	0·21	−1·70	0·30	−0·72	0·20
24 h EE/kg (%)	+4·17	2·83	+2·85	2·20	−0·71	2·38	−0·93	1·75
CHO oxidation (g)	+203·2	38·9	−19·2	30·0	−164·3	86·8	−1·0	19·8
Fat oxidation (g)	−62·3	17·2	+19·1	10·4	+55·6	20·8	−8·2	7·9
Protein oxidation (g)	−10·0	7·7	−0·5	8·6	+14·6	4·5	+0·4	8·0

Weight changes were more marked for changes in energy intake as CHO in both overfeeding ($P<0·05$) and underfeeding ($P<0·001$), probably reflecting changes in CHO stores. The changes in 24 h EE/kg on overfeeding were significantly different from zero ($P<0·05$ for both CHO and fat) but there was no significant difference between CHO and fat. The changes in 24 h EE on underfeeding were not significantly different from zero for either protein or fat. Nutrient oxidation patterns on day 7 were markedly affected by changes in CHO intake, while alterations in fat intake produced little effect. The results suggest that large changes in CHO or fat oxidation due to dietary manipulation have relatively little effect on energy balance after 7 d.

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Energy balance in healthy elderly women. By J. J. REILLY[1]*, A. LORD[1], V. W. BUNKER[1], A. M. PRENTICE[2], W. A. COWARD[2], A. J. THOMAS[1] and R. S. J. BRIGGS[1], [1]*University of Southampton Department of Geriatric Medicine, Southampton General Hospital, Southampton SO9 4XY* and [2]*MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

There is a paucity of data on which to base estimates of the energy requirements of the elderly but, in general, ageing appears to be associated with a reduced energy requirement arising from loss of fat-free mass (FFM) and reduction in physical activity (McGandy *et al.* 1966).

We report measurements of total energy expenditure (TEE) using doubly-labelled water, resting energy expenditure (REE) using a portable indirect calorimeter, the energy expended on physical activity plus thermogenesis (TEE–REE) and energy intake (EI) from 3 d weighed diet record in ten healthy elderly women in Southampton, mean age 73 (SD 3) years. Results are shown in the Table.

	Mean	SD	*n* 10
Wt (kg)	60·0	7·2	
Fat mass (kg)*	21·7	3·9	
FFM (kg)*	38·3	3·6	
EI (MJ/d)	6·71	1·29	
TEE (MJ/d)	9·21	1·48	
REE (MJ/d)	5·11	0·38	
TEE–REE (MJ/d)	4·12	1·19	

* Fat and fat-free mass estimated from total body water.

Rates of TEE and the energy expended on physical activity were higher than those observed in some studies of younger adults in the UK, and higher than the factors currently used to estimate the RDA for energy in the elderly. This supports the hypothesis that ageing has variable effects on body composition, physical activity and, hence, energy requirements.

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