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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at Trinity College, Dublin on Wednesday–Saturday, 15–18 July 1992, when the following papers were presented.

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Faecal losses of retinol and α-tocopherol in subjects with cystic fibrosis and healthy controls. By PENNY J. HALFORD and A. A. JACKSON, *Department of Human Nutrition, University of Southampton SO9 3TU*

It has been presumed that the defective absorption of vitamins A and E in cystic fibrosis (CF) is a direct consequence of fat malabsorption due to pancreatic insufficiency. Few workers have quantified the faecal losses of vitamins A and E in CF children.

Ten children aged between 3–16 years with CF and ten age- and sex-matched healthy controls completed a 7 d weighed food intake and 3 d stool collection between carmine markers. All CF children were stable at the time of study and were receiving pancreatic enzyme replacement therapy and daily supplements of vitamins A and E. Stool samples were analysed in triplicate simultaneously for vitamins A and E using a method based on normal-phase HPLC with UV detection. Faecal fat was measured using the method of Gompertz & Sammons (1963).

	SWt	Faecal retinol		Faecal α-tocopherol	
	(g/d)	(μg/g)	(μg/d)	(mg/g)	(mg/d)
Controls	59	0	0	0·04	5·3
Cystic fibrosis	108*	0·12***	27·6***	0·14*	34·0**

SWt, stool weight.
Significantly different from controls: *$P<0·05$; **$P<0·001$; ***$P<0·0001$.

No significant differences were seen in the energy and fat intakes of the two groups. Of the CF children, 70% had steatorrhoea as assessed by a faecal fat output of greater than 5 g/d. The children with CF were ingesting significantly larger amounts ($P<0·005$) of retinol (2023 *v.* 583 μg/d) and α-tocopherol (105 *v.* 6 mg/d) compared with controls. In CF children, 75% of retinol intake and 95% of α-tocopherol intake was in the form of vitamin supplements. Without vitamin A supplements only 50% of the CF children would have met recommended nutrient intakes. There was a significantly larger percentage of retinol intake ($P<0·0005$) and smaller percentage of α-tocopherol intake ($P<0·05$) appearing in the stools of CF children compared with controls. The CF children lost between 0·2–17% (median 1·3%) of their retinol intake in faeces compared with a virtual absence of retinol in the stools of normal, healthy children. CF children lost 18–82% (median 43%) of α-tocopherol intake in stools compared with 84% in controls.

The results suggest that the greater daily faecal losses of vitamins A and E seen in the CF children are not simply due to a greater faecal output. Subjects with CF have a greater concentration of vitamins A and E/g stool compared with the controls. Vitamins A and E supplementation in children with CF may therefore be important in order to maintain an adequate intake.

Gompertz, S. M. & Sammons, H. G. (1963). *Clinical Biochemistry* **8**, 591–603.

Causes of weight loss in Human Immunodeficiency Virus infection. By C. SUMMERBELL, J. PERRETT and B. GAZZARD, *HIV Unit, Kobler Center, 369 Fulham Road, Chelsea, London SW10 9TH*

Weight loss is a common feature of Human Immunodeficiency Virus (HIV) infection (Raiten, 1990). The aim of this retrospective study was to establish the causes of weight loss in patients with HIV infection.

Of 581 seropositive individuals attending outpatient clinics between June 1990 and June 1991 who had at least three separate weights recorded, 165 were randomly reviewed for potential causes of weight loss. Forty-eight patients were identified as having lost weight over that time (last recorded weight at least 2 kg less than first recorded weight). Primary contributing causes of weight loss were classified into four major categories. Psychosocial causes included a major psychiatric disorder (6), intentional weight loss (1) and marked changes in social circumstances. Drug-related included voluntary cessation of therapy (2) and drug reactions.

	AIDS (CDC IV) (*n* 24)	Symptomatic (*n* 14)	Asymptomatic (*n* 10)
Mean (SD) wt loss (kg)	8·0 (3·6)	5·1 (2·9)	5·2 (2·7)
Mean (SD) rate of wt loss (kg/month)	1·3 (0·7)	1·0 (0·7)	1·4 (1·3)
Mean (SD) resultant BMI	20·7 (3·7)	22·1 (2·5)	23·6 (2·2)
Cause of wt loss:			
Infection	14	4	1
Unexplained	3	3	6
Psychosocial	5	6	2
Drug-related	2	1	1

BMI, body mass index.

The degree of weight loss in AIDS patients was greater than that seen in other groups ($P<0·05$), and most commonly was due to overt infection associated with diarrhoea (10). Indeed, diarrhoea associated with a specific pathogen was associated with a greater weight loss (mean 8·8 (SD 2·6) kg) than any other causal factor. Rate of weight loss was not dependent on stage of disease or cause of weight loss. Only three patients in the AIDS group had no clear explanation for weight loss and in these a diagnosis of lymphoma was made in one patient within 3 months, another had high fevers and the third had marked diarrhoea for which no pathogen could be uncovered.

In general, unexplained weight loss occurred mainly in those patients with a better preserved immune system, and most of these patients had symptoms suggestive of an infection which was not confirmed (fever 2, diarrhoea 2, chest symptoms 3) or had local oral lesions associated with a loss of appetite (3).

It is suggested that the majority of previously reported unexplained weight losses due to HIV infection may be attributed to psychosocial causes and symptoms not investigated in moderately immunocompromised patients.

Raiten, D. J. (1990). *Nutrition and HIV Infection*. Bethesda: Life Sciences Research Office, Federation of American Societies for Experimental Biology.

Oxygen consumption during the acute phase of burn injury in infants and children. By CHARMAINE CHILDS, *University of Manchester, North Western Injury Research Centre and Regional Children's Burns Unit, Manchester M13 9PT*

A marked disturbance in thermoregulation is a characteristic of the early response to burn injury in infants and children. Rectal temperature starts to rise 5–7 h after the burn. It reaches a peak (in excess of 40° in many cases) by 12 h and remains elevated throughout the first 24 h. The rise in rectal temperature is accompanied by an abrupt fall in skin temperature at the extremities and an inhibition of sweating (Childs *et al.* 1990).

The mechanism for the rapid heat storage could involve a reduction in total heat loss or an increase in metabolic heat production, or both. Whilst both mechanisms have been investigated in these patients, it is the change in metabolic activity which will be discussed.

A specially designed system of indirect calorimetry (McGuinness & Childs, 1991) was used for the measurement of oxygen uptake ($\dot{V}_{O_2}$), and it is $\dot{V}_{O_2}$ which is presented in the present paper as an indicator of metabolic activity.

Seventeen patients aged 9 months–10½ years, with burns covering 10–60% of the body surface, were studied bandaged, in a thermoneutral environment, during the first 24 h after the burn. All the patients were given morphine sulphate intravenously (0·2 mg/kg body-weight) for analgesia every 4–6 h. Measurements of $\dot{V}_{O_2}$ were made every 1–2 h during sleep and when the patients were awake (resting). Of the fifty-two healthy children in the control group, thirty-four were studied at rest and eighteen during sleep. The controls were in the same age range as the patients, were similarly bandaged and exposed to similar ambient conditions.

In healthy resting and sleeping children there was a curvilinear relationship between $\dot{V}_{O_2}$ and age. At rest, $\dot{V}_{O_2}$ in children $\leqslant 2$ years was between 12·0–15·0 ml/min per kg, falling to 7–9 ml/min per kg at 5–8 years and during early teens approximating to adult values (4 ml/min per kg). During sleep $\dot{V}_{O_2}$ in healthy children ($\leqslant 2$ years) was in the range 8–9 ml/min per kg and significantly lower than in children of the same age at rest. A log transform of the data and comparison of the regression lines showed that in both groups the lines were significantly different from zero and from each other. Construction of a normal range of $\dot{V}_{O_2}$ for resting and sleeping control groups allowed comparisons with patients. $\dot{V}_{O_2}$ in resting patients was largely within the normal range (within the upper and lower 95% confidence limits), but during sleep $\dot{V}_{O_2}$ was generally well above the upper limit. Further analysis of the data in sleeping patients and their aged-matched controls revealed that $\dot{V}_{O_2}$ rose above the upper limits in most patients during the time of rapid heat storage and was on average 26% above the normal (mean).

These data contribute to our understanding of normal physiology in the child and also show that the disturbance in metabolic activity after burn injury in children is an important contributor to the early rise in deep body temperature.

Childs, C., Stoner, H. B. & Little, R. A. (1990). *Archives of Emergency Medicine* **7**, 303–304.
McGuinness, K. & Childs, C. (1991). *Clinical Physics and Physiological Measurement* **12**, 343–351.

Adipose tissue profiles in inflammatory bowel disease. By B. CORRIDAN, R. COLLINS, C. O'MORAIN and M. J. GIBNEY, *Division of Nutritional Sciences, Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, Dublin 8, Republic of Ireland*

The incidence of inflammatory bowel disease (IBD) is lower in southern Europe than in northern Europe. In southern Europe the diet is rich in monounsaturated fatty acids, while in the north the diet is high in saturated fatty acids. Dietary fat may be a risk factor in the aetiology of IBD. Adipose tissue fatty acid composition is a reliable indicator of the quality of fat consumed and was used in the present study to investigate the relationship between the quality of fat consumed and IBD. Subcutaneous abdominal adipose tissue biopsies were obtained from ulcerative colitis (UC) and Crohn's disease (CD) patients and healthy controls, from Irish and Italian centres. Adipose tissue triacylglycerols were extracted with chloroform:methanol (2:1, v/v), transmethylated with sodium methoxide and analysed by gas liquid chromatography. Twenty fatty acid methyl esters were identified and results for each fatty acid expressed as percentage of total fatty acids.

	Irish			Italian			Irish	Italian
	UC	CD	Control	UC	CD	Control	All	All
n . . .	49	45	35	25	27	35	129	87
Fatty acid								
Saturated:								
C14:0	3·86	3·70	3·44	3·19	3·11	3·40	3·69*	3·25*
C16:0	20·93	21·69	21·25	21·58	22·48	22·14	21·28*	22·08*
C18:0	4·69	4·93	5·00	4·20	4·00	4·42	4·86*	4·23*
Monounsaturated:								
C16:1	5·12	5·10	4·80	3·83	4·39	3·84	5·02*	4·01*
C18:1	46·46	46·35	46·63	50·09	50·92	49·37	46·47*	50·05*
Polyunsaturated:								
C18:2 (*n*-6)	14·89	14·34	14·96	13·63*	11·71*	13·36	14·72*	12·93*
C18:3 (*n*-3)	1·03*	0·90*	1·0	0·69	0·53	0·60	0·97*	0·61*
Others	2·59	2·99	2·92	2·79	2·86	2·90	2·99	2·84

Mean values in the same row were significantly different from each other: *$P<0·05$.

There were no significant differences between IBD patients and controls for any of the major fatty acids found in adipose tissue within either the Irish or the Italian centres. Linoleic acid was significantly lower in the CD group than the UC group in the Italian centre. There were highly significant differences between the entire Irish group and the entire Italian group for all of the predominant fatty acids of adipose tissue and most notably for oleic acid and linoleic acid, which probably reflect geographical differences in fatty acids in the fats consumed. These data tend not to support the hypothesis that dietary fat composition is a significant factor in the aetiology of IBD within either the Irish or Italian centres.

Resting energy expenditure with the ventilated hood and mouthpiece systems in patients with chronic lung disease. By M. K. SRIDHAR[1], R. CARTER[1], J. J. REILLY[2], S. W. BANHAM[1] and M. E. J. LEAN[2], [1]*Department of Respiratory Medicine* and [2]*University Department of Human Nutrition, Glasgow Royal Infirmary, Glasgow G12 8QQ*

In healthy individuals, estimation of resting energy expenditure (REE) by indirect calorimetry using a face mask, mouthpiece and hood are comparable (Segal, 1987).

We compared the estimation of REE by the ventilated hood and mouthpiece systems in patients (five males, five females; mean age 64·6 years) with severe chronic lung disease, when not acutely ill. REE was measured by both methods on two consecutive days following an overnight fast. Before the study the gas analysers of the ventilated hood system (Deltatrac Ltd., Helsinki) were checked by ethanol combustion. Both the ventilated hood system and the mouthpiece system (PK Morgan, Rainham, Kent) were calibrated before each measurement using a certificated gas mixture (BOC special gases). REE was estimated with the patient in the semi-supine posture, first with the ventilated hood and after a 10 min interval with the mouthpiece system. The results are given in the Table.

Mean REE for ten patients (MJ (kcal)/d)

	Hood		Mouthpiece	
	Mean	SEM	Mean	SEM
Day 1	6·08 (1452)	0·30 (71)	7·08 (1693)	0·31 (75)
Day 2	6·14 (1468)	0·30 (71)	6·77 (1618)	0·20 (49)

Data were analysed using Student's t tests.

There was no significant day-to-day variation in REE estimated by either method alone. There was, however, a significant difference between REE estimated by the mouthpiece system compared with the hood ($P<0·01$), with the mouthpiece system tending to provide a higher estimate of REE.

We conclude that, in contrast to the situation in healthy adults, the method of data collection may have an influence on the measurement of energy expenditure in patients with severe lung disease. We believe that this is due to the effect of the mouthpiece apparatus on the breathing pattern of patients (Ashkenazi *et al.* 1980) causing an increase in tidal volume and inspiratory flow rate.

Ashkenazi, J., Silverberd, P. A., Foster, R. J., Hyman, A. I., Milic-Emeli, J. & Kinney, J. M. (1980). *Journal of Applied Physiology* **48**, 577–580.

Segal, K. R. (1987). *American Journal of Clinical Nutrition* **45**, 1420–1423.

The metabolism of amino acids in mice infected with *Schistosoma mansoni*. By DAVID A. BENDER and ELIUD N. M. NJAGI*, *Department of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT*

Previous studies have shown a considerable decrease in the metabolism of [^{14}C] tryptophan in mice infected with *Schistosoma mansoni*, with a reduction in the production of $^{14}CO_2$ over 2 h after injection of [^{14}C]tryptophan to as low as 25% of that seen in uninfected animals. There was no effect on the ability of hepatocytes isolated from infected animals to metabolize tryptophan or on the urinary excretion of tryptophan metabolites (Njagi & Bender, 1990).

The ability of infected mice to metabolize other [^{14}C]amino acids has been determined. Between 42–58 d after infection with 20–40 cercariae of *S. mansoni*, animals received a tracer dose of [^{14}C]amino acid by intraperitoneal injection. Each animal was housed separately, and $^{14}CO_2$ in exhaled air was trapped in 2-methoxymethylamine changed at 10 min intervals. The Table shows the total recovery of $^{14}CO_2$ (dpm/10^3 dpm injected) over 2 h for five animals in each group.

		Control		Infected			
	Day	Mean	SD	Mean	SD	Control/ infected	
Lys	42	663	173	441	193	0·67	NS
Leu	43	1181	321	884	278	0·75	NS
Met	44	597	156	330	105	0·55	**
Glu	48	2130	387	1848	489	0·87	NS
Gly	49	766	111	497	162	0·65	**
Ala	50	2176	412	1776	447	0·82	NS
Trp	51	431	136	223	100	0·52	**
Tyr	52	767	215	509	146	0·66	*
Phe	55	510	151	268	59	0·53	**
Pro	56	1150	218	863	153	0·75	*
Arg	57	497	141	739	196	1·48	*
His	58	591	158	397	86	0·67	*

Significance of differences by *t* test: *$P<0·1$; **$P<0·05$; NS, not significant.

With the exception of arginine, which was metabolized significantly faster, the oxidation of all the amino acids tested were reduced in infected animals. This presumably reflects general impairment of liver function due to accumulation of parasite eggs. The effect of infection on the metabolism of tryptophan, methionine, glycine and phenylalanine was very much more marked, suggesting that the parasites, or their eggs, have a relatively specific requirement for these amino acids.

Njagi, E. N. M. & Bender, D. A. (1990). *Experimental Parasitology* **70**, 43–54.

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A follow-up study to investigate an increase in *Giardia lamblia* infection among Bangladeshi children receiving regular anthelmintic treatment. By E. K. ROUSHAM, *Department of Biological Anthropology, University of Cambridge, Downing Street, Cambridge CB2 3DZ*

A 12-month deworming study was conducted on 123 pre-school Bangladeshi children divided into a treatment group and a control group. Although 2-monthly treatment with a 500 mg dose of mebendazole significantly reduced the prevalence of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm infection, the prevalence of *Giardia lamblia* infection significantly increased in the treatment group from 4% to 30% over 12 months. There was no significant increase in *Giardia* infection in the placebo group. Furthermore, there was no improvement in growth or nutritional status of the treatment group compared with the control group.

To investigate this further, at the end of the 12-month study the sixty-one children in the former placebo group were given mebendazole every 2 months for 6 months. Within 4 months the prevalence of *A. lumbricoides* significantly decreased from 72% to 5%, while the prevalence of *Giardia* infection significantly increased from 18% to 45% ($\chi^2=10{\cdot}76$, $P<0{\cdot}005$).

Finally, a parasitological survey was carried out on a random subsample of children from the same area who had participated in a deworming study for the previous 18 months. In July 1990 faecal samples from 265 of the 1402 children taken showed significantly higher prevalence of *Giardia* in the treatment group than in the placebo group (38% and 21% respectively, $\chi^=17{\cdot}66$, $P<0{\cdot}001$). There was also a significant negative association between *Ascaris* and *Giardia* infections ($\chi^2=17{\cdot}46$, $P<0{\cdot}001$). Again, children who were treated for helminth infection did not show any improvement in growth or nutritional status compared with untreated children.

The follow up study also compared the prevalence of *G. lamblia* in treated and untreated children from the same villages measured in the same month. This was found to be 45% in treated children and 21% in untreated children ($\chi^2=10{\cdot}23$, $P<0{\cdot}005$). The increase in *G. lamblia* cannot, therefore, be explained by either seasonal or environmental effects.

Thus *G. lamblia* infection increased significantly among three groups of children receiving regular anthelmintic treatment. It is difficult to conclude on the adverse effect of *Giardia* infection on child growth because of the highly variable symptoms and duration of the infection.

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The intensity of infection with *Ascaris lumbricoides* and the anthropometric status of children in Bangladesh. By ANDREW HALL, *International Centre for Diarrhoeal Disease Research, PO Box 128, Dhaka 1000, Bangladesh*

The intestinal nematode *Ascaris lumbricoides* is estimated to infect about a quarter of the world's population and it is typical to find that infections are most common and most intense among children. As a part of a study of the intensity of reinfection with *A. lumbricoides* (Hall *et al.* 1992), 249 children aged between 4 and 9 years living in a slum in Dhaka, Bangladesh were treated with a single dose of pyrantel pamoate (11 mg/kg body-weight), and all the worms they expelled were collected in buckets for 48 h after treatment. Participation was by the informed consent of the head of household and the study had been approved by the ethics committee of the ICDDR,B. The worms recovered from each child were counted (worm burden) and weighed (worm biomass). The following anthropometric measurements were made before each child was treated: weight, height, mid-upper-arm circumference (MUAC) and triceps skinfold thickness.

The mean worm burden recovered was 20·9 worms (range 0–187), the mean biomass was 44·2 g (range 0–278·8 g), and 71% of worms were recovered from a third of all children. The subjects were generally undernourished when compared with medians of National Centre for Health Statistics growth standards: the mean Z score of height-for-age (HAZ) was −2·73 (SD 1·40), the mean Z score for weight-for-age (WAZ) was −2·58 (SD 0·92) and the mean Z score of weight-for-height (WHZ) was −1·47 (SD 0·81). Because worms were not normally distributed among hosts, Spearman's rank correlation coefficient was used to examine associations between worm burdens and worm biomass and the nutritional status of children.

All correlations were positive but only the correlations between body weight and the Z score of weight-for-age with worm biomass were statistically significant. In order to examine in more detail these associations, the statistical significance of differences between mean values of nutritional and parasitological parameters was performed for the top and bottom quartiles of children stratified by their worm burden, by worm biomass or by age. There were no differences between children stratified by worm burdens but there were significant differences between children stratified by biomass in terms of body weight, WAZ and WHZ. Stratification by age revealed that younger children were less well nourished than older children and although there were no differences in terms of worm burdens, older children had a significantly heavier worm biomass. This may reflect their older worm burdens or some constraint on worm growth in younger and smaller children.

No evidence was found that the intensity of infection with *A. lumbricoides* was associated with the degree of malnutrition in this study, but nearly all children were infected with *A. lumbricoides*, the duration of their infections was unknown, all children were undernourished, and other parasites and infectious diseases were common in this community. Although this is the largest ever study of *A. lumbricoides* worm burdens, it is clear that cross-sectional studies are unlikely to provide the answer to questions concerning the relationship between the intensity of infection with intestinal parasites and nutritional status.

Hall, A., Anwar, K. S. & Tomkins, A. M. (1992). The intensity of reinfection with *Ascaris lumbricoides* and its implications for parasite control. *The Lancet* **339,** 1253–1257.

Phospholipid-2-9,12,15-linolenic acid in Gambian children with *Plasmodium falciparum* malaria. By J. M. KNOWLES[1], D. I. THURNHAM[1], A. V. S. HILL[2], B. M. GREENWOOD[3] and C. M. TANG[4], [1]*MRC Dunn Nutrition Centre, Milton Road, Cambridge CB4 1XJ*, [2]*Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DZ*, [3]*MRC Laboratories, Fajara, The Gambia* and [4]*Royal Victoria Hospital, Banjul, The Gambia*

Fish oil rich in *n*-3 fatty acids fed to malaria-infected mice slightly improved host survival, but particularly when combined with experimental vitamin E deficiency (Levander *et al.* 1989). We measured plasma phospholipid-2-esterified (PL) fatty acid levels (Iversen *et al.* 1985) in Gambian children with *Plasmodium falciparum* malaria or other diseases and in healthy, student-nurse controls and have found that there is some indication that low 6,9,15-linolenic acid (LLA) levels are proportional to the severity of malaria but this was not significant.

The Gambian diet is mainly cereal-based but sun-dried sea fish is widely consumed in modest amounts. To determine whether the plasma fatty acid composition (indirectly dietary fat) might have any influence on human malaria infection we measured PL-LLA concentrations, and to evaluate what effect LLA might have on *P. falciparum* malaria in children we compared the proportions of children with low and high LLA levels in the different malaria groups with that of the controls (Table).

Several of the children with severe malaria (*n* 16) received transfusions of fresh control blood which may have influenced PL levels so these cases were omitted from further analysis.

	Number of cases*	
	Low *n*-3†	High *n*-3
Student-nurse controls	12	9
Mild malaria	11	7
Severe malaria*	31	9

* Tranfused cases omitted.

† Low and high values separated on basis of cases above and below mean linolenic acid concentration in PL fraction of the control group.

The results suggest that there may be a higher level of PL-LLA in cases of mild malaria than in those with severe malaria. These results could have some relationship to dietary lipid composition but they appear more likely to be a product of the disease process as similar low levels were obtained in Gambian children with other diseases.

Iversen, S. A., Cawood, P. & Dormandy, T. L. (1985). *Annals of Clinical Biochemistry* **22**, 137–140.
Levander, O. A., Ager, A. L., Morris, V. C. & May, R. G. (1989). *American Journal of Clinical Nutrition* **50**, 346–352.

Eating habits, nutrient intake and growth in pre-school children. By J. A. PAYNE and N. R. BELTON, *Department of Child Life and Health, University of Edinburgh, 17 Hatton Place, Edinburgh EH9 1UW* and T. R. KIRK, *Queen Margaret College, Edinburgh EH12 8TS*

The relationship between eating habits, nutrient intake and growth in childhood remains poorly understood. Food supplementation trials in developing countries rarely define basal diets and thus cannot determine whether growth outcomes are related to intake of energy, protein or micronutrients (Golden, 1991). In developed countries there is a paucity of data relating nutrient intake to growth velocity in childhood.

In the present study the nutrient intake of fifty-four randomly selected pre-school children from Edinburgh aged 2–4 years was assessed by the 7 d weighed intake method. Anthropometric parameters including height and weight were measured. The study was repeated 1 year later, providing growth velocity data.

Nutrient intake from the initial survey (S1) was compared to the repeat survey (S2) using the Pearson correlation test.

	S1 (range)	S2 (range)	(*r*)
Age (months)	26–49	39–61	
Energy (MJ/d)	3·0–6·2	3·0–7·3	0·57**
% Energy from starch	11–34	12–31	0·69**
% Energy from sugar	17–41	19–43	0·62**
% Energy from fat	23–48	23–44	0·54**
Vitamin B_1 (mg/d)	0·3–1·1	0·3–1·1	0·53**
Vitamin C (mg/d)	8–171	6–159	0·54**
Vitamin A (retinol equivalent (μg))	108–1597	109–1237	0·35**
Carotenoids (μg)	156–2679	106–3742	0·36**

r, correlation coefficient; $^{**}P<0·001$.

To assess the relationship between nutrient intake and growth velocity the mean nutrient intakes of the combined studies were correlated with height velocity Z scores and height velocity percentiles (Tanner *et al.* 1966). (Both square-root and log base 10 transformations were performed on highly skewed nutrient intake data.) Although nutrient intakes ranged widely, no consistent significant correlations were found with growth velocity data. Curiously, interesting relationships between the square-root transformations of carotenoids intake with height velocity Z scores (r 0·30, $P=0·026$), and with height velocity percentiles (r 0·26, $P=0·057$), were observed.

In conclusion, these results support the concept that eating habits are established at a young age, and have a highly significant bearing on nutrient intake. Despite a wide-ranging intake of nutrients this study found no consistently significant relationship between nutrient intake and growth velocity. However, the possibility of a relationship between carotenoids intake and growth merits further investigation.

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

Golden, M. H. N. (1991). *Acta Paediatrica Scandinavica*, Suppl., **374**, 95–110.
Tanner, T. M., Whitehouse, R. H. & Takaishi, M. (1966). *Archives of Disease in Childhood* **41**, 613–635.

Fruit and vegetable consumption in north Glasgow: some results from the MONICA study of 1986 and 1989. By W. L. WRIEDEN, *School of Food and Accommodation Management, Duncan of Jordanstone College of Art, Dundee DD1 4HT* and C. BOLTON-SMITH, C. A. BROWN and H. TUNSTALL-PEDOE, *Cardiovascular Epidemiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY*

Diets poor in fresh fruit and vegetables have been associated with a higher risk of coronary heart disease (Bolton-Smith *et al.* 1992). Questionnaires, including a food frequency section administered as described previously (Bolton-Smith *et al.* 1991), were completed by over 1000 people in north Glasgow in each of the years 1986 and 1989. It was confirmed that women on average consumed more fruit and vegetable portions (FVP), not including potatoes or pure juices, than men.

Median values for FVP consumed per week in different housing groups in north Glasgow (No. of subjects shown in parentheses)

	Men			Women		
	Owner occupier	Council rented	All	Owner occupier	Council rented	All
1986	13 (191)	11 (386)	12* (623)	17 (176)	12 (376)	14 (579)
1989	14 (245)	11 (324)	12* (621)	17 (270)	14 (401)	15 (705)

Significantly different from all women (Student's t test comparing natural log of (FVP per week + 1)): *$P<0{\cdot}001$.

When the sample was subdivided by age and housing tenure a more positive significant change was seen in women of the 35–44 year age group who lived in council rented accommodation, where an increase from a median (interquartile range) of 11 (8·5) in 1986 to 14 (11) in 1989 was seen ($P<0{\cdot}01$). These figures were still lower than those for owner-occupiers of this age range which were 18 (12) and 16 (10) for 1986 and 1989 respectively (NS). Although the increased FVP consumption by women in the council renters' group is encouraging, it was apparent that fruit and vegetable consumption for the majority of the sample population of north Glasgow was still far below the WHO recommendation of 400 g/d (World Health Organization, 1990). Even amongst women in the owner-occupiers' group (the highest consumers), only 10% were estimated to eat this amount or more, and amongst men in 1989 only 2% of council renters were found to reach the recommendation. In all groups, around half appeared to consume less than 200 g/d.

A further survey of the north Glasgow population is in progress and it will be interesting to see if health education in the last 3 years has brought about an improvement in diet in an area which has one of the highest rates of cardiovascular mortality in Scotland (Registrar General Scotland, 1990).

Bolton-Smith, C., Smith, W. C. S., Woodward, M. & Tunstall-Pedoe, H. (1991). *British Journal of Nutrition* **65,** 321–335.

Bolton-Smith, C., Woodward, M. & Tunstall-Pedoe, H. (1992). *European Journal of Clinical Nutrition* **46,** 85–93.

Registrar General Scotland (1990). *Annual Report 1990.* Edinburgh: H.M. Stationery Office.

World Health Organization Study Group (1990). *Diet, Nutrition and the Prevention of Chronic Diseases.* WHO: Geneva.

A preliminary report on dietary intake and anthropometry of vegetarian children compared with omnivores. By I. NATHAN, S. KIRBY and A. HACKETT, *School of Education and Community Studies, Liverpool Polytechnic, Liverpool L19 6BD*

Concern has been expressed regarding the dietary intake and growth of children on restricted vegetarian diets, (Jacobs & Dwyer, 1988). As part of the longitudinal investigation the nutritional intake and anthropometric measurements of ten lacto-ovo-vegetarian children were compared with ten 'matched' omnivores.

Vegetarian subjects (six female, four male), mean age 9·1 years, were recruited from the Liverpool area. A matching omnivore group was achieved by each vegetarian child introducing a meat-eating friend (of similar age, sex and height) to the study. Dietary intake was estimated using a 3 d diary and follow-up interview. Dietary data were analysed using the Microdiet program (University of Salford).

		Energy (MJ)	Protein (g)	Fat (g)	CHO (g)	Iron (mg)	Fibre (g/MJ)	Weight (kg)	Height (cm)	BMI
Omnivores:	Mean	8·6	62	91	265	10·0	1·7	27·9	131·0	16·2
(*n* 10)	SD	0·62	3·6	7·7	66·9	3·2	0·68	1·3	1·79	1·64
Vegetarians:	Mean	7·6	51	82	240	9·0	2·49	32·3	131·1	18·6
(*n* 10)	SD	0·63	4·4	8·4	62·2	0·66	0·016	0·15	3·71	0·04
P value		0·07	0·09	0·42	0·40	0·66	0·02	0·15	0·98	0·04

BMI, body mass index.

No significant differences in energy, protein, fat, carbohydrate, fibre or iron intakes were found. The fibre intake (g/MJ) of the vegetarians was significantly higher than that of the omnivores. This suggests that the vegetarian children were consuming a diet of lower energy density. There was no significant difference in height between the two groups. However, the BMI of the vegetarians was significantly higher than that of the meat-eaters. This finding may suggest that these vegetarian children have a larger percentage body fat than the omnivores despite reporting slightly lower energy intakes.

Jacobs, C. & Dwyer, J. T. (1988). *American Journal of Clinical Nutrition* **48**, 811–818.

Traditional dietary intakes and serum lipids in Singapore ethnic groups. By N. SAHA, P. Y. TAN, S. P. SOTHY, T. B. NG and C. H. LIM, *Department of Physiology, Faculty of Medicine, National University of Singapore, Singapore 0511*

The mortality rate of Indians in Singapore due to coronary heart disease (CHD) is about four times that of the Chinese (Singapore Registrar-General, 1980). However, dietary intakes of energy, protein, fats, polyunsaturated fats and cholesterol were not significantly different in these ethnic groups (Tan *et al.* 1984) and as such could not explain differential mortality from CHD in Singapore ethnic groups. However, body mass index (BMI) and rate of smoking was significantly higher in Indians than Chinese ($P<0{\cdot}05$).

We investigated the influence of dietary intakes on fasting serum lipid levels in 528 male employees (205 Chinese, 156 Malay, 167 Indians) of the Port of Singapore Authority. Dietary intakes were computed by a 24 h recall method over a period of 1 week. Serum total cholesterol and triacylglycerol levels were estimated in an auto-analyser by the enzymic method reported earlier (Saha, 1987). The influence of dietary intakes was examined by Spearman's rank correlation test (r) and the significance by P test.

Influence of dietary intakes on serum lipids (Spearman's rank correlations: P*)*

Dietary intakes	Total cholesterol	Triacylglycerols
Energy	NS	NS
Vegetable protein	$\leqslant 0{\cdot}004$	NS
Fats	NS	NS
Cholesterol	NS	NS
Polyunsaturated fats	NS	NS
Thiamin	$\leqslant 0{\cdot}0001$	$\leqslant 0{\cdot}0001$
Nicotinic acid	$\leqslant 0{\cdot}002$	$\leqslant 0{\cdot}004$

NS, not significant.

The results in the Table show that the intakes of vegetable protein had a negative correlation with serum total cholesterol levels ($P<0{\cdot}004$), while intakes of both thiamin and nicotinic acid had strong negative correlations with serum total cholesterol and triacylglycerol levels ($P<0{\cdot}0001$ and $P<0{\cdot}005$ respectively). This suggests a possible association between these dietary factors and CHD. This is consistent with earlier observation of higher dietary intakes of thiamin and nicotinic acid in the Chinese having a lower risk of CHD (Tan *et al.* 1984).

Saha, N. (1987). *Atherosclerosis* **68,** 117–121.
Singapore Registrar-General (1980). *Report of the Singapore Registrar-General of Births and Deaths.* Singapore: Government Printing Office.
Tan, P. Y., Ng, T. B. & Saha, N. (1984). *Proceedings of the Nutrition Society* **43,** 135A.

Persuasive power of nutritional messages enhanced by positive and non-technical language. By M. C. MURPHY, A. WISE and A. MCLEISH, *The Robert Gordon University, Queen's Road, Aberdeen AB9 2PG*

Messages were written to include either a positive command or negative command, with either technical or non-technical language. In addition, each message included either guilt-evoking or neutral ideas relating to whole family or child-related responsibilities. There were eight messages, combining each of these three opposing characteristics for four foods: chips, bread, soups, and soft drinks. Messages suggested changes such as frying straight chips instead of crinkle-cut (positive), or just not eating chips (negative). Bias was excluded by Latin-square design. Mothers (n 100) were asked to rank the messages (1–8) for each food in order of persuasiveness. Ranks were summed for each individual for each component of the message and expressed as percentages of the total ranks (minimum 27%, maximum 73%), and the distribution of scores between these values are given in the Table which also shows the results of 1000 random simulations.

% Score	Random	Technical	Guilt-evoking	Positive
67–73	0	2	2	4
55–67	18·5	9	23	42
45–55	63	30	53	52
35–45	18·5	41	22	2
27–35	0	18	0	0

The distribution of persuasiveness of guilt-evoking statements did not differ from what was obtained from random rankings, but the lack of persuasiveness of technical language was significant ($P<0·001$), in contrast to the persuasiveness of positive language ($P<0·001$). These results are different to those of Randall *et al.* (1992) who found no difference in preference for positive language. However, the design of their study included equal numbers of suggestive and command messages so that half of their negative messages included a suggestion, whereas in the present study all negative messages were commands only and excluded suggestions. It is concluded that nutritional messages should be worded in positive language, or that negative language should include suggestions. The use of technical language is not supported. Results for guilt-evoking language do not show any advantage of this approach.

The authors wish to thank the Bon Accord Shopping Centre, Aberdeen for permission to survey mothers in their Food Court.

Randall, L., Wise, A. & McLeish, A. (1992). *Journal of Human Nutrition and Dietetics* (In the Press).

Food sources of fat and sugar in the Northern Irish diet. By M. E. BARKER, *Centre for Human Nutrition, University of Sheffield S5 7AU* and K. A. THOMPSON, *Centre for Health and Social Research, University of Ulster*

Recent dietary recommendations for the UK (COMA, 1991) advocated population reductions in fat and sugar intakes. In this regard, the dietary information from a large (*n* 592) cross-sectional epidemiological study of adults (Barker *et al.* 1989) was used to compare the food and nutrient intake characteristics of low- and high-fat consumers. These two groups were differentiated by the criterion of 35% total energy (including alcohol) as fat. Dietary intake was measured using the 7 d weighed inventory and described in terms of twelve food groups. Statistical testing was by independent *t* tests.

Men in the low-fat group relied on alcohol and carbohydrate (particularly sugar) as opposed to fat as an energy source. A similar effect was observed in women, but with greater emphasis on carbohydrate.

The major food sources of fat were meat and meat products (23%), cereal products including cakes, biscuits and puddings (19%), fat spreads and oils (18%), and milk and milk products (13%). The major food sources of sugar were cereal products (29%), sugar, confectionery and preserves (25%), milk and milk products (16%), and beverages (15%). The Table shows the food sources of fat that differed significantly between the two groups. It is evident that consumption of meat and meat products was a major discriminator between the two fat groups in men but not in women. In contrast, consumption of chips and milk and milk products discriminated between the two fat groups in women but not in men. Cereal products, egg and egg products and spreading fats and oils consistently discriminated between the low- and high-fat groups.

Mean fat contribution (g/10 MJ) of various food groups in low- and high-fat consumers

	Males		Females	
Food source	Low-fat (*n* 45)	High-fat (*n* 213)	Low-fat (*n* 53)	High-fat (*n* 281)
Cereal products	15·7	20·1**	17·0	22·1**
Milk and products	12·5	13·8	11·6	14·9**
Egg and products	3·1	5·4**	3·6	5·5**
Meat and products	17·3	26·1***	20·4	22·8
Chips	9·1	10·4	5·0	10·8***
Spreading fats and oils	15·2	19·0*	14·2	18·9**

Significantly different from low-fat group: *$P<0·05$; **$P<0·01$; ***$P<0·001$.

From these data it is clear that dietary advice to effect reductions in fat and sugar intakes should emphasize reduced consumption of cakes, biscuits and puddings. These foods are major sources of dietary fat and sugar and, along with spreading fats and oils, consistently discriminate between low- and high-fat consumers.

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Barker, M. E., McClean, S. I., McKenna, P. G., Reid, N. G., Strain, J. J., Thompson, K. A. Williamson, A. P. & Wright, M. E. (1989). *Diet, Lifestyle and Health in Northern Ireland.* Coleraine: University of Ulster.

Committee on Medical Aspects of Food Policy (1991). *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom.* London: H.M. Stationery Office.

Ireland: a comparison of food and nutrient trends in the national surveys. By MARY MOLONEY, *Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8*

Two national nutrition surveys have been undertaken in Ireland, 1946–48 (*n* 14 640) and 1990 (*n* 1214). Two different methodologies were used in estimating food and nutrient intakes. In the 1946–48 survey the investigator weighed and measured the amounts of all foods in the house at the start and end of the 1-week survey period. The housewife recorded the amounts of all foods, including home-grown, brought into the house in the interim. This allowed the amounts of food eaten and wasted by the family as a whole to be estimated. In 1990, a 7-day diet history, made with the aid of a photographic atlas, was used. The pattern of macro- and micronutrient intakes/capita per d (excluding alcohol) has altered in the period between the 1946–48 and the 1990 surveys (Department of Health (Dublin), 1946–48; Irish Nutrition and Dietetic Institute, 1990).

Year	Energy (MJ)	Protein (%)	Fat (%)	CHO (%)	Ca (mg)	Fe (mg)
1946–48	13·04	13	29	58	1369	20·0
1990	9·79	15	36	49	1075	12·7

The higher nutrient intakes in the first national nutrition survey can possibly be explained by the higher energy expenditure of the population such as: greater physical labour, less transport, few labour-saving devices, and poorer quality housing, insulation and heating. The increase in fat (as a percentage of energy), of which saturated fat formed a major proportion, is significant, especially in a country that has such a high mortality from ischaemic heart disease.

In the 1946–48 survey, butter and sugar were rationed to approximately 6 oz per diet head per week and 4 oz per diet head per week respectively. The consumption of each food (per capita per d) in 1946–48 compared with 1990 is noteworthy, e.g. milk (whole, skimmed, low-fat) 569 ml *v.* 426 ml, eggs 43 g *v.* 24 g, cheese 3·3 g *v.* 6·1 g, meat 125 g *v.* 153 g, fish 14 g *v.* 15 g, potatoes 549 g *v.* 225 g,, vegetables 125 g *v.* 59 g and jams 18 g *v.* 9 g. The higher consumption of milk can be attributed to the high percentage of farming families (*n* 951), i.e. 32% of those surveyed, who had an average daily consumption of 747 ml milk. The overall markedly higher potato intake made a significant contribution of approximately 15% to the higher energy intake (compared with 8·5% in the 1990 survey). The findings of these two surveys question whether or not dietary trends have improved in the intervening 42 years.

Department of Health (Dublin), National Nutrition Survey (1946–1948) Part VI 8–30, Part V 6–16.
Irish Nutrition and Dietetic Institute. Irish National Nutrition Survey (1990).

Maternal diet and social class in the East End of London. By W. DOYLE and M. A. CRAWFORD, *Institute of Brain Chemistry and Human Nutrition, Hackney Hospital, Homerton High Street, London E9 6BE*

Undernutrition adversely affects development and growth. Women (513) living in Hackney, East London, recorded their food intakes for 1 week during the first trimester of their pregnancies (Doyle *et al.* 1990; Wynn *et al.* 1991). The survey was carried out between 1983 and 1989. Social class (Registrar-General's classification) and diet were found to be independently correlated with the birth weight of the baby. The correlation between energy intake and social class was not statistically significant. In contrast, there were significant correlations between a range of micronutrients and social class. Significantly fewer women in the lower social class groups achieved the levels of intake of those mothers whose babies were born in the optimum birth weight range of 3·5–4·5 kg (Table).

Mean birth weight, percentage low birth weight (≤2500 g) and nutrient intakes of women in different social class groups

Social classes . . .	I & II (*n* 80)	IIIn & IIIm (*n* 182)	IV & V (*n* 251)	*P*=2-tailed*
Birth wt (g)	3439	3281	3165	<0·001
Low birth wt (%)	0	5·5	7·2	0·006†
Energy (MJ)	8·39	8·35	7·90	0·013
Fibre (g)	22·4	19·1	15·8	<0·001
Nutrients:				
Thiamin (mg)	1·33	1·21	1·08	<0·001
Niacin (mg)	17·5	16·3	14·1	<0·001
Pyridoxine (mg)	1·59	1·54	1·34	<0·001
Folate (μg)	247	202	174	<0·001
Vitamin C (mg)	129	100	80·3	<0·001
Carotene (mg)	2476	2510	1792	<0·001
Mg (mg)	334	281	245	<0·001
Fe (mg)	14·6	13·2	11·2	<0·001
Zn (mg)	11·7	10·3	9·21	<0·001
Ca (mg)	1031	933	867	<0·001
K (mg)	3158	2989	2797	0·001

* Mann-Whitney U test.
† Chi-square test.

We suggest that some mothers in this inner city area of London may not be consuming a diet of sufficient quality to satisfy optimum reproductive requirements. The discrepancy between intakes was associated more with nutrient density than energy intake. Many of these nutrients reflect consumption of vegetables, fruit and wholegrain cereals which are, regardless of reproductive status, associated with general health unconnected with pregnancy.

Doyle, W., Crawford, M. A., Wynn, A. H. A. & Wynn, S. W. (1990). *Journal of Nutritional Medicine* **1**, 9–17.
Wynn, A. H. A., Crawford, M. A., Doyle, W. & Wynn, S. W. (1991). *Nutrition and Health* **7**, 68–88.

The plate model for dietary education. By J. ARMSTRONG, *Department of Human Nutrition and Dietetics, The Queen's College Glasgow G3 6LP* and M. E. J. LEAN, *Department of Human Nutrition, University of Glasgow G12 8QQ*

A 'plate model' system has been suggested as an aid to dietary education for patients with diabetes and other groups (Community Nutrition Group (BDA), 1987; Karlstrom *et al.* 1989; Lean *et al.* 1991). Various models have been published without scientific validation.

To illustrate how a plate model could be established, eight meals were prepared to conform to the current EASD/British Diabetic Association guidelines (Lean *et al.* 1991) for energy (i.e. >50% energy from carbohydrate, <35% from fat, <10% from saturated fat). Each meal comprises a main dish served with an apple and one large slice of wholemeal bread with 7 g of polyunsaturated margarine. Total energy for the complete meals was 2·66–2·90 MJ (638–694 kcal) to represent approximately one-third of average daily intake. The recipes were those used in McCance and Widdowson (Paul & Southgate, 1978).

Foods for the main dish were arranged as segments of a circle on a plate with diameter 25·4 cm. The dishes consisting of two components (i.e. chicken curry and spaghetti bolognese) were examined and gave similar angles on the plate: staple 233–236°, sauce 124–127°. The remaining three component dishes resulted in segments: potatoes 97–146°, vegetables 124–158° and meat or fish 82–110°.

The same meals were then arranged to fit a standard plate model with angles: meat or fish 90°, staple 125° and vegetables 145°, or with a two component meal: staple 235°, meat and vegetable sauce 125°. The components were weighed and mean nutrient content of the meals (including the apple and wholemeal bread with 7 g of polyunsaturated margarine) are shown in the Table.

Component	Mean	SD
Energy: MJ	2·74	0·09
kcal	653	24·8
Per cent of energy as:		
Protein	14·7	1·67
Fat	33·2	2·92
Carbohydrate	52·0	2·83
Saturated fats	7·86	2·52
Dietary fibre (g)	12·1	0·88

It is proposed that a standard plate model should be explored to provide an illustrated guide to a balanced meal as an adjunct to conventional dietary education.

Community Nutrition Group of British Dietetic Association (1987). *Nutrition Guidelines for School Meals*, Information sheet No. 14.

Karlstrom, B., Vessby, B. & Eliasson, M. (1989). In *Diabetes 1988*, pp. 923–925 [R. Larkins, P. Zimmett and D. Chisholm, editors]. Amsterdam: Elsevier.

Lean, M. E. J., Brenchley, S., Connor, H., Elkeles, R. S., Govindji, A., Hartland, B. V., Lord, K., Southgate, D. A. T. & Thomas, B. J. (1991). *Journal of Human Nutrition and Dietetics* **4**, 393–412.

Paul, A. A. & Southgate, D. A. T. (1978). *McCance and Widdowson's 'The Composition of Foods'* (4th ed). London: H.M. Stationery Office.

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How effective is healthy eating advice for women from different socio-economic groups?

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While there is evidence that intensive dietary advice is effective at improving dietary habits (Retzlaff *et al.* 1991), the efficacy of simple healthy eating advice is largely unknown. Irish adult women were found to be nutritionally vulnerable in a recent national nutrition survey. Another recent Irish study suggests that women from low income groups may be particularly at risk (Gibney & Lee, 1990). The present study was undertaken to examine the effectiveness of standard healthy eating advice on working women from socially advantaged (socio-economic (SE) classes 1 and 2) and disadvantaged (SE classes 5 and 6) backgrounds.

A total of eighty-three healthy women were recruited from their workplaces, forty-three from SE classes 1 and 2 and forty from SE classes 5 and 6. The groups were comparable with regard to age but differed in body mass index, being on average 1·85 kg/m^2 lighter in SE classes 1 and 2 than in SE classes 5 and 6. The women were interviewed twice: a baseline interview (period 1), and one at 4 weeks (period 2) following receipt of healthy eating advice in the form of a standard leaflet (Irish Heart Foundation 'Eating for a Healthy Heart', 1991). At baseline, dietary information was collected using the diet history method and minimal explanation of the leaflet was given. The follow-up interview used the same diet history method. The results are presented in the Table as mean values (standard deviation).

SE classes . . .	1 and 2		5 and 6		ANOVA	
Period . . .	1	2	1	2	SE group	Period
Energy (MJ)	8·5 (1·5)	7·9 (1·2)	9·4 (1·9)	8·7 (1·9)	P=0·001	P=0·02
Protein (g)	72 (13)	69 (10)	84 (21)	80 (13)	P=0·000	NS
Fat (g)	93 (21)	86 (19)	100 (23)	91 (23)	NS	P=0·02
Carbohydrate (g)	213 (48)	204 (38)	249 (72)	228 (71)	P=0·001	NS
Dietary fibre (g)	21·5 (7·3)	21·6 (5·8)	19·7 (5·8)	19·9 (5·4)	NS	NS
% Energy as fat	41·6 (5·1)	40·7 (4·9)	40·2 (5·3)	39·0 (5·7)	P=0·05	NS

NS, not significant.

With the exception of a reduction in total energy and total fat intakes, all the differences between baseline and follow-up were accounted for by differences between the SE groups and not due to healthy eating advice. Although a significant reduction in fat intake took place, it was small and counterbalanced by a reduction in carbohydrate leading to no net change in % energy as fat. While this study is limited by the fact that it only examined a small number of adult women, it questions the usefulness of this type of healthy eating advice leaflet as a tool to improve dietary habits. It also suggests that the effectiveness of healthy eating advice cannot be truly assessed without accounting for the effect of SE status on diet.

Supported by a grant from the Mater College for Postgraduate Education and Research, Dublin 7.

Gibney, M. J. & Lee, P. (1990). *Journal of Human Nutrition and Dietetics* **4**, 21–29.

Retzlaff, B. M., Dowdy, A. A., Walden, C. E., McCann, B. F., Gey, G., Cooper, M. & Knopp, R. M. (1991). *American Journal of Clinical Nutrition* **53**, 890–898.

Effect of myofibrillar muscle proteins on *in vitro* biolysability of iron. By F. M. KIRWAN, I. O'CONNOR, P. A. MORRISSEY and A. FLYNN, *Department of Nutrition, University College Cork, Republic of Ireland*

Non-haem Fe absorption from meals is enhanced by meat (Cook & Monsen, 1976). However, the nature of the enhancing factor in meat is unknown. The present study was carried out to compare the effect of myofibrillar muscle proteins on the *in vitro* bioavailability of Fe in an attempt to identify the 'meat factor'.

Rabbit skeletal muscle was fractionated and whole muscle (WM), myofibrillar protein (MP), myosin (M) and actin (A) were isolated. M was subjected to selective proteolysis with chymotrypsin (*EC* 3.4.21.1), and heavy meromyosin (HMM), light meromyosin (LMM), rod region (RR), and head region (HR) were prepared. Protein fractions (1 g) were incorporated into 100 g semi-synthetic liquid meal containing dextrose (11·5 g), corn oil (5·9 g), $CaHPO_4$ (0·11 g), KH_2PO_4 (0·22 g) and Fe (0·6 mg) as $FeCl_3$, the meals were labelled with ^{59}Fe and *in vitro* bioavailability of Fe was determined by the method of Miller *et al.* (1981). This method measures the release of soluble, low molecular weight (dialysable) Fe after digestion with pepsin (*EC* 3.4.4.1) and pancreatin under simulated gastrointestinal conditions. Egg albumen (EA) was used as the reference protein. Results were expressed as % dialysable Fe.

The effect of myofibrillar proteins on in vitro *Fe bioavailability (*n *6)*

	EA	WM	MP	A	M	LMM	HMM	RR	HR
Mean	5·2	8·1*	12·2*	8·0*	16·0*	3·9*	16·1*	13·0*	13·1*
SEM	0·2	0·2	0·2	0·2	0·4	0·3	0·5	0·5	0·4

Significantly different from EA: *$P<0·01$.

When compared with EA, all protein fractions significantly enhanced Fe availability, except for LMM which was slightly inhibitory. M had a greater enhancing effect than A and, within M, the enhancing effect was greatest for the HMM fraction. The enhancement coincides with the known distribution of cysteine residues in myofibrillar proteins: A 5; M 42 (all in HMM and none in LMM).

Incorporation of the sulfhydryl blocking agent, N-ethylmaleimide (NEM) (0–15 mM), into meals containing M reduced Fe bioavailability in a dose-related manner, but NEM had only a small effect in meals containing A. Incorporation of cysteine (0–30 mM) into meals containing A increased Fe bioavailability in a dose-related manner with a plateau reached at 15 mM.

The present results suggest that the enhancement of non-haem Fe absorption by meat is due, at least in part, to cysteine residues in myofibrillar proteins, particularly M.

Cook, J. D. & Monsen, E. R. (1976). *American Journal of Clinical Nutrition* **29,** 859–867.
Miller, D. D., Schricker, B. R., Rasmussen, R. R. & Van Campen, D. (1981). *American Journal of Clinical Nutrition* **34,** 2248–2256.

Effect of tannic acid on iron and zinc absorption from a model food system in sucking rats.

By K. CASHMAN and A. FLYNN, *Department of Nutrition, University College, Cork, Republic of Ireland*

Polyphenols are strong inhibitors of non-haem Fe absorption but their effect on the absorption of other trace elements is less well understood. The present study compares the effect of added polyphenol (tannic acid) on absorption of Fe and Zn in a model food system (infant formula) using the sucking rat model which we have previously described (Cashman *et al.* 1991).

Tannic acid was added at concentrations of 0–10 mM to reconstituted cow's milk-based infant formula (SMA Gold, Wyeth Laboratories) containing 6·7 mg Fe/l and 5·0 mg Zn/l. Formulas were extrinsically labelled with both ^{59}Fe and ^{65}Zn, (1μCi/ml each) and allowed 24 h for isotopic equilibrium to be attained. Wistar rats (16-d-old), fasted for 16 h, were given 0·2 ml formula by gavage. Animals were killed 8 h later and the stomach, small intestine (SI) and caecum–colon removed. SI was perfused with 6 ml 0·15 M NaCl. ^{59}Fe and ^{65}Zn were determined in a well gamma counter. Absorption of ^{59}Fe and ^{65}Zn (% dose) was calculated as:

$$\text{Absorption (\%)} = 100 - (\text{stomach} + \text{SI perfusate} + \text{caecum–colon})(\%).$$

Effect of tannic acid on ^{59}Fe and ^{65}Zn absorption (% dose) from infant formula in 16-d-old sucking rats

		^{59}Fe		^{65}Zn	
Tannic acid (mM)	*n*	Mean	SEM	Mean	SEM
0·00	6	96·8	0·8	94·8	1·1
0·01	4	92·7	0·4	92·3	1·3
0·02	4	81·3	2·3	92·1	0·6
0·05	4	54·2	5·9	87·8	1·7
0·10	4	45·2	6·3	82·3	3·5
0·20	6	44·2	3·9	79·9	2·9
0·60	5	40·4	4·5	67·5	1·9
5·00	4	39·1	2·1	59·4	3·2
10·00	5	44·3	2·7	49·6	4·1

Over 90% of ^{59}Fe and ^{65}Zn was absorbed from infant formula alone. Addition of tannic acid to formula reduced the absorption of ^{59}Fe and ^{65}Zn in a dose-dependent manner. This inhibitory effect was much more marked for ^{59}Fe than for ^{65}Zn.

These results show that tannic acid inhibits the absorption of both non-haem Fe and Zn, although its effect on Zn is much weaker than on Fe.

Cashman, K., Flynn, A. & Harrington, M. (1991). *Proceedings of the Nutrition Society* **50**, 185A.

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The effect of dietary fibres on iron and zinc absorption in sucking rats. By M. HARRINGTON and A. FLYNN, *Department of Nutrition, University College, Cork, Republic of Ireland*

The practice of enrichment of foods with dietary fibre is becoming more widespread. While some dietary fibre fractions have potential beneficial effects on health, some components could have potentially adverse effects on nutrition, e.g. reduced absorption of trace elements. In this study the effect of a range of dietary fibres on absorption of Fe and Zn was investigated using a sucking rat model which we have previously described (Cashman *et al.* 1991).

Samples of fibre from apple, orange, pea, sugar beet, oat, barley and wheat were obtained from SOFALIA (France). Fibre samples (5 g) were incorporated into a slurry (5 g/100 g) of steam-cooked rice (Milupa Baby Rice) in water. All meals were extrinsically labelled with both ^{59}Fe and ^{65}Zn (1μCi/ml each) and 0·2 ml was given by gavage to 16-d-old Wistar rats previously fasted for 18 h. Animals were killed 14 h later and stomach, small intestine (SI) and caecum–colon removed. SI was perfused with 6 ml 0·15 M NaCl. Absorption of ^{59}Fe and ^{65}Zn (% dose) was calculated as:

$$\text{Absorption (\%)} = 100 - (\text{stomach} + \text{SI perfusate} + \text{caecum–colon})(\%).$$

Effect of dietary fibres on ^{59}Fe and ^{65}Zn absorption (% dose) in sucking rats

		^{59}Fe		^{65}Zn	
Fibres	*n*	Mean	SEM	Mean	SEM
Control	6	64·7	3·6	59·4	3·1
Apple	7	58·4	2·9	56·5	2·4
Orange	7	82·7***	1·1	63·2	1·8
Pea	6	80·3**	3·4	65·2	3·7
Sugar beet	7	76·4**	2·3	83·3***	1·8
Oat	6	41·7**	2·5	38·0**	2·1
Barley	5	75·3*	1·3	46·6*	3·8
Wheat	7	47·6**	1·5	28·2**	2·7

Significantly different from control: *$P<0·05$; **$P<0·01$; ****$P<0·001$.

^{59}Fe absorption from the rice meal was significantly reduced by oat and wheat fibres, but was enhanced by orange, pea, sugar beet and barley fibres, and was unaffected by apple fibre. ^{65}Zn absorption was significantly reduced by oat, barley and wheat fibres, was enhanced by sugar-beet fibre, and was unaffected by apple, orange and pea fibres.

The results show that the effect of dietary fibre preparations on bioavailability of trace elements is quite variable and probably reflects the balance of the influences of inhibitors (e.g. phytate) and enhancers (e.g. ascorbic acid, organic acids) present in these preparations.

Cashman, K., Flynn, A. & Harrington, M. (1991). *Proceedings of the Nutrition Society* **50**, 185A.

Assessment of exchangeable pools of zinc using the stable isotope ^{70}Zn. By M. J. JACKSON[1], SUSAN J. FAIRWEATHER-TAIT[2], T. E. FOX[2], S. GABRIELLE WHARF[2], J. EAGLES[2] and P. C. CROGHAN[3], [1]*Department of Medicine, University of Liverpool, Liverpool L69 3BX*, [2]*AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA* and [3]*School of Biological Sciences, University of East Anglia, Norwich*

The assessment of Zn status in man is complicated by the lack of a reliable biochemical indicator, thus Zn requirements for optimal health and function have not been clearly established (Gibson, 1989). Isotopic techniques may provide an answer to this problem and one potential approach is to study plasma Zn kinetics following an intravenous injection of a Zn isotope. Detailed studies of Zn kinetics have been carried out using ^{65}Zn in man (Wastney *et al.* 1986), with preliminary studies of the use of stable isotopes to study Zn kinetics in man (Miller *et al.* 1991; Lowe *et al.* 1992). We have, therefore, investigated various models for studying Zn kinetics in man following an injection of stable ^{70}Zn.

Two adults were given intravenous injections of either 0·29 or 1·36 mg ^{70}Zn at the beginning of a 10 d metabolic balance study. Blood samples were taken at defined times throughout the 10 d period. Blood plasma, urine and faecal samples were analysed for Zn isotope ratios by thermal ionization mass spectrometry.

The isotopic ratio data for plasma samples were analysed using two-compartment and four-compartment models, and the size of the mobilizable Zn pool was calculated according to the method of Johnson *et al.* (1991).

Both two- and four-compartment models demonstrate that isotopic Zn equilibrates with plasma Zn and with a rapidly exchangeable pool, probably located within the liver. The size of this latter pool in the two subjects was 7·5 mg (subject 1) and 5·6 mg (subject 2), using the two-compartment model, compared with 6·2 and 10·4 mg respectively, calculated from the four-compartment model. The later decay of the plasma isotopic Zn enrichment appears to be dictated by exchange with a pool of approximately 200 mg. In contrast, the 'mobilizable' Zn pool was found to be unreliable, varying throughout the study (285–465 mg Zn in subject 1 and 593–770 mg Zn in subject 2).

These data indicate that both two- and four-compartment models provide similar data concerning the size of the exchangeable pools with which isotopic Zn exchanges and may be suitable for use in studies of Zn status in man. The measurement of a 'mobilizable' Zn pool by the method of Johnson *et al.* (1991) does not appear to be suitable for this purpose.

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Gibson, R. S. (1989). *Progress in Food and Nutrition Science* **13**, 67–111.

Johnson, P. E., Vanderpool, R. A., Milne, S. K., Mahajan, S. K., Prasad, A. S. & Muller, L. K. (1991). In *Trace Elements in Man and Animals* – 7, pp. 4.6–4.7 [B. Momcilovic, editor]. Zagreb: IMI.

Lowe, N. M., Rhodes, J. M., Green, A. & Jackson, M. J. (1992). *Proceedings of the Nutrition Society* **51**, 59A.

Miller, L. V., Fennessey, P. V., Friel, J. K., Hang, Z., Noake, V. L., Westrott, J. C. & Hambidge, K. M. (1991). *FASEB Journal* **5**, A921.

Wastney, M. E., Aamodt, R. L., Rumble, W. F. & Henkin, R. I. (1986). *American Journal of Physiology* **251**, R398–R408.

Zinc supplementation in young Gambian children. By P. H. EVANS, C. J. BATES, P. G. LUNN, C. A. NORTHROP-CLEWES, S. HOARE, T. J. COLE and P. J. AGGETT, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

The effects of inadequate Zn nutrition on growth and morbidity in children are well recognized. Evidence from dietary and biochemical studies suggests that marginal Zn deficiency may occur in The Gambia. In the present study, effects of Zn supplementation on anthropometric, immunological and biochemical variables were examined in rural Gambian weanling infants (Bates *et al.* 1992).

This double-blind longitudinal controlled intervention study had two matched groups, stratified for age and sex. The children were aged 6 months to 2·5 years, and the Zn dose provided 20 mg Zn/d, as Zn gluconate in a fruit drink, for 1·25 years. The control group received the fruit drink alone.

No significant effects of Zn supplementation on body weight or length were observed. Analyses of blood plasma, urine and hair showed no differences in Zn concentration, and no changes in Zn-dependent plasma alkaline phosphatase (*EC* 3.1.3.1). Immunological assays revealed no Zn effect on plasma C3 complement or on CD4:CD8 lymphocyte ratios. However, a significant reduction in the urinary lactulose:creatinine ratio, and a transient increase in the acute-phase plasma protein, α1-antichymotrypsin, were seen (Table).

	Zinc			Placebo			
	n	Median	95% CI	*n*	Median	95% CI	
Lactulose:creatinine (molar ratio) at 8 weeks	43	0·35	0·25–0·48	39	0·56	0·46–0·68	($P<0·02$)
	n	Mean	SEM	*n*	Mean	SEM	
α1-Antichymotrypsin (g/l) at 2 weeks	41	1·02	0·053	41	0·84	0·043	($P<0·01$)

The reduction in gut permeability suggested by lowered lactulose excretion appears to resemble a recent observation by Roy *et al.* (1991), who similarly gave a Zn supplement to Bangladeshi children with diarrhoeal disease.

We conclude that Gambian weanling children may, in some circumstances, benefit from Zn supplementation, and this deserves further study.

Bates, C. J., Evans, P. H., Dardenne, M., Prentice, A., Lunn, P. G., Northrop-Clewes, C. A., Hoare, S., Cole, T. J., Horan, S. J., Longman, S. C., Stirling, D. & Aggett, P. J. (1993). *British Journal of Nutrition* **69**, 243–255.

Roy, S. K., Haider, R., Tomkins, A. & Behrens, R. H. (1991). *Proceedings of the Nutrition Society* **50**, 60A.

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Effects of dietary lactose and sucrose on antioxidant status in copper-deficient diabetic rats. By B. M. MCDERMOTT, J. J. STRAIN, K. J. MCADAM and P. R. FLATT, *Human Nutrition and Diabetes Research Groups, University of Ulster at Jordanstown, Newtownabbey, Co Antrim BT37 0QB*

Cu deficiency and the consumption of diets rich in lactose (compared with sucrose) can impair antioxidant enzyme activities (Lynch & Strain, 1989). Alterations in antioxidant defence mechanisms occur in diabetes (Strain, 1991). The aim of the present study was to investigate the effects of lactose or sucrose consumption on antioxidant enzyme activities in diabetic rats fed a low Cu (0·82–1·02 mg Cu/kg) diet.

Two groups (*n* 12) of male weanling Sprague-Dawley rats were housed individually and fed diets containing either 300 g sucrose/kg or 300 g lactose/kg for 35 d. Animals consuming the sucrose diet were pair-fed against those fed the lactose-based diet, while deionized water was supplied *ad lib*. Six rats from each group were injected with streptozotocin (STZ; 50 mg/kg body weight) to induce diabetes. These rats were then pair-fed against control rats in the same dietary group. After 16 d the rats were killed. Organs were removed for analysis of tissue Cu status, cytochrome c oxidase (CCO; *EC* 1.9.3.1), catalase (CAT; *EC* 1.11.1.6), glutathione S-transferase (GST; *EC* 2.5.1.18) and glutathione peroxidase (GSHPx; *EC* 1.11.1.9).

	Lactose				Sucrose				ANOVA results		
	Control		Diabetic		Control		Diabetic				INT
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	CHO	Db	CHO × Db
Body wt (g)	279	8·60	207	6·30	282	10·8	196	7·20	NS	***	NS
Plasma glucose (mg/l)	13·9	1·54	44·3	1·39	12·7	1·46	40·5	4·21	NS	***	NS
Liver:											
Cu (μg/g)	2·30	0·29	4·85	0·42	3·44	0·72	6·75	1·10	NS	**	NS
CAT (U/mg protein)	0·19	0·02	0·13	0·02	0·21	0·03	0·12	0·02	NS	**	NS
GST (U/mg protein)	0·24	0·02	0·15	0·02	0·18	0·02	0·16	0·03	NS	*	NS
Kidney:											
Cu (μg/g)	9·11	0·93	10·6	0·19	8·92	0·58	13·7	0·75	NS	***	*
CCO (U/mg protein)	1·33	0·14	1·23	0·01	1·47	0·11	1·63	0·06	*	NS	NS
CAT (U/mg protein)	0·20	0·01	0·13	0·01	0·21	0·02	0·17	0·01	NS	***	NS
GSHPx (mU/mg protein)	9·76	2·47	6·97	0·61	13·9	3·63	24·4	6·43	*	NS	NS

NS, not significant; SE, standard error; INT, interaction; Db, Diabetes; CHO, carbohydrate; ANOVA, two-way analysis of variance.

Levels of significance: **P*<0·05; ***P*<0·01; ****P*<0·001.

Results from the present study suggest that while the diabetic state appears to decrease antioxidant enzyme activity, consumption of sucrose, compared with lactose, increases Cu status in diabetes.

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Lynch, S. M. & Strain, J. J. (1989). *British Journal of Nutrition* **61**, 345–354.
Strain, J. J. (1991). *Proceedings of the Nutrition Society* **50**, 591–604.

The effect of dietary copper deficiency on endothelium-dependent relaxation of rat aorta *in vitro*. By D. P. MEGAW, J. M. ALLEN and J. J. STRAIN, *Biomedical Sciences Research Centre, University of Ulster, Coleraine BT52 1SA*

Dietary Cu deficiency is known to produce cardiovascular abnormalities in several animal species (e.g. Kitano, 1980). It was the purpose of the present study to determine if dietary-induced Cu deficiency altered endothelium-dependent and -independent relaxation of rat aorta *in vitro*.

Male Wistar rats were fed on an American Institute of Nutrition purified rat diet containing either 2·5 or 7·5 mg Cu/kg. Between week 6 – week 9, individual animals were killed by intraperitoneal injection of a lethal dose of anaesthetic (Equithesin; 0·9 ml/400 g body weight). A blood sample was obtained by cardiac puncture and the descending thoracic aorta (DTA) and liver were excised. The DTA was cleaned of surrounding connective tissue and placed in ice-cold (5°) Krebs solution. The vessel was divided into proximal and distal portions and rings (3 mm length) were cut from both. These rings were mounted in a water-jacketed organ bath (37°) for the measurement of isometric tension, and placed under 30 mN tension. The bath was constantly perfused with oxygenated Krebs solution. The preparations were allowed to equilibrate for 90 min before being precontracted with noradrenaline (NOR; 10^{-6}M). Relaxation in response to either carbachol (CARB; 10^{-9}–10^{-7}M) or sodium nitroprusside (NANP; 10^{-10}–10^{-6}M) was examined by cumulative addition of the drug to the perfusing solution. Results were expressed as a percentage of the NOR-induced tone, and relaxations to either the endothelium-dependent CARB or the endothelium-independent NANP were not found to differ significantly ($P>0·05$; ANOVA) between dietary groups. For example, in proximal rings taken from control animals (n 12), CARB (10^{-7}M) induced 45·3 (SEM 6·6)% relaxation as compared to 43·9 (SEM 7·5)% in rings from animals fed Cu-deficient diets (n 12). Hepatic Cu levels, as measured by atomic absorption spectrophotometry, differed significantly ($P<0·05$) between control (17·5 (SEM 2·3) μg/g dry weight) and Cu-deficient animals (12·0 (SEM 1·0) μg/g dry weight).

It is concluded that, in these mildly Cu-deficient rats, neither endothelium-dependent or -independent relaxation of aortic rings to the exogenous addition of CARB or NANP is impaired. This is in contrast to the situation in more severely Cu-deficient animals where endothelium-dependent relaxations of distal aortic segments were significantly decreased compared with control animals (e.g. Megaw *et al.* 1992).

This work was supported by a grant from the Northern Ireland Chest, Heart and Stroke Association.

Kitano, S. (1980). *Circulation Research* **46,** 681–689.
Megaw, D. P., Allen, J. M. & Strain, J. J. (1992). *Irish Journal of Medical Sciences* (In the Press).

Immunological function in copper deficiency. By K. K. TONG, D. P. MEGAW, J. M. ALLEN, J. J. STRAIN and B. M. HANNIGAN, *Biomedical Sciences Research Centre, University of Ulster, Coleraine, BT52 1SA*

While clinical Cu deficiency in humans is rare, several reports (e.g. Sandstead, 1988) indicate that sub-clinical deficiency may be more prevalent. Cu is involved in many critical metabolic functions, e.g. reactions catalysed by the Cu-dependent enzymes Cu/Zn superoxide dismutase (*EC* 1.15.1.1) and cytochrome c oxidase (*EC* 1.9.3.1). With regard to immune function, nutritional Cu deficiency in humans is characterized by recurrent infections and sepsis, symptoms reversible by dietary Cu supplementation (Lukasewycz *et al.* 1987) although the precise biochemical and cellular mechanisms are ill-defined.

Cytokines are protein mediators of the response to infection. One such cytokine, tumour necrosis factor-α (TNF), initiates a rapid inflammatory response to micro-organisms. The aim of the present study was to determine whether TNF production from rat peritoneal macrophages could be altered by *in vivo* Cu deficiency.

		Hepatic Cu (μg/g dry wt)		TNF level (pg/μg cell protein)		
Diet	*n*	Mean	SE	Mean	SE	Median
Trial 1:						
Cu-adequate	4	17·5	2·3	1739	856	1565
Cu-deficient	4	12·0*	1·0	1429	1289	173
Trial 2:						
Cu-adequate	8	18·7	1·68	2490	952	1756
Cu-deficient	6	6·5*	0·96	1434	639	1049

* Significantly different from Cu adequate at $P<0·05$ level (Student's *t* test).

Male weanling Wistar rats were maintained on Cu-adequate (7·5 mg Cu/kg) or Cu-deficient (2·5 mg Cu/kg) diets for a minimum of 6 weeks. Two trials were carried out. In Trial 1, eight rats were studied; in Trial 2, fourteen rats were studied. Cu deficiency was confirmed in rats at euthanasia by measurement of hepatic Cu. This was significantly decreased in the Cu-deficient relative to the Cu-adequate groups in both trials. Peritoneal macrophages were harvested at euthanasia, purified by adherence and washing, and maintained as monolayers. TNF levels in monolayer supernatants were measured by L929 fibroblast bioassay and the results expressed as pg of TNF activity per μg cell protein. There was no significant difference in TNF level between deficient and adequate groups. A trial is currently under way using a larger animal population to validate this new finding.

Lukasewycz, O. A., Kolquist, K. L. & Prohaska, J. R. (1987). *Nutrition Research* **7**, 43–52.
Sandstead, H. H. (1988). *American Journal of Clinical Nutrition* **35**, 809–814.

Effect of changes in environmental temperature on self-selection of ascorbic acid in coloured feeds by broiler chicks. By H. R. KUTLU and J. M. FORBES, *Department of Animal Physiology and Nutrition, The University of Leeds, Leeds LS2 9JT*

Increased demand for ascorbic acid (AA) has been demonstrated in birds in high environmental temperature due to lowered AA synthesis (Coates, 1984). It has been shown that broiler chicks can associate different levels of AA in feeds by means of their colour and they can adjust the proportion of AA-supplemented and unsupplemented feeds eaten to meet the requirements for AA according to environmental temperature (Kutlu & Forbes, 1992). However, it is not known (1) how quickly birds can associate dietary supplemental AA with colour and (2) how chicks respond to environmental changes in adjusting the proportion of AA-supplemented and unsupplemented feed intakes.

Unsupplemented red (R−), 0·2 g AA/kg supplemented red (R+), unsupplemented green (G−) and 0·2 g AA/kg supplemented green (G+) coloured standard starter diets were prepared. Thirty-six 1-week-old female broiler chicks were divided into four groups. Following an 8 d training period at a constant 28°, the chicks were given a choice between either G+ and R− or G− and R+ for 10 d under either environment 1 (heated: 10 h, 37°; 14 h, 26° per d from day 1 to 5 and unheated: constant 26° from day 6 to 10) or environment 2 (unheated from day 1 to 5 and heated from day 6 to 10).

Total daily food intake tended to be depressed by heat stress (75·4 *v.* 70·2 g/d over days 1 to 5; 87·4 *v.* 82·8 over days 6 to 10). Changes in environmental temperature had significant ($P<0·05$) effects on the ratio of intakes of AA-supplemented and unsupplemented feeds while not significantly affecting total intakes. Birds kept under environment 1 increasingly consumed more AA-supplemented feed than unsupplemented feed irrespective of its colour during the first 5 d, and daily intakes of AA-supplemented feed as a proportion of total intake were 0·56, 0·62, 0·66, 0·67, 0·66 ($P<0·05$ from day 3). When the birds were transferred to the unheated condition for the second 5 d the chicks consumed reducing proportions of supplemented feed irrespective of its colour and the proportions were 0·53, 0·45, 0·37, 0·34, 0·35 ($P<0·05$ from day 8). Food choice under environment 2 was affected by environmental temperature in a similar manner as those under environment 1. During the first 5 d birds increasingly consumed more unsupplemented feed than supplemented feed and daily intakes of supplemented feed as a proportion of total intake were 0·42, 0·37, 0·38, 0.30, 0.34 ($P<0·05$ from day 7). When the birds were moved to the heated condition the proportion changed to 0·46, 0·54, 0·60, 0·59, 0·58 ($P<0·05$ from day 8).

The results show that chicks can associate AA with colour within 3 d. As a result of this birds are able to adjust the proportion of AA-supplemented and unsupplemented feeds eaten to meet their AA requirements according to the environmental temperature.

Coates, M. E. (1984). Metabolic role of the vitamins. In *Physiology and Biochemistry of the Domestic Fowl*, vol. 5, pp. 27–36 [B. W. Freeman, editor]. London: Academic Press.

Kutlu, H. R. & Forbes, J. M. (1992). *Proceedings of the Nutrition Society* **51,** 55A.

Cholecystokinin octapeptide suppresses feeding and conditions colour aversion in chickens. By M. COVASA and J. M. FORBES, *The University of Leeds, Department of Animal Physiology and Nutrition, Leeds LS2 9JT*

The gastrointestinal hormone cholecystokinin (CCK) has been reported to inhibit feeding in various species, including chickens (Savory & Gentle, 1983), and to act as a satiety signal (Smith & Gibbs, 1979), although it may act through malaise. We investigated whether CCK can condition a colour preference or aversion, and, if so, how long it lasts and how easily it is reversed.

Twenty-four, 3-week-old female broiler chickens, housed in individual cages, were accustomed to coloured foods (red and green) and tested for their natural colour preference. During the experimental period, one colour, the conditioning stimulus (CS+), was paired with intraperitoneal injections of CCK-octapeptide (CCK-8; 14 μg/kg); the other colour was paired with injections of saline. For 2 weeks, injections of CCK-8 or saline were made alternately on Monday, Tuesday, Thursday and Friday, and coloured food preference was tested on Wednesday and Saturday. The injections were given after 1 h of food deprivation and coloured food was offered for the following 2 h. The chickens had free access to the uncoloured food for the rest of the day. Colour preferences were assessed in two-colour choice tests, and the first pecking time recorded. The conditioning effect was measured for another 2 weeks after injections ceased and the effect became dissociated. In the second part, the CS+ was paired with food of the other colour, three injections of CCK-8 and three of saline were given and preferences were assessed.

CCK-8 produced a significant suppression in food intake ($P<0{\cdot}05$). While chickens showed no significant colour preference before conditioning ($P=0{\cdot}21$), a clear aversion emerged for the coloured food previously paired with CCK-8 during conditioning ($P<0{\cdot}005$). In the second part of the experiment, chickens shifted their aversion in response to CCK-8 being paired with the different coloured foods ($P<0{\cdot}005$). The association of the CS+ aversion became significant after two injections (day 3) in the first part of the experiment, but significance was attained more gradually in the second part (day 6, $P=0{\cdot}02$; day 9, $P=0{\cdot}007$). After conditioning the aversion to CS+ was no longer present by day 3 in the first part, but had not disappeared until day 9 in the second part.

CCK-8 can produce long-lasting effects upon conditioned behaviour after the peptide had dissipated and was no longer biologically active. The results suggest that intraperitoneal injections of CCK-8 decrease feeding and can act as an aversive stimulus during conditioning and further support a role for CCK in learned food aversion.

Savory, C. J. & Gentle, M. J. (1983). *Appetite* **4**, 165–176.

Smith, G. P. & Gibbs, J. (1979). In *Progress in Psychobiology and Physiological Psychology*, vol. 8, pp. 179–243 [J. M. Sprague and A. N. Epstein, editors]. New York: Academic Press.

Poorer cognitive performance associated with dieting and high levels of dietary restraint.

By MICHAEL W. GREEN and PETER J. ROGERS, *Consumer Sciences Department, AFRC Institute of Food Research, Reading Laboratory, Reading RG6 2EF*

Dieting or slimming is a major preoccupation of UK consumers. However, while the behavioural aspects of dietary restraint and the metabolic and nutritional consequences of food restriction have been studied extensively, the possible effects of dieting on cognitive (mental) efficiency have received very little attention. In the present study women undergraduates of normal weight-for-height were assessed for their dieting behaviour and their concerns about eating in relation to body weight (dietary restraint scale from van Strien *et al.* 1986). Advertisements were placed specifically to recruit dieters (i.e. subjects who were currently dieting to lose weight) as well as non-dieters. All subjects also completed a battery of tasks assessing cognitive performance.

	Not dieting				Current dieters			
	Low-to-medium restraint (*n* 28)		High restraint (*n* 17)		Wt loss <1 kg (*n* 7)		Wt loss >1 kg[a] (*n* 18)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Dietary restraint (max=5)	2·2	0·1	3·5*	0·1	3·2*	0·4	4·0*†	0·2
Bakan task (% targets detected during first 3 min)	50	4	42	4	49	6	37*	4
Simple reaction time (ms)	334	13	346	14	384	25	382*	18
Immediate memory (no. of words recalled, max=20)	10·9	0·7	9·8	0·8	9·6	1·3	8·6*	0·9
Heart rate (pulse/min)	78	1	76	3	79	4	73*	2

[a] Weight loss for subjects in this group varied between 1·4 and 5·0 kg during 7 to 65 d on their current diet.
* Different from low-to-medium restraint, $P<0·05$ (2-tail).
† Different from high restraint and current dieters with weight loss <1 kg, $P<0·05$ (2-tail).

The Table shows that the current dieters were relatively impaired on a rapid information processing task (Bakan task), simple reaction time and immediate memory. Performance was poorest in subjects who had lost the most weight since the start of their current diet, while the group of highly restrained but non-dieting subjects tended to perform at a level intermediate between the low-to-medium restrainers and the current dieters. The results for heart rate fail to support the suggestion that the impairment in cognitive performance is due to over-arousal associated with the stressful effects of maintaining dietary restraint (Rogers & Green, 1992). Lowered heart rate is, however, typical of a chronic state of undernutrition (Keys *et al.* 1950).

The above findings indicate a sizeable impairment of cognitive performance related to both dietary restraint and weight loss. Controlled, prospective studies of dieting will be carried out to dissociate these different factors, although the evidence already points to substantial effects of food restriction *per se*.

Keys, A., Brozek, J., Henschel, A., Mickelsen, O. & Taylor, H. F. (1950). *The Biology of Human Starvation*, vol. 1. Minneapolis: University of Minnesota Press.

Rogers, P. J. & Green, M. W. (1992). *British Journal of Clinical Psychology* (In the Press).

van Strien, T., Frijters, J. E. R., Bergers, G. P. A. & Defares, P. B. (1986). *International Journal of Eating Disorders* **5**, 295–315.

The acute effects of lunches of differing fat and carbohydrate content on mood and cognitive performance. By HELEN M. LLOYD, MICHAEL W. GREEN, PETER J. ROGERS and DAVID J. MELA, *AFRC Institute of Food Research, Reading Laboratory, Reading RG6 2EF* and ANN F. WALKER, *Department of Food Science and Technology, University of Reading, Reading RG6 2AP*

There has been considerable interest in recent years regarding the effects of diet on performance efficiency and mood. Many of the studies carried out have concentrated on the influence of lunch, and post-lunch impairments in performance efficiency have been observed both under laboratory conditions and in real-life settings (see Craig, 1986 for review). Concomitant changes in mood have also been observed (Smith *et al.* 1991). The effects of meal composition on the 'post-lunch dip' have been studied, although attention has focused mainly on the effects of carbohydrate (CHO) *v.* protein (Pr) content. This study was designed to investigate the acute effects of iso-caloric meals of differing fat and CHO content on mood and cognitive performance.

Ten young healthy volunteers (eight female, two male) were fed low-fat (23 g fat, 100 g CHO, 26 g Pr), medium-fat (35 g fat, 79 g CHO, 21 g Pr) and high-fat (50 g fat, 47 g CHO, 24 g Pr) iso-caloric lunches in random order on three different days, each separated by a 1 week interval. The medium-fat lunch was similar in composition to that normally taken by the subjects. Subjects could not detect any differences in sensory qualities between the meals. The subjects were asked to complete mood and hunger ratings and also to carry out a variety of performance tests pre-lunch and during the 3 h period following lunch. These tests were designed to determine the nature and extent of any subjective and behavioural changes.

Simple reaction time in ms expressed as the difference from baseline measurements

	30 min post-lunch		90 min post-lunch		150 min post-lunch	
	Mean	SD	Mean	SD	Mean	SD
Low fat	6·06	7·27	5·64	10·0	6·69	10·9
Medium fat	−10·5	10·9	−19·7*	7·6	−15·5	6·46
High fat	−2·65	10·9	19·2	12·6	4·9	6·9

* Significantly different from low- and high-fat meals, $P<0·05$.

An example of the results on cognitive performance is shown in the Table. These results revealed a post-lunch dip in reaction time performance (i.e. a longer reaction time) with the low- and high-fat lunches, although this appeared to occur at a later stage and to a greater extent with the high-fat meal. There was an increase in 'drowsiness' post-lunch, with the high-fat lunch having a significantly greater effect than either of the other meals ($P<0·05$). Other mood scales indicated that both the high-fat and low-fat meals differed from the medium-fat meal, with subjects rating themselves as less 'calm' and less 'muddled' after the medium-fat lunch ($P<0·05$).

Taken together, these findings indicate that the macronutrient content of lunch, independent of energy value, can have substantial effects on subsequent mood and cognitive performance. In particular, higher than usual proportions of fat or carbohydrate at lunch appear to impair cognitive efficiency.

Craig, A. (1986). *Nutrition Reviews* **44,** 163–171.
Smith, A., Ralph, A. & McNeill, G. (1991). *Appetite* **16,** 85–91.

Effect of chronic energy deprivation on 24 h activity of lean and obese mice. By C. M. MURPHY and J. F. ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Republic of Ireland*

Reduced activity may contribute to the sustained positive energy balance which leads to obesity in the genetically obese mouse (Dauncey & Brown, 1987). Reduced activity is one response to sustained energy deprivation in lean mice. Is this so in obese mice? Aston strain obese mice were studied and compared with their lean litter-mates (*n* 8). Animals were fed standard mouse pellets (Nutec Ireland Ltd.), and were maintained at thermoneutrality (30°) and a 16 h light–8 h dark cycle. *Ad lib.* energy intake was determined for each group for 1 week. Animals were then given a reduced diet of 50% by weight of their *ad lib.* intake. Body weight, metabolic rate (indirectly as O_2 consumption), and gross activity (by microwave doppler system) were measured weekly before and during deprivation, for 24 h periods in undisturbed animals. The results are given in the Table.

	Animal	*Ad lib.*-fed		50% fed					
				Week 1		Week 2		Week 3	
	(*n* 8)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Wt (g)	Lean	34·6	1·0	33·3**	1·1	31·7**	1·2	30·0**	1·2
	Obese	60·4	1·8	58·4**	1·8	54·9**	1·6	52·1**	1·7
O_2 consumed (24 h mean; ml/g per h)	Lean	2·21	0·10	2·00**	0·09	2·00**	1·1	1·96**	1·0
	Obese	1·56	0·07	1·55	0·07	1·58	0·07	1·48**	0·06
Activity (24 h mean; movements/h)	Lean	8·8	1·9	6·2*	1·6	6·2*	1·9	5·5**	1·4
	Obese	3·7	0·8	3·6	0·8	4·5	2·0	1·2**	0·3

Significantly different from *ad lib.*-fed (paired *t* test): *$P<0·05$; **$P<0·01$.

The results confirm that *ad lib.*-fed obese animals do have a reduced activity. Food restriction caused a steady significant weight loss in both groups. O_2 consumption was markedly reduced from the outset in lean animals but only in the 3rd week in the obese. On the energy-reduced diet, lean animals decreased their activity somewhat in the first 2 weeks and markedly so in the 3rd week. Only in the 3rd week was there a very marked reduction in activity in the obese. Examining light *v.* dark period activity, the marked effect of low energy intake occurred during the awake dark phase. In the lean animals some diminution could be seen in the light (inactive) phase, however, as activity was almost zero in the light phase for the *ad lib.*-fed obese animals, no further diminution was possible. In conclusion, reduced activity could not be an important agent of resistance to weight loss in the obese, as they started from such a low base.

Dauncey, M. J. & Brown, D. (1987). *Quarterly Journal of Experimental Physiology* **72**, 549–559.

Food behaviour evolution in anorexia nervosa. By G. MORANDE and M. MONTENEGRO, *Hospital Central de la Cruz Roja de Madrid, Avda Reina Victoria 23, 28003 Madrid, Spain* and P. VARELA and A. MARCOS, *Instituto de Nutrición y Bromatología, Facultad de Farmacia, Ciudad Universitaria, 28040 Madrid, Spain*

Fashion has been shown to act as a model for food behaviour, often leading to severe damage in young people. Thus, eating disorders acquire great interest among which anorexia nervosa is considered a psychosomatic syndrome. Anthropometric measurements are the most common and simple guidelines followed to evaluate nutritional recovery in malnourished subjects. Moreover, attitudes towards eating and weight, and psychological and behavioural characteristics associated with eating disorders have been assessed by using the Eating Attitudes Test (EAT), while the Beck Depression Inventory (BDI) and General Health Questionnaire (GHQ) are useful tools to measure depressive and anxiety symptoms respectively. Therefore, the purpose of the present study was to test interrelationships between anthropometry and psychological recovery after nutritional and psychiatric therapies.

To this end, nineteen female patients suffering from anorexia nervosa were chosen and divided into two groups: group A, patients submitted to less than six months of therapy and group B, patients submitted to more than two years of therapy. Anthropometric measurements (weight, height, body mass index (BMI) and ideal body weight (IBW)), EAT, BDI and GHQ were evaluated.

Anthropometric and psychological measurements in patients suffering from anorexia nervosa

	Group A (*n* 8)		Group B (*n* 11)	
	Mean	SEM	Mean	SEM
Age (years)	15·00	0·73	15·18	0·48
Wt (kg)	45·50	2·63	45·05	1·19
Height (cm)	161·00	0·02	156·00*	0·01
BMI (kg/m^2)	17·58	0·80	18·43	0·54
IBW (%)	86·87	4·03	90·48	4·61
EAT	63·88	6·63	25·91*	3·64
GHQ	31·00	4·40	8·33*	4·58
BDI	29·33	1·09	13·00*	3·05

* Significantly different from group A: $P<0·05$.

Psychological health was significantly improved in group B, while no differences were found between height, BMI or IBW. Moreover, most of the patients of group B did not show menarche, while amenorrhea took place in 75% of the patients in group A. This outcome may suggest that psychological recovery precedes somatic recovery in the periods evaluated in this work.

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The effect of covert manipulation of dietary fat and energy density on *ad lib.* food intake in humans. 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*

Recent energy studies suggest that energy imbalances are primarily due to differences in intake rather than expenditure. Dietary macronutrient composition is important in determining the fuel mix being oxidized or stored, and may also have a large influence on food intake.

The present study assessed whether food intake in man is primarily regulated by monitoring energy balance, or carbohydrate (CHO) or fat balances, or both. Six men (age 41·8 (SD 11·5) years, weight 75·1 (SD 4·5) kg, height 1·76 (SD 0·02) m) were studied during three separate 7 d periods of whole-body indirect calorimetry, preceded by 2 d maintenance energy intake (1·4 × BMR). Whilst in the calorimeter subjects had *ad lib.* access to one of three covertly manipulated diets. Energy expenditure, substrate intakes and oxidations were continuously measured during this time. The diets were low-fat (LF; fat:CHO: protein as percentage energy 20:67:13); medium-fat (MF; 40:47:13); high-fat (HF; 60:27:13). Energy density increased with percentage fat. Diets were offered on a 3 d rotating menu and the order was randomized across subjects.

Results, given as balances of energy and macronutrients (intake − oxidation) are shown in the Table.

Diet . . .	20% fat (LF)				40% fat (MF)				60% fat (HF)			
Day	Energy (MJ)	Fat (MJ)	CHO (MJ)	Protein (MJ)	Energy (MJ)	Fat (MJ)	CHO (MJ)	Protein (MJ)	Energy (MJ)	Fat (MJ)	CHO (MJ)	Protein (MJ)
1	−1·61	−2·11	0·94	−0·26	0·03	−0·78	0·88	−0·03	1·71	1·31	0·43	0·0
2	0·29	−0·95	1·34	0·07	1·11	0·19	1·06	−0·01	2·74	2·11	0·49	0·26
3	−0·16	−0·96	0·96	0·01	0·77	0·53	0·50	−1·16	2·07	1·97	0·16	0·07
4	−0·67	−0·81	0·36	−0·03	0·50	0·34	0·07	0·10	1·35	1·46	−0·18	0·19
5	0·09	−0·42	0·65	0·05	0·93	0·79	0·15	0·10	5·35	4·12	0·85	0·51
6	−0·01	−0·43	0·54	0·05	0·98	1·07	−0·08	0·08	1·79	2·10	−0·40	0·15
7	−1·07	−0·36	−0·47	−0·06	0·54	0·62	−0·14	0·08	2·26	2·62	−0·27	0·03
Total	−3·14	−6·03	4·33	−0·17	4·87	2·76	2·44	0·16	17·27	15·68	1·07	1·30

Increasing the energy density and fat content of the diet led to a significantly greater voluntary energy intake. Relative to MF, the energy balance on LF was negative (t= −3·843, P=0·0012), the balance on HF was positive (t=5·06, P=0·0001) (stepwise multiple regression). Dietary manipulation accounted for 65% of the variation. This suggests that energy intake is not indiscriminately regulated and may be perturbed by altering dietary energy derived from fat. From these data we conclude that higher-CHO, lower-energy diets have a greater satiety effect (energy ingested/time) than low-CHO, high-energy diets. However, there was no evidence of a specific need to eat any physiologically-determined, absolute level of CHO intake, since mean daily CHO intakes were 372 (SEM 11·3) g, 313 (SEM 2·7) g and 226 (SEM 10·0) g on the low-, medium- and high-fat diets respectively.

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Behaviour of stunted children and the relationship to development. By J. M. MEEKS GARDNER, S. M. GRANTHAM-MCGREGOR and S. M. CHANG, *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, Minnesota, USA*

The mechanism by which undernutrition in young children leads to poor development is unclear. It is possible that certain behaviours such as the quality of the child's exploration may play a role. In the present study we examined the relationship between behaviour and development in stunted children. It was carried out as part of a larger intervention study on the effects of nutritional supplementation and psychosocial stimulation on stunted children's development (Grantham-McGregor *et al.* 1991). Poor neighbourhoods in Kingston, Jamaica were surveyed. Seventy-eight children aged 12–24 months with height-for-age < (NCHS standards −2 SD) were selected for this study and were randomly assigned to three groups: control, supplemented, and both (supplemented and stimulated). A group of matched controls (*n* 26) was also studied. Children were observed at home for 8 h on enrolment and for 5 h after a further 6 months. Behaviours were measured using time sampling and ratings. An activity score was computed from time and motion measurements. Development quotients (DQ) were measured on enrolment and after 6 (+6), 12 (+12) and 24 (+24) months of intervention.

On enrolment, the stunted children showed significantly more apathy, and displayed less enthusiasm when exploring the environment than non-stunted children. Caretakers of the stunted children had poorer quality of vocalizations to them compared with caretakers of non-stunted children. Factor analysis of the variables was carried out and the stunted children had significantly lower scores on the 'exploring/happy/chatting/active' factor. There were no significant intervention effects on behaviour.

At 6 months the stunted children continued to show less enthusiasm in exploring. They were also less happy, fussed or cried more often, and showed less variety in their types of exploration. Scores on the 'unhappy child' factor were significantly higher in the stunted children. The quality of vocalizations of caretakers to the stunted children continued to be poorer than those of caretakers to non-stunted children.

Multiple regression analyses showed that on enrolment the 'happy child' factor predicted change in DQ scores from enrolment to +12 months. The 'unhappy' factor at +6 months did not predict subsequent DQ change.

In conclusion, several significant differences were detected between the behaviours of stunted and non-stunted children, and their caretakers. Some of the behaviours which differed between the groups on enrolment predicted later change in development.

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Grantham-McGregor, S. M., Powell, C. A., Walker, S. P. & Hines, J. H. (1991). Nutritional supplementation, psychosocial stimulation, and mental development of stunted children: the Jamaican study. *Lancet* **338**, 1–5.

The effect of meal size on the cardiovascular responses to food ingestion. By M. B. SIDERY and I. A. MACDONALD, *Department of Physiology and Pharmacology, University of Nottingham Medical School, Clifton Boulevard, Nottingham NG7 2UH*

Food ingestion leads to substantial cardiovascular changes, including vasodilatation in the splanchnic bed. The aim of the present study was to assess the effects of meals of differing energy content.

Six healthy women (mean age 24 (range 21–26) years; mean body mass index (BMI) 23·8 (range 21·4–25·2) kg/m^2) were studied on three occasions, before and after the ingestion of high-carbohydrate meals (84% carbohydrate) containing 1, 2, or 3 MJ energy. The 1 MJ meal (cornflakes and skimmed milk) contained 54% of total carbohydrate as starch and 29% as sugar; the 2 MJ meal (cornflakes, skimmed milk, bread and honey) contained 48% of total carbohydrate as starch and 36% as sugar; the 3 MJ meal (cornflakes, skimmed milk, sugar, bread and honey) contained 45% of total carbohydrate as starch and 44% as sugar. Measurements of cardiac output (CO; indirect Fick), superior mesenteric artery blood flow (SMABF: Duplex Ultrasound), calf blood flow (CBF; venous occlusion plethysmography), heart rate (HR) and blood pressure (SBP; oscillometry) were made during fasting and for 120 min postprandially. Arterialized venous blood samples were used to measure whole blood glucose.

After the 1 MJ meal CO increased by 1·24 l/min (95% confidence interval of the change (CI) 0·99 to 1·49 l/min; $P<0·01$), HR by 9 beats/min (b/min) (CI 6·2 to 12·4 b/min; $P<0·01$), SBP by 5 mmHg (CI 0·6 to 10 mmHg; $P<0·05$), SMABF by 450 ml/min (CI 200 to 698 ml/min; $P<0·02$) and blood glucose by 2·5 mmol/l (CI 2·0 to 3·0 mmol/l; $P<0·001$). CO and SMABF had returned to baseline values by 90 min but HR did not return to baseline until 120 min.

The only different responses after the 2 MJ compared with the 1 MJ meal were a significant rise in CBF of 1·35 ml/100 ml per min, peaking at 90 min (CI 0·2 to 2·5 ml/100 ml per min; $P<0·05$) and no change in SBP.

Following the 3 MJ meal, the increases in HR (14·5 b/min (CI 10·5 to 18·5 b/min; $P<0·001$)) and SMABF (850 ml/min (CI 609 to 1090 ml/min; $P<0·0001$)) were significantly greater when compared with the 1 MJ meal ($P<0·02$ and $P<0·001$ respectively). CBF fell significantly within the first 30 min (CI −2·1 to 1·0 ml/100 ml per min; $P<0·02$), and failed to rise above baseline after this. In contrast to the 1 MJ meal, HR was still significantly higher at 120 min ($P<0·05$), CO was still elevated at 90 min ($P<0·04$), and SMABF was still elevated at 90 min ($P<0·001$). Neither SBP or DBP changed after the 3 MJ meal.

Thus, increasing the size of a high-carbohydrate meal produces more substantial responses of some, but not all, cardiovascular variables. As one might expect, increasing meal size produced cardiovascular responses of longer duration.

Glucagon-like peptide-$1_{(7-36)}$ amide and glucose-dependent insulinotropic polypeptide in the hyperinsulinaemia of obese hyperglycaemic (*ob/ob*) mice. By J. M. E. KNAPPER, *School of Biological Sciences, University of Surrey, Guildford GU2 5XH*, C. J. BAILEY, *Department of Pharmaceutical Sciences, Aston University, Birmingham B4 7ET* and P. R. FLATT, *Department of Biological and Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland BT52 1SA*

Exaggerated plasma insulin responses to the gastrointestinal hormone glucose-dependent insulinotropic polypeptide (GIP) have been observed in *ob/ob* mice. This study evaluated the involvement of a newer gastrointestinal hormone glucagon-like peptide-$1_{(7-36)}$ amide (GLP-$1_{(7-36)}$ amide) in the hyperinsulinaemia of *ob-ob* mice. Porcine GIP or human GLP-$1_{(7-36)}$ amide were administered by intraperitoneal injection at equimolar doses (40 μg/kg and 33 μg/kg respectively) in a saline (9 g NaCl/l) or glucose (33·1 kJ/kg; 2 g/kg) vehicle to 18 h-fasted adult Aston *ob/ob* and lean (+/+) mice.

	GLP-$1_{(7-36)}$ amide				GIP			
	Total content (pmol/intestine)		Concentration (pmol/g wet wt)		Total content (pmol/intestine)		Concentration (pmol/g wet wt)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
ob/ob mice	93·7*	8·8	54·1*	5·2	169·4*	26·1	107·1*	14·5
+/+ mice	34·6	4·3	25·8	3·3	64·5	12·1	48·3	9·3

Significantly different from the +/+ group when assessed by Student's unpaired *t* test: *$P<0·01$, for groups of 5–6 animals.

In a saline vehicle, neither GIP nor GLP-$1_{(7-36)}$ amide demonstrated any significant effects on glucose homeostasis or plasma insulin concentrations, revealing the importance of glucose in modulating the responsiveness of the pancreatic β-cells to the hormones. When administered with glucose, both GIP and GLP-$1_{(7-36)}$ amide significantly improved glucose tolerance ($P<0·01$; one-way analysis of variance and Duncan's range test) and increased insulin secretion ($P<0·01$). The hormones were equipotent in their actions in lean and *ob/ob* mice. Measurements of GLP-$1_{(7-36)}$ amide and GIP in intestines demonstrated marked enhancement in the concentration and total content of these hormones in *ob/ob* mice.

We conclude, therefore, that GLP-$1_{(7-36)}$ amide is a potent incretin in mice and may, like GIP, contribute to the marked hyperinsulinaemia of the *ob/ob* syndrome.

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Postprandial lipid and lipoprotein response to a fish-oil test meal. By E. DALY and M. J. GIBNEY, *Division of Nutritional Sciences, Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, Dublin 8, Republic of Ireland*

Plasma lipid and lipoprotein changes were monitored over a 12 h period in eight subjects (four male, four female) fed a single meal rich in *n*-3 fish oils (0·5 g MaxEPA oil/kg body-weight providing about 0·1 g C20:5 and 0·05 g C22:6/kg body-weight). Although the mean plasma triacylglycerol (TAG) response was monophasic with the peak occurring 5 h after the meal, 50% of the subjects exhibited either a biphasic or triphasic response. It would appear that both chylomicrons and very-low-density lipoproteins (VLDL) contribute to all plasma TAG peaks, even those occurring at later time points in the postprandial phase. Although the magnitude of postprandial lipeamia varied considerably (range 4·3–11·2 mmol/l plasma 12 h TAG area) a correlation was seen with fasting plasma TAG concentration (r 0·98, P=0·002). Following a decrease (P<0·05) immediately after the meal, the plasma non-esterified fatty acid (NEFA) concentration rose steadily to give a fasting concentration 12 h after the meal that was higher (P<0·01) than the initial fasting value. The increase in VLDL TAG concentration could not be explained by an increase in the concentration of the plasma NEFA substrate entering the liver because the mean VLDL TAG peaked at 5 h, and at this stage the plasma NEFA had only barely recovered to its fasting level.

Following isolation of the chylomicrons and VLDL by density gradient ultracentrifu gation, the lipids were separated by thin-layer chromatography and their fatty acid patterns analysed by gas chromatography (using a glass column packed with 10% silar 10C). They tended to assume the composition of the dietary fat in the order: chylomicron TAG>plasma TAG>VLDL TAG>plasma NEFA>plasma phospholipid, with the increase in percentage eicosapentaenoic acid (EPA; C20:5 *n*-3) and docosahexaenoic acid (DHA; C22:6 *n*-3) occurring primarily at the expense of C16:0, C18:1 and C18:2. The DHA:EPA ratio was lower in the test meal (0·5) than in any of the lipid fractions, but particularly so in the plasma NEFA fraction (1·71) which would indicate alternative metabolic pathways for EPA. Although the exact partitioning of newly-liberated NEFA is uncertain, our results suggest that the majority are directed to adipose tissue with the VLDL TAG receiving exogenous fatty acids mainly from chylomicron remnants in the postprandial phase.

E.D. is the recipient of a postgraduate scholarship from McDonnells (Flora).

Lunchtime dietary choice of young teenagers in Tayside. By WENDY L. WRIEDEN, *School of Food and Accommodation Management, Duncan of Jordanstone College of Art, University of Dundee, Dundee DD1 4HT*

Secondary school pupils (*n* 488) aged 14–15 years were asked to complete a questionnaire during the course of an exhibition in Dundee in 1991. Almost 90% (437, of which 189 were boys and 248 were girls) gave a single answer for where they obtained their lunch and could, therefore, be divided into four groups namely: home, packed meal, local shop, and school canteen. The proportion of children using a local shop at lunchtime (27% of boys and 15% of girls) and going home for lunch (35%) was higher than that calculated from the report on the diets of British schoolchildren (Department of Health, 1989), with a consequent fall in the percentages using school canteens or taking packed lunches.

Two elements of the diet were compared between the meal types. Pupils who consumed some fruit, salad or vegetables (not including chips) were classed as FSV. Pupils were put into a category of high sugar (HS) if they normally consumed chocolate or cakes as well as a fizzy drink or squash. This combination could total 60 g sugar, the daily maximum amount of sugar recommended by the Committee on Medical Aspects of Food Policy (COMA; Department of Health, 1991).

Percentage of pupils in each meal group eating FSV and HS

	Home	Packed meal	Local shop	School canteen
n . . .	152	79	88	118
FSV	40	59	24	29**
HS	34	29	44	21*

Within rows, groups are significantly different by chi-square test: *$P<0{\cdot}05$; **$P<0{\cdot}01$.

The large percentage of teenagers estimated to be eating HS coupled with the low percentage consuming FSV in the local shop group is of concern, especially as a quarter of boys may obtain their lunch from this source. It would appear that it is the packed lunch group that had the highest percentage of teenagers eating FSV, and this group also had the second lowest percentage for HS. If these two variables can be taken as an indication of the nutritional quality of a meal then the practice of taking a packed lunch to school should be encouraged.

Department of Health (1989). *Report on Health and Social Subjects* no. 36. London: H.M. Stationery Office.

Department of Health (1991). *Report on Health and Social Subjects* no. 41. London: H.M. Stationery Office.

The effect of non-starch polysaccharide on bacterial contribution to faecal mass. By A. J. COSTELLO, J. L. MURPHY and S. A. WOOTTON, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

Non-starch polysaccharide (NSP) is fermented in the gut resulting in an increase in faecal bacterial mass. The addition of fermentable substrate (bananas) to an NSP-free diet was shown to increase colonic bacterial cell growth and faecal mass (Murphy *et al.* 1991). The aim of the present study was to determine whether ispaghula husk increased bacterial contribution to faecal mass.

Six males (aged 22–43 years) consumed (1) an NSP-free diet of Ensure and Maxijul providing 78 mg N, 486 mg lipid and 124 kJ/kg body weight per d and (2) the same diet supplemented with 21 g ispaghula husk per d. Stools were collected in the final 3 d of the two 5 d study periods between carmine markers. Faecal bacteria were isolated (Stephen & Cummings, 1980) and freeze-dried stools analysed for energy (bomb calorimetry), N (Kjeldahl) and lipid. The results expressed as medians and ranges are summarized in the Table.

	SWW (g/d)	SDW (g/d)	FE (kJ/d)	FL (g/d)	FN (g/d)	Bm (g/d)
NSP-free	57 (46–78)	15 (12–19)	326 (281–380)	1·5 (0·9–2·9)	0·9 (0·6–1·1)	3·5 (3–6·1)
Ispaghula	117·8* (68–156)	24·4* (11–31)	556 (237–727)	1·2 (0·9–1·9)	0·9 (0·6–1·9)	4·5 (2·7–6·6)

SWW, stool wet weight; SDW, stool dry weight; FE, faecal energy; FL, faecal lipid; FN, faecal nitrogen; BM, bacterial mass.

Significantly different from NSP-free diet (Wilcoxon Rank Sum): $*P<0·05$.

Supplementation resulted in greater stool mass and faecal energy, but did not alter faecal lipid, N or bacterial mass. This suggests that ispaghula husk does not support the growth of colonic microflora; rather it passes through the gut non-fermented, bulking out the stool and increasing faecal energy.

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

Murphy, J. L., Wootton, S. A., Langran, M. & Jackson, A. A. (1991). *Proceedings of the Nutrition Society* **50**, 56A.

Stephen, A. M. & Cummings, J. H. (1980). *Journal of Medical Microbiology* **13**, 45–56.

Modest-but-adequate food budgets for five household types. By M. NELSON and A. B. MAYER, *Department of Nutrition and Dietetics, King's College, London W8 7AH*

Food budget standards provide a basis for assessing dietary adequacy in relation to cultural and economic as well as physiological needs, and for setting scale rates for social security and child care. Modest-but-adequate implies a budget which reflects current food consumption patterns modified to conform to healthy eating guidelines, far enough above subsistence levels to allow for individual variations in taste and choice and waste, and based on low but not minimum prices. Previously (Nelson & Peploe, 1990) we described the basis for calculating modest-but-adequate food budgets for households with two adults and one child. The present report outlines our revised assumptions, and gives modest-but-adequate food budgets for five household types.

The five household types were: two adults (male and female) aged 18–45 years (2A); two adults and two children (one under 5) (2A,2C (younger)); two adults and two children (one aged 11–16) (2A,2C (older)); one adult and two children (1A,2C); and single male aged 26–50 (1A). Budgets were calculated as follows: (1) National Food Survey (NFS) data from 1983–1987 were used to describe food purchasing patterns at a modest-but-adequate income level for each household type. The modest-but-adequate income level was taken to be the income band at which 35% of household expenditure (not including housing costs) was spent on food and fuel (based on Family Expenditure Survey (FES) data). (2) The NFS data do not include sweets, soft drinks, or alcoholic beverages. Contributions from sweets and soft drinks (using FES data) and alcoholic beverages (using Health Education Authority guidelines) were added into the food profile. (3) The adequacy of the diet (% Reference Nutrient Intake (RNI)) was determined, allowing for waste, consumption of food by visitors, and foods eaten away from home. (4) Food purchasing patterns were adjusted to conform to Department of Health guidelines for healthy eating. (5) The total diet was adjusted to provide the Estimated Average Requirement for energy, and at least 100% RNI for all other nutrients. (6) The purchases were costed using Sainsbury food prices at March, 1992, with contributions for foods eaten away from home based on FES data. This approach re-establishes a scientific basis for food budget methodology in Britain. The results suggest that households on income support are unable to afford a modest-but-adequate and healthy diet.

Cost (£ per household per week) of modest-but-adequate food purchases in five household types, at March 1992 prices

Household type . . .	2A	2A,2C (younger)	2A,2C (older)	1A,2C	1A
n . . .	316	235	101	65	86
Home food supply	26·82	45·67	53·92	33·80	12·18
Soft drinks	1·02	1·57	1·97	1·06	0·56
Sweets and chocolate	0·69	2·04	1·80	0·99	0·60
Food eaten away from home	10·86	9·51	12·05	3·56	12·84
Total food budget	39·39	58·79	69·74	39·41	26·18
Alcohol	12·86	12·86	12·86	5·36	7·50

Nelson, M. & Peploe, K. A. (1990). *Journal of Human Nutrition and Dietetics* **3**, 121–140.

Hepatic drug-metabolizing enzymes in spontaneously obese hyperglycaemic (*ob/ob*) mice.

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It has been established that both chemically-induced and spontaneously occurring insulin-dependent diabetes mellitus cause marked alterations of the hepatic cytochrome P-450 proteins that participate in the metabolism of both endogenous and exogenous substrates (Barnett *et al.* 1990; Ioannides & Parke, 1990). The present study was undertaken to investigate whether non-insulin-dependent diabetes causes similar effects in hepatic cytochrome P-450 activity and in glutathione conjugation. Six male spontaneously obese hyperglycaemic (*ob/ob*) mice and six lean litter-mates, all 16 weeks of age, were obtained from Aston University. Blood samples were taken at the time of death, the livers were immediately excised and microsomal and cystolic fractions prepared.

The obese mice were hyperglycaemic with plasma glucose concentrations double those of the lean animals (lean 5·6 (SEM 0·2) mM, obese 11·2 (SEM 1·4) mM; $P<0·05$). The plasma ketone body levels (sum of acetoacetate and 3-hydroxybutyrate concentrations) did not differ between the two groups (lean 0·40 (SEM 0·01) mM, obese 0·38 (SEM 0·01) mM). Cytochrome b_5 and total cytochrome P-450 levels (nmol/mg microsomal protein) in obese mice were not significantly different from those in the lean animals (lean 0·21 (SEM 0·05), 0·45 (SEM 0·1) respectively; obese 0·19 (SEM 0·01), 0·51 (SEM 0·1) respectively). Furthermore, the activities of the cytochrome P-450 1A, 2B, 2E, 3A and 4A proteins towards selective substrates were also comparable in the two groups. However, glutathione S-transferase (*EC* 2.5.1.18) activity (μmol 1-chloro-2,4-dinitrobenzene conjugated/min per mg cytosolic protein) was markedly lower in obese mice when compared with the lean animals. The present study demonstrates that non-insulin-dependent diabetes, as manifested in the obese hyperglycaemic (*ob/ob*) mouse, decreases glutathione conjugation capacity but, in contrast to insulin-dependent diabetes, it does not modulate the hepatic microsomal cytochrome P-450-dependent mixed-function oxidase system.

Barnett, C. R., Flatt, P. R. & Ioannides, C. (1990). *Chemico-Biological Interactions* **74,** 281–289.
Ioannides, C. & Parke, D. V. (1990). *Drug Metabolism Reviews* **22,** 1–85.

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Dietary factors and vertebral bone density in perimenopausal women from a general medical practice in Northern Ireland. By J. EATON-EVANS[1], E. M. MCILRATH[2], W. E. JACKSON[3], P. BRADLEY[3] and J. J. STRAIN[1], [1]*Human Nutrition Research Group, University of Ulster at Coleraine BT52 1SA,* [2]*Royal Victoria Hospital, Belfast BT12 6BA* and [3]*Skegoneill Health Centre, Belfast BT15 3LL*

Low bone density in middle age is a risk factor for osteoporosis in the elderly. Gradual bone loss is part of the ageing process, with an increase in the rate of trabecular bone loss in the 5 years after the menopause. Diet throughout life is thought to be a contributing factor to osteoporosis in old age.

Seventy-seven Caucasian women volunteers aged 46–56 years (mean 50·8, SD 3·26 years) were recruited from a General Practitioner's Health Centre. Trabecular bone density of the lumbar vertebra (L2) was measured by computerized tomography (CT) scan (X-ray exposure equivalent to 1–6 cGy). Data on social factors, lifestyle and the frequency of eating certain foods were collected by interview questionnaire, and anthropometric measurements were made.

The women's mean vertebral trabecular bone density was 122·6 (SD 30·7) mg/cm^3. Seven (9%) women had mild or moderate osteoporosis as defined by a bone density of less than one SD below the mean for age and sex of a reference population (Kallendar *et al.* 1989). Twenty-two women (28·5%) had a bone density of less than 100 mg/cm^3 and, using the criteria of Cann *et al.* (1985) and Odvina *et al.* (1988), were considered to be at increased risk of fracture or compression of the spinal vertebrae. Bone density declined with age (r −0·37, $P<0·001$) and years since menopause (r −0·27, $P<0·01$). In addition, those who considered themselves post-menopausal had lower bone densities than those who considered themselves pre- or perimenopausal. Step-wise multiple regression analysis of lifestyle and the frequency of eating selected foods suggested a negative association of bone density with age, and consumption of eggs and alcohol, and a positive association of bone density with vegetable consumption and parity:

bone density=316·9−4·3 age−6·6 eggs+1·5 vegetables−7·7 alcohol+15·0 parity

where age is in years, eggs is the number of eggs eaten in the previous week, vegetables is the frequency of eating a variety of vegetables (excluding potatoes and dried pulses) in the previous week, alcohol consumption is categorized as (1) never, (2) sometimes or (3) often and parity categorized as (1) nulliparous or (2) mono- and multiparous (multiple correlation coefficient r 0·66). There were no statistically significant correlations between bone density and body mass index or reported frequency of consuming milk, dairy foods and meat or recall of the amount of milk drunk in childhood.

It is suggested that the factors, including diet, which have been associated with osteoporosis in the elderly (Griffin, 1990) may be different to those associated with low vertebral trabecular bone density in perimenopausal women.

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Cann, C. E., Genant, H. K., Kolb, F. O. & Ettinger, B. (1985). *Bone* **6**, 1–7.

Griffin, J. (1990). *Osteoporosis and the Risk of Fracture*, pp. 22–47. London: Office of Health Economics.

Kallendar, W. A., Felsenberg, D., Louis, O., Lopez, P., Klotz, E., Osteaux, M. & Fraga, J. (1989). *European Journal of Radiology* **9**, 75–80.

Odvina, C. V., Wergedal, J. E., Libanah, C. R., Schulz, E. E. & Baylink, D. J. (1988). *Metabolism* **37**, 221–228.

Raised metabolic demands for energy may contribute to weight loss in institutionalized psychogeriatric patients. By R. M. SUTHERLAND and S. A. WOOTTON, *Moorgreen Hospital and Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

Many psychogeriatric patients, particularly those with senile dementia of Alzheimer's type (SDAT) and multi-infarct dementia (MID), are underweight on admission to long-stay institutions and lose weight during hospitalization (Asplund *et al.* 1981). The aim of the present study was to determine the extent to which poor food intake or altered metabolic demands for energy could contribute to weight loss in female psychogeriatric patients in long stay care.

Weighed food intake was recorded over 7 d in ten weight-losing SDAT/MID patients (WL; >10% weight loss over preceding 12 months; age 77 (SE 2) years, weight 43·1 (SE 1·9) kg, fat-free mass (FFM) 35·7 (SE 1·4) kg, body mass index (BMI) 18·4 (SE 0·7)) and ten weight-stable SDAT/MID patients from the same institution (WS; weight within 0·5 kg over preceding 3 months; age 76 (SE 3) years, weight 57·7 (SE 2·7) kg, FFM 37·4 (SE 1·2) kg, BMI 23·9 (SE 1·1)). Energy intake (EI) was estimated from a computerized food composition database. Basal metabolic rate (BMR) was determined by indirect calorimetry using a ventilated hood (Datex Deltatrac) and predicted from age, sex and body-weight (Department of Health, 1991).

The BMR of WL patients was greater than that of WS patients whether expressed in absolute units (5·05 (SE 0.27) *v.* 4·23 (SE 0·16) MJ/d; $P<0·02$), relative to FFM (142 (SE 6) *v.* 113 (SE 4) kJ/kg FFM per d; $P<0·001$) or as a percentage of predicted BMR (114 (SE 5) *v.* 84 (SE 3) %; $P<0·001$). Although WL patients tended to be served and to consume less dietary energy than WS patients (served 6·31 (SE 0·38) *v.* 7·36 (SE 0·43) MJ/d; consumed 5·67 (SE 0·55) *v.* 6·37 (SE 0·50) MJ/d), these differences did not attain statistical significance. When EI was expressed as a ratio EI:BMR, WL patients were served and consumed approximately 29% less than WS patients (served 1·26 (SE 0·07) *v.* 1·75 (SE 0·11); $P<0·01$; consumed 1·11 (SE 0·09) *v.* 1·52 (SE 0·12); $P<0·02$).

These results suggest that food provision to these weight-losing psychogeriatric patients was insufficient to satisfy the greater metabolic demands for energy, in contrast to that observed in weight-stable patients in the same institution. Further studies are required to establish the determinants of this raised metabolic demand and whether increased food provision could alter the decline in body weight and cognitive function.

Asplund, K., Normark, M. & Petterson, V. (1981). *Age and Ageing* **10**, 87–94.
Department of Health (1991). *Reports on Health and Social Subjects* no. 41. London: H.M. Stationery Office.

Assessment of nutritional status of hospitalized general surgical patients in Ibadan, Nigeria. By O. O. KESHINRO, J. C. O. OMOKO and P. U. EGELE, *Department of Human Nutrition, University of Ibadan, Nigeria* and A. M. LAOYE, *Department of Nursing, College of Medicine, University of Ibadan, Nigeria*

Surgery, trauma or severe infection, singly or in combination, initiate a complex series of events in man known as the metabolic response to injury. Badoe *et al.* (1986) illustrated how nutrient requirements are altered in illness. Studies in developed countries showed that malnutrition exists in hospitalized patients, including vitamin deficiences (Hill *et al.* 1977), and they are often largely unrecognized and untreated.

An investigation was carried out at the University College Hospital, Ibadan, Nigeria where the nutritional status of twenty-six surgical patients was studied. The variables examined included anthropometric and biochemical measurements and a 4 d weighed food intake. There was a decrease in the anthropometric measurements which continued with longer hospital stay. Weight loss correlated significantly with loss of triceps skinfold thickness within the 1st week after surgery and, more vividly, 2 weeks after surgery, ($P<0{\cdot}05$) as shown in the Table.

	Before surgery		3 d post-surgery		7 d post-surgery		14 d post-surgery	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body wt (kg) (cumulative)	57·6	4·6	57·2	4·5	56·7	3·9	56·1	4·0
Triceps skinfold thickness (mm)	10·8	2·5	10·6	2·1	10·3	2·0	10·0	2·0
Arm circumference (cm)	26·6	1·7	26·5	1·8	26·0	1·7	25·8	1·6
Packed cell volume	39·4	5·2	35·9	5·1	35·0	4·9	32·5	5·5
Serum albumin (g/100 ml)	3·6	0·4	3·2	0·5	3·1	0·2	3·0	0·0
Plasma ascorbate (mg/100 ml)	0·9	0·2	0·6	0·2	0·4	0·0	0·4	0·0

The mean consumption of energy, protein and vitamin C (% recommended dietary allowance; FAO/WHO, 1970/72/73) was quite low, being 84%, 70%, 67% and 53% respectively.

The present study shows the development of severe malnutrition in hospitalized patients. Dietary intakes are severely lacking and emphasize the need to develop appropriate hospital feeds which will overcome blandness of taste and anorexia due to illness.

Badoe, E. A., Archampong, E. O. & Jaja, M. O. A. (1986). *Principles and Practice of Surgery. Nutrition in Surgery* **117**, 88–101.

Food and Agriculture Organization/World Health Organization (1970/72/73). *Nutrient Requirements Study 28*. WHO Monograph Series no. 61. Geneva: WHO.

Hill, G. L., Blackett, R. L., Pickford, I., Burkinshaw, L., Young, G. A., Warren, J. V., Schorah, C. J. & Morgan, D. B. (1977). Malnutrition in surgical patients: an unrecognized problem. *Lancet* **i**, 689–692.

Linoleic and α-linolenic acid oxidation during undernutrition: are they important in energy metabolism? By S. C. CUNNANE, Z.-Y. CHEN and J. YANG, *Department of Nutritional Sciences, University of Toronto, Toronto, Canada*

[1-^{14}C]linoleic acid (18:2*n*-6) and α-linolenic acid (18:3*n*-3) are readily oxidized both in vivo and in vitro but little is known of their whole-body partitioning between: (1) storage or structural lipids, (2) longer chain products (LCP) or (3) oxidation. We have shown that 18:2*n*-6-enriched triacylglycerols are selectively retained in liver and serum during energy deficit (Chen & Cunnane, 1992), suggesting that undernutrition may affect the partitioning and, hence, oxidation of 18:2*n*-6 and 18:3*n*-3. The fatty acid balance method was used to assess apparent oxidation of 18:2*n*-6 and 18:3*n*-3 during pregnancy compromised by chronic or acute undernutrition. In the balance method,

apparent oxidation=intake−(accumulation+excretion).

Chronic undernutrition was induced in rats by feeding a low-zinc diet from day 8 of pregnancy to term (Zn[–] 3 mg/kg, compared with 35 mg/kg in controls). In a separate study, acute undernutrition was induced by fasting for 24 h or 48 h during days 13–15 of pregnancy followed by refeeding to term. Daily fatty acid intake and excretion were measured and groups were killed at the beginning and end of each study period to determine fatty acid accumulation. Organ and carcass fatty acid analysis was done by conventional methods (Chen & Cunnane, 1992) and apparent fatty acid oxidation calculated. In the Zn[–] study, food intake and weight gain were decreased towards term and overall intake of 18:2*n*-6 and 18:3*n*-3 was reduced by 39% ($P<0{\cdot}01$). Net whole-body accumulation of both 18:2*n*-6 and 18:3*n*-3 was negative, and *n*-6 and *n*-3 LCP accumulation was significantly less in the Zn[–] rats. In the Zn[–] group at term, apparent whole-body oxidation of both 18:2*n*-6 and 18:3*n*-3 was 50–60% higher (mg/d) or eight–ninefold higher (mg/g body weight gained) than in the controls. In the acute undernutrition study, apparent whole-body oxidation of 18:2*n*-6 and 18:3*n*-3 was lower during fasting relative to that of saturates and monounsaturates. However, during refeeding to term, apparent oxidation of 18:2*n*-6 was increased 304% and 18:3*n*-3 oxidation was increased 203% relative to *ad lib*.-fed controls ($P<0{\cdot}01$). In fact, relative to the start of the study (day 13), there was a net whole-body loss of total *n*-6 and total *n*-3 fatty acids in rats fasted between day 13–15 of pregnancy but then refed to term. Thus, during the acute undernutrition period, there was relative conservation of 18:2*n*-6 and 18:3*n*-3 but during recovery from fasting with *ad lib*. accesss to food, pregnant rats preferentially oxidized 18:2*n*-6 and 18:3*n*-3 while accumulating the equivalent of the entire dietary intake of saturates and monounsaturates consumed during the refeeding period. We conclude that dietary or whole-body stores, or both, of 18:2*n*-6 and 18:3*n*-3 are not only important as precursors for *n*-6 and *n*-3 LCP, but their oxidation may also be considerable, particularly during undernutrition.

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Chen, Z.-Y. & Cunnane, S. C. (1992). *American Journal of Physiology* (In the Press).

The importance of dietary fat in maintaining energy balance in a rural subsistence farming community. By B. J. SONKO[1], A. M. PRENTICE[1], A. A. PAUL[1], A. PRENTICE[1], Y. SCHUTZ[2], E. JEQUIER[2] and R. G. WHITEHEAD[1], [1]*Dunn Nutrition Unit, Keneba, The Gambia* and [2]*Institute of Physiology, University of Lausanne, Switzerland*

Poor diets in developing countries tend to have a very low fat content. These may be associated with raised metabolic requirements for fat due to a high physical workload or infection, or both. Rural Gambian women lose up to 50% of their body fat during each year's hungry (wet) season when a deterioration in diet quality and availability coincides with intense farm work. We hypothesized that the dietary fat intake may be so low that this seasonal weight loss may be driven by a negative fat balance, and that this would occur even if sufficient food were available to meet the full energy requirements.

Ten non-pregnant, non-lactating women, after an overnight fast, performed simulated wet season field work (5×45 min at 3×BMR during 8·5 h) in a whole-body respiratory chamber. A meal providing 0·6×BMR was provided after the second exercise. Net fuel oxidation rates were calculated from non-protein respiratory quotient. Fat oxidation (24 h) was computed by adding values for rest and sleep (15·5 h) from an existing data-base for similar women. Fat oxidation rates were as follows: simulated field work, 0·098 (SD 0·022) g/min or 50·0 g/8·5 h; rest and sleep 0·042 (SD 0·010) g/min or 39·1 g/15·5 h; total 89·1 g/24 h. Total energy expenditure for the simulated day was 9700 kJ/24 h.

The fat contents of wet and dry season diets were estimated from extensive food records to be 15·1% and 26·4% by energy. Even if the women could obtain sufficient food to cover their energy needs this would only provide 37 g fat/24 h when diet quality was poorest. The estimated negative fat balance (intake−net utilization) of −52 g/24 h supports the initial hypothesis and matches the observed rate of weight loss. Theoretical calculations based on the higher fat content of the diet and the lower workload during the dry season indicate that positive fat balance would occur. Current evidence suggests that *de novo* fat synthesis is of minor significance in humans on a Western (relatively high-fat) diet. It may be more important in subjects living on high carbohydrate diets, but the obligatory biochemical costs associated with the process would make it undesirable when energy is already limiting.

The work suggests that under certain circumstances the daily oxidation rates of fat may exceed fat intake by a wide margin. The deficit can only be made up by fat synthesis or by consuming excessive energy intakes. The latter strategy would create problems with both energy and carbohydrate disposal and would probably inhibit uptake. The results emphasize the need to consider diet quality as well as quantity in the provision of energy from marginal diets in the developing world.

The effect of dietary fatty acid composition on mRNA for lipoprotein lipase in the rat. By M. C. MURPHY, S. M. PUDDICOMBE, L. M. MORGAN & C. M. WILLIAMS, *Nutritional Metabolism Research Group, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

Although there have been a number of studies of effects of diet and hormones on lipoprotein lipase (LPL) activity and levels of LPL mRNA (Raynolds *et al.* 1990), there have been no studies which have investigated effects of different dietary fatty acids on LPL gene expression. In the present study male Wistar albino rats were pair-fed diets containing 50 g fat of different fatty acid composition/kg for 2 weeks. The diets fed were (1) a mixed oil (42% saturated fatty acid, 43% monounsaturated fatty acid, 16% polyunsaturated fatty acid) (*n* 8), (2) corn oil (*n* 8) or (3) fish oil (*n* 8). Animals were killed and RNA was extracted from liver and perirenal and epididymal fat pads, separated on denaturing agarose gels and blotted onto nylon membranes using a 'Northern methodology'. Samples were hybridized to a human cDNA probe for LPL supplied by Oka (Gotoda *et al.* 1989). Two transcripts were identified in epididymal and perirenal adipose tissue, which were approximately 3·7 and 1·7 kilobases in size, in the tissues studied. The results suggest that (1) in fish oil-fed animals there is greater production of LPL mRNA in epididymal fat compared with corn oil-fed animals ($P<0·05$), (2) corn oil-fed animals had significantly greater production of LPL mRNA in perirenal adipose tissue compared with the other dietary groups ($P<0·05$), (3) there was insignificant expression in liver and (4) in fish and mixed oil-fed animals there was greater production of LPL mRNA in epididymal compared with perirenal fat. The significance of these findings to the control of LPL activity by different dietary fatty acids will be the subject of further investigation.

	Fish oil		Mixed oil		Corn oil	
	Mean	SEM	Mean	SEM	Mean	SEM
LPL expression:						
Epididymal	69 400*	15 188	51 723	11 641	31 285	5366
Perirenal	15 045	4758	15 155	4734	37 361†	9144
LPL activity ($\times 10^6$) (μmol oleate/min per mg at 37°)	3·97	1·4	2·02	0·26	2·64	0·61

* Significantly different from corn oil: $P<0·05$.
† Significantly different from other dietary groups: $P<0·05$.

Gotoda, T., Senda, M., Gamou, T., Furuchi, Y. & Oka, K. (1989). Nucleotide sequence of human cDNA coding for a lipoprotein lipase (LPL) cloned from placental cDNA library. *Nucleic Acids Research* **17**, 2351.

Raynolds, M. V., Awald, P. D., Gordon, D. F., Gutierrez-Hartman, A., Rule, D. C., Wood, W. M. & Eckel, R. H. (1990). Lipoprotein lipase gene expression in rat adipocytes is regulated by isoproterol and insulin by different mechanisms. *Molecular Endocrinology* **4**, 1416–1422.

Effect of heat treatment and enzyme supplementation of barley-based diets on performance of broiler chicks. By K. J. MCCRACKEN[1,2], R. URQUHART[1] and M. R. BEDFORD[3], [1]*Food and Agricultural Chemistry Department, The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX*, [2]*Department of Agriculture for Northern Ireland* and [3]*Finnfeeds International Ltd., Market House, High Street, Marlborough, Wiltshire SN8 1AA*

Heat pasteurization of poultry diets is increasingly practised but there is a dearth of published information on the effects of this process on nutrient availability. Feed enzymes have proved effective in improving nutrient availability of mash and pelleted diets based on barley and rye (Pettersson & Aman, 1989; Pettersson *et al.* 1991) but there is little information on the stability of feed enzymes at the temperatures being used for heat pasteurization against salmonella.

Four diets based on the same formulation, containing 400 g barley/kg and a total cereal content of 560 g/kg, were fed to male, broiler chicks in individual cages from 4 to 25 d of age (*n* 25). The dietary treatments were: (1) no heat treatment, no enzyme; (2) no heat plus enzyme; (3) heat treatment (15 min, 85°), no enzyme; (4) heat treatment plus enzyme. The enzyme mixture contained β-glucanase (*EC* 3.2.1.4) and xylanase (*EC* 3.2.1.32) (Avizyme SX) and was included at 1 kg/tonne. After treatment, all four diets were pelleted and crumbed to provide a uniform feed for presentation. During the last 9 d complete collections of excreta were made from six birds on each treatment for determination of metabolizable energy (ME). Viscosity was measured in ileal contents after the experiment was completed (*n* 5).

The average composition of the diets was (g/kg dry matter (DM)): crude protein 275, oil 71, ash 66, gross energy 19·6 MJ/kg. The mean initial bird weight was 77 g. Two mortalities occurred with diet 3.

Mean DM intakes (g/d) for diets 1 to 4 were respectively 57·5, 58·0, 60·6, 58·9 (SEM 1·04; P=0·16). The corresponding values for live-weight gain were 43·0, 44·0, 45·0, 45·6 (SEM 1·13; P=0·40) and for feed conversion ratio (FCR; g feed/g gain) were 1·35, 1·33, 1·35, 1·30 (SEM 0·022; P=0·21). The mean metabolizability of energy (ME:GE) values were 0·734, 0·760, 0·713, 0·747 (SEM 0·0064; P<0·001). Excreta DM values for the bulked 9 d samples tended to be lower for the birds fed on diet 3 than for the other treatments (372, 388, 338, 396 g/kg for diets 1 to 4 respectively), and the viscosity measurements tended to be higher for diet 3 (6·2, 4·8, 11·0, 5·3 centipoise; SEM 1·66; P=0·066).

The results suggest that the relatively severe conditions of heat treatment imposed, in terms of temperature and time, reduced the apparent digestibility, increased the viscosity of gut contents and reduced faecal DM content compared with the unheated diet. Addition of enzymes improved nutrient availability of unheated and heat-treated diets to similar extents and eliminated the undesirable side-effects of heat pasteurization. This establishes the stability of the feed enzymes at the temperature employed.

It is, therefore, concluded that the use of a stabilized enzyme supplement such as Avizyme SX is effective in eliminating potential side-effects of heat pasteurization of barley-based diets for broiler chicks.

Pettersson, D. & Aman, P. (1989). *British Journal of Nutrition* **62,** 139–149.
Pettersson, D., Graham, H. & Aman, P. (1991). *Animal Feed Science and Technology* **33,** 1–14.

Basal and hormone-stimulated rates of lipogenesis in liver and adipose tissue of rats fed on corn oil and fish oil diets. By C. M. WILLIAMS, J. M. BEETY, A. ZAMPELAS, N. FURLONGER, and L. MORGAN, *The Nutritional Metabolism Research Group, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

Although feeding fish oil reduces circulating triacylglycerol levels, the mechanism of this effect has not been fully established. In the present investigation we have studied the effects of feeding fish oil on basal and hormone-stimulated rates of lipogenesis in adipose tissue and liver in the rat. Two groups of eight Wistar albino rats were pair-fed diets containing 10% fat (as energy) either as corn oil or fish oil for a period of 2 weeks. Animals were killed in the fed state after overnight meal feeding, and blood was collected by cardiac puncture. In the liver slices the rates of basal, insulin (4 nM)-, gastric inhibitory polypeptide (GIP) (6 nM)- and GIP+insulin (6 nM+4 nM)-stimulated ^{14}C-glucose incorporation, and in adipose tissue explants the rates of basal and insulin (2 nM)-stimulated ^{14}C-acetate incorporation, were measured.

	Basal		Insulin		GIP		Insulin+GIP	
Additions	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Adipose tissue (nmol acetate incorporated/mg):								
Corn oil	1·34	0·21	3·38	0·05	—		—	
Fish oil	2·23*	0·12	6·75*	1·00	—		—	
Liver (nmol glucose incorporated/mg):								
Corn oil	0·83	0·08	1·25	1·46	0·92	0·14	1·65†	0·24
Fish oil	0·62	0·09	0·96	0·21	0·81	0·10	1·35†	0·18

* Significantly different from corn oil-fed rats: *$P<0·01$.
† Significantly different from basal-fed rats: †$P<0·05$.

Rates of basal and insulin-stimulated acetate incorporation were significantly higher in adipose tissue of fish oil-fed animals ($P<0·01$, both cases). In the liver, GIP or insulin alone had no effect on glucose incorporation into lipids, but GIP in the presence of insulin resulted in a significantly increased incorporation of glucose into lipids (corn oil $P<0·01$; fish oil $P<0·05$).

It is concluded that in fish oil-fed animals there is increased sensitivity to the effects of insulin in adipose tissue. This effect may be mediated through enhanced receptor sensitivity resulting from changes in membrane phospholipid fatty acid composition.

Effects of triacylglycerol structure on postprandial chylomicron triacylglycerol clearance.

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Since studies in rats have shown the position of saturated fatty acids on the triacylglycerol molecule may be an important determinant of chylomicron clearance (Redgrave *et al.* 1988), a human study was designed in order to compare chylomicron triacylglycerol clearance after consumption of two separate test meals differing in their triacylglycerol structure. Sixteen male volunteers consumed test meals consisting of 84 g powdered meal replacement (protein 15·12 g; carbohydrate 60·48 g), and 40 g of test oil on two separate occasions. The test oils had the same fatty acid compositions but differed in that palmitic acid was located in positions sn-2 (OPO), and sn-3 (OOP) respectively. The concentrations in plasma of chylomicron triacylglycerol, insulin and GIP are shown in the Table.

Time after feeding (min) . . .	0	30	60	90	120	180	240	300	360
Chylomicron triacylglycerol (mmol/l):									
OOP: Mean	0·47	0·66	0·69	0·75	0·80	0·82	0·79	0·92	0·80
SD	0·21	0·50	0·48	0·34	0·42	0·39	0·36	0·44	0·38
OPO: Mean	0·48	0·56	0·67	0·78	0·82	0·92	0·86	0·94	0·91
SD	0·19	0·21	0·23	0·30	0·29	0·40	0·30	0·44	0·44
Insulin (pmol/l):									
OOP: Mean	7·5	49·9	40·7	39·9	26·6	11·4	7·7	7·2	5·5
SD	5·5	25·8	20·0	23·8	20·0	7·5	4·5	3·7	2·7
OPO: Mean	7·9	47·9	49·9	33·9	28·0	11·9	8·2	7·4	7·0
SD	3·9	26·5	27·5	17·4	32·4	6·9	5·4	3·4	4·5
GIP (pg/ml):									
OOP: Mean	234	1636	1720	1575	1228	1067	1063	976	522
SD	145	774	487	441	390	320	304	400	313
OPO: Mean	261	1596	1651	1589	1200	1155	1048	796	530
SD	213	593	474	483	439	555	402	258	262

The results show no difference between the two meals in clearance of chylomicron triacylglycerol, plasma insulin or plasma GIP. With both meals, the plasma polypeptides rose to peak values (insulin approximately 13 pmol/l; GIP approximately 1800 pg/ml at 45 min and declined to baseline levels after 240 min (insulin) and 360 min (GIP). Measurement of chylomicron triacylglycerol clearance does not provide a measure of chylomicron remnant clearance, and it is the remnants which have been implicated in atherogenesis. Availability of an assay for apoB-48, the chylomicron-specific apolipoprotein B, would enable effects of diet on chylomicron remnant clearance to be determined.

Redgrave, T. G., Kodali, D. R. & Small, D. M. (1988). *Journal of Biological Chemistry* **263,** 5118–5123.

The relationship between changes in metabolic rate, haemodynamic variables and plasma catecholamine levels during acute starvation. By J. WEBBER and I. A. MACDONALD, *Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH*

Sympathoadrenal pathways are thought to play a major role in adaptations to over- and undernutrition. Noradrenaline turnover is reduced during fasting and increased during overfeeding in animals (Landsberg & Young, 1978), whilst in man there is a component of glucose-induced thermogenesis which can be reduced by β-blockade (Acheson *et al.* 1983). We set out to examine the changes in plasma adrenaline and noradrenaline during acute starvation in man, and whether these were related to alterations in resting metabolic rate (RMR) and haemodynamic status.

Twenty-three healthy (nine male, fourteen female), non-obese subjects aged 19–30 years were recruited. They attended on three occasions having fasted for 12, 36 or 72 h. During this period they were allowed water *ad lib.* and consumed 80 mmol sodium chloride and 50 mmol potassium chloride/24 h. Arterialized venous blood samples for plasma catecholamines were taken after the subject had been supine for at least 90 min. RMR was measured by indirect calorimetry. Heart rate and blood pressure were recorded with an automatic cuff, and forearm blood flow (FBF) was determined by venous occlusion plethysmography.

Hours of starvation	RMR (J/kg per min)		Noradrenaline (nmol/l)		Adrenaline (nmol/l)		Heart rate (beats/min)		FBF (100 ml per min)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
12	69·7	1·9	0·73	0·04	0·20	0·02	64·2	1·7	3·5	0·2
36	76·0**	2·0	0·69	0·04	0·24	0·03	70·0**	2·0	5·2**	0·4
72	73·1	1·9	0·87**	0·07	0·32**	0·05	70·3**	2·0	6·3**	0·5

Significantly different from value at 12 h: $^{**}P<0{\cdot}01$.

Plasma catecholamines are unchanged at 36 h of starvation, but are elevated at 72 h. Heart rate and FBF are already significantly increased at 36 h, with the latter rising further by 72 h. RMR, on the other hand, is maximal at 36 h. That changes in catecholamines are maximal at 72 h whilst RMR is falling shows that there is no simple relationship between sympathoadrenal activity and changes in RMR during starvation in humans.

Acheson, K., Jequier, E. & Wahren, J. (1983). *Journal of Clinical Investigation* **72,** 981–986.
Landsberg, L. & Young, J. B. (1978). *New England Journal of Medicine* **298,** 1295–1301.

Cholesterol oxidation in porcine muscle as influenced by pig diet. By F. J. MONAHAN[1], J. I. GRAY[2], D. J. BUCKLEY[1] and P. A. MORRISSEY[3], *Departments of* [1]*Food Technology* and [3]*Nutrition, University College, Cork, Republic of Ireland* and [2]*Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824, USA*

Lipid oxidation products, particularly cholesterol oxides, have been implicated in atherosclerotic lesion formation, the initial stage in a sequence of events that can lead to coronary or cerebral thrombosis (Hubbard *et al.* 1989). Previous studies have shown that, in cooked pork, lipid oxidation is influenced by the unsaturated fatty acid content and by the α-tocopherol content of the pig's diet (Monahan *et al.* 1992). The present study investigated the effect of oxidized dietary oil and dietary α-tocopherol on cholesterol oxidation in pork.

Seventy-two Landrace × Yorkshire pigs (barrows and gilts, 80–90 d old) were divided into six groups of twelve and balanced with respect to litter-mate, body weight and sex. For 10 weeks before slaughtering, pigs were fed on diets containing either oxidized (4·5 meq peroxide/kg diet) or unoxidized corn oil with 10, 100 or 200 mg α-tocopheryl acetate/kg diet. After slaughter, *longissimus dorsi* muscle samples were cooked, stored at 4° and assessed for cholesterol oxidation at 48 h intervals. Cholesterol oxides were measured by capillary gas chromatography.

Effect of dietary oil and α-tocopheryl acetate supplementation on cholesterol oxide content (μg/g) of cooked pork during storage at 4°

Dietary oil	Dietary α-tocopherol (mg/kg diet)	Day 2						Day 4					
		β-epoxide		7β-OH		7-keto		β-epoxide		7β-OH		7-keto	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Oxidized:	10	5·29[c]	0·14	4·49[b]	0·26	7·70[b]	0·25	7·21[b]	0·60	5·35[b]	0·41	10·92[b]	0·75
	100	4·20[b]	0·44	3·21[a]	0·42	5·37[a]	0·56	4·93[a]	0·49	4·41[a]	0·26	9·31[a]	0·71
	200	3·22[a]	0·18	2·57[a]	0·08	4·03[a]	0·12	5·65[a]	0·28	4·28[a]	0·46	8·41[a]	0·71
Unoxidized:	10	5·05[b]	0·22	3·94[b]	0·31	7·17[b]	0·25	5·67[a]	0·38	5·07[b]	0·45	9·79[b]	0·75
	100	3·65[a]	0·41	3·10[a]	0·24	5·09[a]	0·44	5·64[a]	0·28	4·54[a,b]	0·31	7·85[a]	0·51
	200	3·42[a]	0·59	2·68[a]	0·17	4·06[a]	0·20	5·15[a]	0·50	4·05[a]	0·45	8·79[a]	1·17

[a,b,c] For each oil, mean values in the same column with unlike superscripts are significantly different (Fisher's LSD test): $P<0·05$.

Cholesterol oxides were present only in trace amounts immediately after cooking. However, after 2 and 4 d of refrigerated storage cholestan-5β, 6β-epoxy-3β-ol (β-epoxide), cholest-5-ene-3β, 7β-diol (7β-OH) and 5-cholesten-3β-ol-7-one (7-keto) were detected in all samples. Analysis of variance of the data revealed that cholesterol oxides were significantly influenced ($P<0·01$) by dietary α-tocopherol but not by dietary oil. Cholesterol oxide levels were significantly higher ($P<0·05$) in pork from pigs fed 10 mg α-tocopheryl acetate/kg diet compared with pigs fed 100 or 200 mg/kg after 2 and 4 d of refrigerated storage. Cholesterol oxide levels in pork from pigs fed the latter two diets were not significantly different.

The results show that cholesterol oxidation in pork is greatly accelerated during storage after cooking and that dietary α-tocopherol supplementation reduces levels of potentially harmful cholesterol oxides in meat products.

Hubbard, R. W., Ono, Y. & Sanchez, A. (1989). *Progress in Food and Nutrition Science* **13**, 17–44.
Monahan, F. J., Buckley, D. J., Morrissey, P. A., Lynch, P. B. & Gray, J. I. (1992). *Meat Science* **31**, 229–241.

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The effect of triacylglycerols on lymphocyte proliferation. By PHILIP C. CALDER and ERIC A. NEWSHOLME, *Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU*

We have reported the inhibitory effects of a variety of non-esterified fatty acids, particularly polyunsaturated fatty acids (PUFA) upon mitogen-stimulated lymphocyte proliferation and interleukin-2 production *in vitro* (Calder *et al.* 1991, 1992; Calder & Newsholme 1992*a*,*b*). Caution must be exercised in extending the findings of such studies to the *in vivo* situation since dietary PUFA are transported in the circulation as triacylglycerols (TAG) rather than in the non-esterified form. Therefore, it was considered important to investigate the effect of TAG on lymphocyte proliferation.

Rat lymph node lymphocytes were prepared and cultured in the presence of the T-cell mitogen concanavalin A as described elsewhere (Calder *et al.* 1991, 1992). The cell culture medium was supplemented with an emulsion containing dioleyl phosphatidylcholine, bovine serum albumin and various TAG. Lymphocyte proliferation was determined as the incorporation of [^{3}H]thymidine over the final 18 h of a 66 h culture period.

In the absence of added emulsion, [^{3}H]thymidine incorporation (mean (SEM), *n* 6) was 109 420 (8246) disintegrations/min. Thymidine incorporation was not affected by addition of emulsion which did not contain TAG (mean (SEM) incorporation was 110 650 (5197) disintegrations/min). TAG containing saturated or monounsaturated fatty acids (myristate, palmitate, stearate, oleate) did not affect lymphocyte proliferation except for trimyristin and tripalmitin at the highest concentration used (100 μM). In contrast, TAG containing PUFA (linoleate, α-linolenate, arachidonate) were inhibitory at concentrations as low as 10 or 30 μM. At 100 μM such TAG inhibited lymphocyte proliferation by up to 85%.

	[^{3}H]thymidine incorporation (disintegrations/min per well)							
	10 μM		30 μM		50 μM		100 μM	
TAG	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Trimyristin	104 400	4575	98 819	6340	95 776	6864	87 956*	5941
Tripalmitin	100 160	4499	99 206	9253	92 082	8541	84 386**	5743
Tristearin	100 489	4163	94 471	5650	93 420	7574	94 323	8133
Triolein	99 109	4185	98 113	7357	94 477	9342	97 707	5280
Trilionlein	91 509	7626	67 602***	4320	59 242***	9983	31 859***	4341
Tri (α) linolenin	72 306***	5139	51 149***	6042	35 733***	4215	24 392***	4042
Triarachidonin	61 861***	5758	41 140***	5049	26 591***	6137	14 411***	3267

Significantly different from thymidine incorporation with no triacylglycerol: *P<0·02; **P<0·01; ***P<0·001 (*n* 6).

These results indicate that TAG containing PUFA, but not saturated or monounsaturated fatty acids, are potent inhibitors of lymphocyte proliferation *in vitro*. This is the first report that such TAG can directly affect immune functions. The effect on lymphocyte proliferation may be partially responsible for the immunosuppression caused by PUFA-rich diets which makes such diets useful for therapy in inflammatory disorders. This study also suggests that infusion of lipid emulsions, particularly those containing PUFA, could compromise host defence.

Calder, P. C., Bevan, S. J. & Newsholme, E. A. (1992). *Immunology* **75**, 108–115.
Calder, P. C., Bond, J. A., Bevan, S. J., Hunt, S. V. & Newsholme, E. A. (1991). *International Journal of Biochemistry* **23**, 579–588.
Calder, P. C. & Newsholme, E. A. (1992*a*). *Clinical Science* **82**, 695–700.
Calder, P. C. & Newsholme, E. A. (1992*b*). *Mediators of Inflammation* **1**, 107–112.

The influence of acetate and nutrient balance on ouabain-sensitive respiration in ovine liver. By N. S. JESSOP, *Institute of Ecology and Resource Management, The University of Edinburgh, Edinburgh EH9 3JG* and R. A. LENG, *Department of Biochemistry, Microbiology and Nutrition, The University of New England, Armidale, NSW 2351, Australia*

Diets which differ in metabolizability are utilized with differing efficiency. The cause of this is a matter for debate although it has been proposed that oxidation through wasteful metabolism of acetate is one major factor (Leng, 1990). The present study investigates the effects of acetate and nutrient balance on a major energy-consuming process, Na^+,K^+-ATPase activity.

Twelve sheep (Merino × Border Leicester) of approximately 40 kg body weight were penned individually and allocated to receive 0·85 kg dry matter (DM)/d of one of three diets, each based on oaten chaff and sucrose (metabolizable energy concentration (MJ/kg DM) of 8·5, effective rumen degradable protein:fermentable metabolizable energy ratio of 4·0) with additions of urea or fish meal, or both, such that the supply of rumen undegradable protein varied (calculated to be 5, 40 or 75 g/d). After 4 weeks on the diet sheep were slaughtered and liver snips prepared within 3 min of exsanguination. Liver snips were incubated at 37° in Minimal Essential Medium containing glucose (5 mM), and propionate (1·25 mM) at acetate concentrations of 0, 1, 2·5 and 5 mM. Oxygen uptake was measured polarographically in the absence and presence of 10^{-4} M ouabain. Analysis of variance for split-plot designs was carried out using Genstat 5.

Total respiration (5·74 (SD 1·11) nmol O_2/min per mg protein) did not vary between diets or with changing acetate concentration. Ouabain-sensitive respiration increased linearly on all diets between 0 and 2·5 mM acetate (% inhibition = 16·6 + 4·05 × acetate concentration (mM); r^2 0·65, $P<0·001$), but at 5 mM acetate it fell to levels similar to those measured without acetate (% inhibition of 16·2 (SD 2·3)). Between 0 and 2·5 mM acetate there was a significant effect of diet ($P<0·01$) such that supply of UDP lowered the proportion of total respiration attributable to sodium pump activity by a constant amount of each acetate level (mean % inhibition adjusted for acetate concentration for diets 1 to 3 were 23·6, 21·3 and 18·6 (SED 0·95) respectively; diet 1 provided the least UDP and diet 3 the most). There was no such difference at 5 mM acetate.

Thus, increasing the imbalance of nutrients, either by addition of acetate within the physiological range to the incubation medium or by omission of UDP from a diet thus reducing the ratio of protein to energy in the nutrients absorbed, increased the rate of sodium pumping and hence the energy expenditure on this process. Since total respiration did not change this would result in less substrate being available for other, more productive purposes.

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Leng, R. A. (1990). *Nutrition Research Reviews* **3**, 277–303.

Phytase activity in wheat bran subjected to simulated gastric digestion. By J. H. MITCHELL and A. WISE, *The Robert Gordon University, Queen's Road, Aberdeen AB9 2PG*

The possibility that phytase in bran could hydrolyse phytate in the stomach, as suggested by Sandberg & Andersson (1988), was investigated by simulating gastric conditions, either with high (HA) or low acidity (LA). Bran (1 g, containing 48 mg phytate) was incubated at 37° with 10 ml skimmed milk (SM) or casein (CA) solution (31·5 g/l) and the phytate content of the whole mixture analysed (Vaintraub & Lapteva, 1988) at 0, 30, 60, 120 or 180 min. During the incubation, a solution of 20 mM KCl, pepsin (1 g/l) and HCl (148 or 280 mM) was added continuously to half of the tubes (Expt) at 0·2 ml/min for 60 min. The pH reached was 4·0 and 1·6 for LA and HA, respectively. The experiment was repeated three times. The remaining phytate (% original) in each tube is given in the Table.

	CA-LA		CA-HA		SM-LA		SM-HA	
Time (min)	Control	Expt	Control	Expt	Control	Expt	Control	Expt
0	100	100	100	100	100	100	100	100
30	92	78	94	72	97	89	97	91
60	84	61	83	71	95	76	97	86
120	74	43	77	70	92	60	95	80
180	66	30	67	72	90	52	91	82

Control incubations with casein lost phytate three times as fast as control incubations with skimmed milk ($P<0·001$). Analysis of variance showed significant differences at all incubation times due to adding acid ($P<0·01$). There were significant ($P<0·01$) interactions due to the concentrations of acid added at 120 and 180 min. Simulated gastric digestion that reached pH 4 (LA) stimulated phytate hydrolysis which continued throughout the incubation, but at higher acidity (HA) an initially-stimulated activity stopped. It is concluded that phytase in bran can hydrolyse phytate under simulated gastric conditions, but the effect depends on how low the pH falls and the composition of the meal.

Sandberg, A. S. & Andersson, H. (1988). *Journal of Nutrition* **118,** 469–473.
Vaintraub, I. A. & Lapteva, N. A. (1988). *Analytical Biochemistry* **175,** 227–230.

Vitamin A levels in a sample of Irish animal livers. By YVONNE E. FINNEGAN, M. RUSSELL and P. M. MATHIAS, *Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Republic of Ireland*

Recently, concern has been expressed at the unexpectedly high levels of vitamin A in the livers of commonly-consumed herbivorous animals (Steadman, 1990). Coupled with these findings are reports of the potential teratogenic effects of high vitamin A intakes in humans (Bielsalski, 1989). To date, no comparable data on vitamin A content of Irish animal livers are available, and hence no information on which to base risk assessment of liver consumption. This was the aim of the present study.

Two sources of animal livers were obtained. First, whole livers from lambs, cows and pigs were obtained on the day of slaughter from local abattoirs, and whole chicken livers obtained from local retail outlets. Portions (20 g) were taken from the various lobes for analysis. Second, representative samples of lamb, beef and pig livers were obtained from local butchers. Analysis of vitamin A in the livers was carried out by a method developed in these laboratories. Liver samples were thinly sliced or homogenized and 1 g portions placed in test tubes and frozen. Samples were then dried under vacuum for 7 h. Vitamin A was extracted in 3 ml hexane or chloroform:methanol (2:1, v/v) and assayed by high performance liquid chromatography with a fluorescence detector (Ex.330 nm, Em.475 nm). Retinol palmitate and retinol were the only forms of vitamin A quantified. Results, expressed in terms of total retinol activity, are shown in the Table.

	Lamb		Pig		Beef		Chicken	
Source	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Abattoir:								
Total retinol activity (mg/100 g)	59·2	6·3	14·8	1·2	10·9	1·4	10·8	1·1
Range:	51·5–67·4		10·9–19·5		8·7–13·0		6·2–14·2	
n	6		12		8		8	
Butchers:								
Total retinol activity (mg/100 g)	22·8	5·0	10·5	1·6	4·6	1·4	–	
Range:	15·1–31·5		6·7–15·1		2·3–5·6			
n	10		10		10			

These results are similar to animal liver vitamin A concentrations reported in other European countries, although levels of vitamin A in beef and pig liver are lower than those in the UK and Germany (Pascal, 1991). However, extracts from lamb and beef liver contained appreciable amounts of an unidentified vitamin A ester (probably retinol stearate) which accounted for up to 30% total vitamin A activity. Thus, the above figures can be considered to be conservative. The difference in vitamin A levels found between livers obtained from abattoirs and local butchers could be explained by seasonal variation in feeding and supplementation practices.

This study shows that in Ireland the consumption of animal livers could present the same potential risk to selected consumers as is the case in other European countries.

Bielsalski, H. K. (1989). *Toxicology* **57**, 117–161.

Pascal, G. (1991). Commission of the European Communities Scientific Committee for Food. CS/Vit/10 April.

Steadman, S. W. (1990). Commission of the European Communities Scientific Committee for Food. CS/VitA/3.

A conceptual approach to the determination of upper safe limits for the consumption of micronutrients. By D. H. SHRIMPTON, *Council for Responsible Nutrition, 63 Hampton Court Way, Thames Ditton, Surrey KT7 0LT*

That particular patterns of eating can influence health is widely accepted (Hurren & Black, 1991) and a major element of a nutrition policy involves 'the direction of food consumption to achieve a balance of nutrient intake commensurate with optimum health' (Gibney, 1991).

However, optimal intakes are difficult to quantify because 'RDAs are neither minimal requirements nor necessarily optimal levels of intake' (National Research Council, 1989). Further, for individuals, 'there is uncertainty about the relevance of many biological markers – as evidence of an individual's status for that nutrient' (Department of Health, 1991). Consequently, the optimal intake of a nutrient for an individual may differ substantially from the population RDI and may vary from time to time according to the circumstances of the individual's metabolism, eating habits, activity and environmental circumstances.

When an intake of a nutrient is deemed to be optimal, it is implicit that levels below and above are sub-optimal; but only when clinical disorder is diagnosed can there be certainty that the intake was deficient or excessive. Because micronutrients can be consumed as supplements of high nutrient density, excessive consumption is a possibility, but there is no conceptual basis for determining an upper safe limit.

It is proposed that: any micronutrient may be safely consumed in any amount that is less than that for which a contra-indication has been reported from either the scientific literature or from responsibly-monitored practice. It is comprehensive, requiring neither exceptions nor special pleading for particular micronutrients, and is simple, objective and able to take account of new data without modification of the principle.

The approach can be applied in the EC to regulating the implementation of hypotheses currently under debate, for example, the generalized proposition that when oxidative processes involving free radicals become imbalanced the health of the individual may be impaired and that this can be countered by the consumption of diets rich in micronutrients with a known antioxidant property, specifically carotenoids, vitamins A, C, E and Se (Diplock, 1991). Contra-indications on a basis of daily consumption for vitamins C and E and Se are, respectively: 1 g (Olsen, 1987), 800 mg (Bendich & Machlin, 1988), and 200 μg (Matru *et al.* 1989). There is comparable data for most micronutrients (Martindale, 1989).

Bendich, A. & Machlin, L. J. (1988). *American Journal of Clinical Nutrition* **48**, 612–619.
Department of Health (1991). *Report on Health and Social Subjects* no. 41. London: H.M. Stationery Office.
Diplock, A. J. (1991). *American Journal of Clinical Nutrition* **53**, 189s–193s.
Gibney, M. J. (1991). In *Food Policy Trends in Europe*, pp. 19–32 [H. Deelstra, M. Fondu, W. Ooghe, and R. van Havere, editors]. London: Ellis Horwood.
Hurren, C. & Black, A. (editors) (1991). *The Food Network*. London: Smith-Gordon & Company Ltd.
Martindale (1989). *The Extra Pharmacopoeia*, 29th ed. [J. E. F. Reynolds, editor]. London: The Pharmaceutical Press.
National Research Council (1989). *Recommended Dietary Allowances*. Washington, DC: National Academy Press.
Olsen, J. A. (1987). *American Journal of Clinical Nutrition* **45**, 693–703.

Effects of iodine and retinol supplementation on thyroid function in pre-school children in the Morogoro Region of Tanzania. By M. J. CRESSWELL, T. C. CHAN and J. J. STRAIN, *Human Nutrition Research Group, University of Ulster at Coleraine BT52 1SA*, N. C. ROLLINS and J. A. DODGE, *Department of Child Health, The Queen's University of Belfast BT12 6BA* and F. MHANDO and K. MTEBE, *Sokoine University of Agriculture, Morogoro, PO Box 3006, Tanzania*

It has been suggested on the basis of epidemiological data that vitamin A deficiency might be a goitrogenic factor (Ingenbleek & De Visscher, 1979). The aim of the present study was to investigate the effects of I or retinol supplementation, or both, on thyroid function in children from a region of Tanzania where signs of vitamin A deficiency, shown by means of conjunctival impression cytology, were common and where there was also endemic goitre (Gray *et al.* 1993).

A total of 733 children aged 2–7 years were randomly allocated into four groups and administered the following supplements: (A) placebo capsule; (B) retinol and I; (C) retinol; and (D) I.

Retinol (60 mg) was taken in the form of an oral capsule and I (400 mg) as iodized oil (1 ml) by intramuscular injection (Lipiodol, Laboratoire Guerbet, France). A 2 ml blood sample was taken (1) before supplementation and (2) at 8 weeks post-supplementation. Serum samples were frozen at −20° before analyses. Thyroxine (T_4), triiodothyronine (T_3) and thyroid stimulating hormone (TSH) were measured in the serum to assess thyroid function. Results are shown in the Table.

	T_4 (nmol/l)		T_3 (pmol/l)		TSH (mIU/l)	
Group	Mean	SD	Mean	SD	Mean	SD
A: (1)	108	19·2	2·66	0·54	2·47	1·98
(2)	106	24·5	2·73	0·66	3·19**	1·84
	(*n* 109)		(*n* 123)		(*n* 106)	
B: (1)	108	19·8	2·70	0·41	2·54	1·78
(2)	122***	24·3	2·54*	0·44	2·44	1·81
	(*n* 110)		(*n* 111)		(*n* 107)	
C: (1)	104	20·6	2·65	0·45	2·74	1·75
(2)	106	19·8	2·72	0·46	3·16*	1·77
	(*n* 117)		(*n* 110)		(*n* 104)	
D: (1)	109	17·7	2·69	0·49	2·56	1·69
(2)	119***	25·3	2·58	0·72	2·43	2·03
	(*n* 118)		(*n* 119)		(*n* 107)	

Significantly different from baseline (1) measurement (paired *t* test): $*P<0·05$, $***P<0·001$.

Two-way analysis of variance showed no interaction between retinol and I supplementations with respect to thyroid function. I supplementation improved thyroid function; T_4 was increased and T_3 was decreased. This is consistent with the I-sparing effect observed in populations with insufficient I. The significant increase in TSH in groups which were not given I supplementation may indicate the appearance of a goitrogenic factor during the experimental period.

Gray, A. M., Eaton-Evans, J., McKenna, P. G., Strain, J. J., Rollins, N. C., Kategile, P., Mosha, T. & Mtebe, K. (1993). *Proceedings of the Nutrition Society* **52,** 109A.

Ingenbleek, Y. & De Visscher, M. (1979). *Metabolism* **28,** 9–19.

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Relationships between plasma, gastric tissue and gastric juice ascorbic acid levels in subjects with and without gastritis. By A. J. WARING, C. J. SCHORAH, G. M. SOBALA and D. LYNCH, *Department of Clinical Medicine, University of Leeds, Leeds LS2 9JT*

In normal individuals levels of gastric ascorbic acid are on average higher than plasma levels suggesting that active secretion of ascorbic acid probably occurs between plasma and gastric juice. However, gastric pathology, associated with increased risk of cancer, considerably reduces gastric ascorbic acid levels (Sobala *et al.* 1989). We have investigated this secretion and how it is modified by disease by examining the relationship between plasma, gastric tissue and gastric juice ascorbic acid levels in normal subjects and in patients where chronic gastritis has led to a significant decrease in gastric juice ascorbic acid levels. With ethics committee approval ascorbic acid levels in plasma, gastric juice and gastric mucosa were measured by high performance liquid chromatography, after extraction into metaphosphoric acid, in samples taken from fasted patients attending an endoscopy clinic (Sobala *et al.* 1991). Histological examination of biopsy samples allowed classification of patients into normal and chronic gastritis groups.

Pearson product moment correlation coefficients for forty-three subjects (eleven normal histology, thirty-two chronic gastritis) calculated on square root transformed (normalized) data

	Gastric juice ascorbic acid (dependent variable)			Plasma ascorbic acid (independent variable)		
	Total	Normal histology	Chronic gastritis	Total	Normal histology	Chronic gastritis
Stomach antrum ascorbic acid	0·320*	0·393	0·388*	0·707***	0·662*	0·739***
Stomach body ascorbic acid	0·113	0·088	0·242	0·669***	0·793**	0·671***
Plasma ascorbic acid	0·365*	0·326	0·322	—	—	—

Significance of correlation: $*P<0·05$; $**P<0·01$; $***P<0·001$.

The Table shows that ascorbic acid in gastric juice was neither closely dependent upon the levels in mucosal cells nor on the concentration in plasma, but there was a strong relationship between plasma ascorbic acid and tissue levels. These correlations were unaffected by gastritis, in contrast to the gastric juice ascorbic acid concentrations which were considerably reduced (86·9 *v.* 16·8 μmol/l median values; normal *v.* gastritis respectively). Thus, in both the normal and the diseased human stomach, factors other than plasma and gastric mucosal levels of ascorbic acid are important in determining the concentration of gastric juice ascorbic acid.

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Sobala, G. M., Pignatelli, B., Schorah, C. J., Bartsch, H., Sanderson, M., Dixon, M. F., Shires, S., King, R. F. G. & Axon, A. T. R. (1991). *Carcinogenesis* **12,** 193–198.

Sobala, G. M., Schorah, C. J., Sanderson, M., Dixon, M. F., Tompkins, D. S., Godwin, P. & Axon, A. T. R. (1989). *Gastroenterology* **97,** 357–363.

Antioxidant protection of the anoxic–reoxygenated heart. By S. O'FARRELL and M. J. JACKSON, *Department of Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX*

Oxygen-derived free radicals are thought to play an important role in myocardial ischaemia and reperfusion injury. Evidence from both *in vivo* dietary studies and *ex vivo* organ studies suggest that endogenous antioxidants, like vitamin E, play an important role as a defence mechanism against oxygen free radical-mediated lipid peroxidation. We have previously reported that polyunsaturated fatty acid supplementation does not have an exacerbating effect on damage to the isolated rat heart, induced by a period of anoxia and reoxygenation (O'Farrell & Jackson, 1992). These earlier studies were undertaken using a conventional Langendorff isolated heart system in which the flow of perfusion medium is regulated by hydrostatic pressure (Langendorff, 1895). Reanalysis of control data indicates that release of creatine kinase (CK, *EC* 2.7.3.2; an index of damage to the heart) is significantly related to flow rates of perfusion media ($r-0{\cdot}89$) and, hence, dependent upon vascular resistance in addition to damage to the heart. We have therefore modified the isolated heart system to standardize the perfusion rate and re-examined the effect of antioxidants on anoxia and reoxygenation-induced damage.

Isolated rat hearts were subjected to 30 min anoxia followed by 120 min reoxygenation, with perfusion at a constant flow rate of 8 ml/min. Anoxia and reoxygenation caused a significant increase in the mean creatine kinase efflux at 15 min post-anoxia (117 (SEM 28) mU/min per g), compared with control normoxic hearts (32 (SEM 5) mU/min per g). Pharmacological treatment with desferrioxamine (0·61 mM) was found to exacerbate damage (192 (SEM 22) mU/min per g), in contrast to previous data using a non-flow-regulated system (Badylak *et al.* 1987). However, vitamin E treatment (α-tocopherol 30 μM) had some protective effect on the anoxic–reoxygenated heart (60 (SEM 16) mU/min per g).

These data indicate that some previous experiments intended to study the effects of antioxidants in the anoxic–reoxygenated heart may actually be demonstrating modification of perfusion rates, rather than protection of the cardiac tissue. However, vitamin E appears to display cardio-protective effects in a perfusion-independent system.

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

Badylak, S. F., Simmons, A., Turek, J. & Fabbs, C. F. (1987). *Cardiovascular Research* **21**, 50–56.
Langendorff, O. (1895). *Physiologie des Menschen und der Tiere* **61**, 291–332.
O'Farrell, S. & Jackson, M. J. (1992). *Proceedings of the Nutrition Society* (In the Press).

The validation of a food frequency and amount questionnaire for assessing dietary antioxidant intakes amongst older adults. By C. M. O'BRIEN and M. NELSON, *Department of Nutrition and Dietetics, King's College, Campden Hill Road, Kensington, London W8 7AH*

A self-administered food frequency and amount questionnaire (FAQ) has been devised and validated for use in an ongoing case–control study on the role of dietary antioxidants in the aetiology of peripheral arterial disease (PAD).

The principal aim of the FAQ is to assess vitamin C, E, retinol and carotene intakes, as well as energy and fat intakes based on a list of 133 food items. Foods included in the list are those with a high concentration of the vitamins and those consumed regularly by the study population group. Sets of black and white photographs showing small, medium and large portions (Crawley, 1988) were used to aid subjects in their estimation of portion sizes. Fifteen females and fourteen males aged 55–74 years participated. Subjects filled out the questionnaire before being interviewed, and then kept weighed records of food and drink consumption for 7 d. Twenty-three of the original subjects completed the questionnaire again 12 months later as a measure of repeatability.

Nutrient intakes for questionnaires and weighed records were estimated using food composition tables (Paul & Southgate, 1985).

The Table shows Pearson correlation–coefficients for the weighed records (R) *v.* the original questionnaire (Q), and the weighed records *v.* the average of the two questionnaires (AV).

	Male				Female			
	R *v.* Q (*n* 14)		R *v.* AV (*n* 11)		R *v.* Q (*n* 15)		R *v.* AV (*n* 12)	
Nutrient	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Energy: (MJ)	0·37	0·198	0·57	0·067	0·62	0·014	0·61	0·037
Fat (g)	0·58	0·029	0·60	0·049	0·51	0·052	0·52	0·046
Vitamin C (mg)	0·78	0·001	0·85	0·001	0·28	0·304	0·28	0·376
Vitamin E (mg)	0·33	0·247	0·61	0·046	0·61	0·015	0·46	0·130
Retinol equivalent ($\log_e$ mg)	0·70	0·005	0·78	0·005	0·17	0·560	0·35	0·267
Retinol ($\log_e$ mg)	0·70	0·005	0·73	0·011	0·25	0·360	0·38	0·218
Carotene ($\log_e$ mg)	0·58	0·030	0·77	0·006	0·53	0·040	0·60	0·038

In general, correlations are better for men than for women, possibly due to increased compliance in the male group. Correlations improved when the average questionnaire values were compared to weighed intakes, except for vitamin E for females.

Analysis of within- to between-subject variance ratios predicted correlations of the average questionnaire values with the 'truth', ranging from 0·61 for energy to 0·99 for retinol equivalents (males), and 0·38 for vitamin C to 0·77 for carotene (females). The results presented here predict that for females, carotene intakes will be correctly classified by thirds in > 67·9% of cases, and vitamin C, which has the lowest correlation, will be correctly classified > 51·4% of the time. Males fared better, with > 81% of subjects being correctly classified into thirds for vitamin C, retinol equivalents, retinol and carotene intakes, > 67·9% of subjects correctly classified for vitamin E intake and 63·2% correctly classified for energy and fat intakes.

Crawley, H. (1988). *Food Portion Sizes.* Ministry of Agriculture, Fisheries and Food Publication. London: H.M. Stationery Office.

Paul, A. A. & Southgate, D. A. T. (1985). *McCance and Widdowson's The Composition of Foods*, 4th ed. London: H.M. Stationery Office.

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Influence of illness on serum retinol of children in northern Ghana. By S. M. FILTEAU[1], S. MORRIS[2,3], R. A. ABBOTT[1], A. M. TOMKINS[1], B. KIRKWOOD[2], P. ARTHUR[2,3], D. ROSS[2,3], J. GYAPONG[3] and J. RAYNES[2], [1]*Centre for International Child Health, Institute of Child Health, 30 Guilford Street, London WC1N 1EH*, [2]*London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT* and [3]*Ghana VAST Child Health Project*

Serum retinol concentration is often used for assessing human vitamin A status. However, since serum retinol decreases during systemic infections it lacks specificity as a measure of vitamin A status in infected individuals or in populations where there is a high prevalence of infection. This study was undertaken to quantify the contribution of infection to deficient values of serum retinol in such a population.

Blood samples (drawn 4 months after vitamin A supplementation or placebo administration) and morbidity data were taken from the Ghana Vitamin A Supplementation Trial Child Health Study. Children aged 6–59 months, (ninety-one from the vitamin A group and eighty-seven from the placebo group) were selected based on assessments of their health reported by mothers to fieldworkers in the 2 weeks before blood sampling. Serum levels of retinol, serum amyloid A (SAA) and α_1-acidglycoprotein (AGP) were measured. Significant negative correlations were seen between log values of serum retinol and each of the acute phase proteins (AGP: r –0·35, $P<0·001$; SAA: r −0·20, $P=0·004$). Serum retinol was lower but not significantly so in children with fever or vomiting compared with healthy children or those with mild diarrhoea. AGP levels and the proportion of children with raised levels were higher in the fever and vomiting groups than in the healthy and mild diarrhoea groups. SAA showed similar but non-significant trends. Fifty-seven per cent of clinically healthy children had AGP >1 g/l, selected as a cut-off between normal and acute phase values, suggesting that subclinical infections may have induced an acute phase response with depressed serum retinol levels. In all groups (AGP >1 g/l was associated with decreased serum retinol concentration.

	AGP <1 g/l	AGP ≥1 g/l
Mean serum retinol μmol/l (95% CI)	0·63 (0·56–0·72)	0·48 (0·44–0·53)
Serum retinol range (% of children):		
<0·35 μmol/l	9	26
0·35–0·7 μmol/l	53	52
≥0·7 μmol/l	38	22

These results suggest caution in using serum retinol alone to assess vitamin A status in populations with a high prevalence of infection, in particular when relating vitamin A status to morbidity and mortality. Measurement of AGP, which is negatively correlated with serum retinol and which distinguishes healthy children from those with systemic illnesses (present study and Pressac *et al.* 1990), may help interpretation of serum retinol data in the absence of detailed morbidity data.

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Pressac, M., Vignoli, L., Aymard, P. & Ingenbleek, Y. (1990). *Clinica Chimica Acta* **188,** 129–136.

Risk factors for intra-uterine growth retardation in Campinas, Brazil. By PATRICIA RONDO, *State University of Campinas* and *Catholic University of Campinas, Campinas, Brazil* and ANDREW TOMKINS, *Centre for International Child Health, University of London, London WC1N 1EH*

Low birth weight (<2·5 kg) is present in approximately 13% of deliveries in Campinas, an urban area of Brazil in São Paulo state. Much of this low birth weight is due to intra-uterine growth retardation (IUGR). This has important implications on the early and late development of the infant. IUGR is associated with an increased risk of development of infection due to a lowered level of immunity, and may also be associated with the development of disease processes in middle age and later life.

Several studies of IUGR have emphasized the importance of nutrition and, indeed, much of the current programme in antenatal care clinics revolves around promotion of dietary intake, particularly in relation to micronutrients. Others have emphasized the importance of shorter maternal stature, cigarette smoking, illness and hypertension.

The present study was performed as a case–control study of 356 live births with IUGR and 362 live births with normal nutritional status. IUGR was defined according to the classification of Lubchenco *et al.* (1966). Infants and mothers were recruited from four maternity centres in Campinas between February 1991 and February 1992. On the same day that an infant with IUGR was identified, a well-nourished infant was identified for comparison.

Cord blood index	% of cases		Odds ratio	Statistical significance
	IUGR	Controls		
Packed cell volume ≤48%	18·0	27·0	0·64	$P<0·05$
Haemoglobin ≤15 g/dl	25·3	36·0	0·60	$P<0·05$

This study shows that there were significant differences in cord blood packed cell volume and haemoglobin between IUGR and controls but no significant differences for cord or maternal folate (erythrocytes) and ferritin. The raised IUGR cord haemoglobin and packed cell volume could be explained by a compensatory mechanism to overcome the known lower O_2 supply to the fetus.

IUGR in this population is associated with maternal size (height, leanness), primiparity, previous history of low birth weight, smoking, alcohol or coffee intake, low socio-economic status and number of visits to antenatal care services. However, IUGR seems not to be associated to Fe or folate deficiencies.

Further analyses of this extensive data set are indicated but these data show that, in this population at least, concentration on improving micronutrient status of women during pregnancy as a strategy to improve birth weight may have limited impact unless there is much wider attention towards other risk factors.

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Lubchenco, L. O., Hansman, C. & Boyd, E. (1966). *Paediatrics* **37**, 403–408.

Effects of potassium depletion and repletion on growth and resting energy expenditure in the growing rat. By R. HAILWOOD, A. JONES and S. S. WOOTTON, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

The exclusion of a specific nutrient from an otherwise adequate diet is thought to result in a decreased efficiency of energy utilization (Kleiber, 1945). In a previous study (Hailwood *et al.* 1992), restricted growth following exclusion of K from the diet was associated with an increase in total energy expenditure as determined by carcass analysis. The aim of the present study was to determine the effects of excluding and replacing K in the diet on resting energy expenditure (REE) and growth in the rat.

Eighteen male Wistar rats (initial body weight 100 g) were randomly allocated to either (1) a K-adequate diet (108 mmol K^+/kg diet) for 28 d (KAD), (2) a K-free diet for 14 d followed by a K-adequate diet for 14 d (KDEF) or (3) a K-adequate diet pair-fed to energy intake of KDEF animals for 28 d (KADPF). Food intake and body weight were recorded daily and REE was determined weekly by indirect calorimetry.

	Gain in body wt (g)		REE (kJ/100 g/d)				
Day . . .	0–14	15–28	0	7	14	21	28
KAD (*n* 6): Mean	105·8[a]	89·3[a]	79·1	72·4[a]	64·1	59·5	51·8[a]
SEM	3·0	3·7	2·0	2·2	1·7	1·2	1·6
KDEF (*n* 6): Mean	9·2[b]	133·6[b]	74·1	70·1[a]	59·0	64·5	62·4[b]
SEM	1·8	4·4	3·5	2·0	1·0	2·1	1·6
KADPF (*n* 6): Mean	15·9[c]	125·4[c]	76·6	63·0[b]	56·7	62·4	57·9[a,b]
SEM	3·0	4·9	3·9	2·4	1·3	1·9	1·9

[a,b,c] Mean values in the same column with unlike superscript letters were significantly different (ANOVA): $P<0·05$.

The exclusion of K from an otherwise adequate diet initially results in an increased metabolic demand for energy at rest in rats in the face of attempts to conserve energy in response to food restriction. These changes in energy expenditure and rates of growth suggest a decreased economy of energy utilization at rest which may contribute to an elevated total energy expenditure.

Kleiber, M. (1945). *Nutrition Abstracts and Reviews* **15,** 207–222.
Hailwood, R., Wootton, S. A. & Jackson, A. A. (1992). *Proceedings of the Nutrition Society* **51,** 34A.

Effects of a 4-week dietary fat feeding trial on the metabolic responses to tumour necrosis factor α in rats. By HILDA MULROONEY and R. F. GRIMBLE, *Department of Human Nutrition, Southampton University, Southampton SO9 3TU*

The length of time over which a dietary fat feeding trial must be carried out in order to change metabolic responses to tumour neurosis factor α (TNFα) is unclear. We carried out a 4-week feeding trial with various fats and examined the effects upon responses to TNFα.

Sixty male weanling Wistar rats (weight 61 (SD 2) g) were fed on either standard rat chow (27 g fat/kg diet) or synthetic diets (100 g total fat/kg diet); each synthetic diet contained 10 g maize oil/kg to prevent essential fatty acid deficiency, the remaining fat was either maize oil (rich in linoleic acid), coconut oil (low in linoleic acid, high in short-chain saturates), fish oil (low in linoleic acid, high in eicosapentaenoic acid), or butter (rich in oleic acid, low in linoleic acid).

Half of each group was injected intraperitoneally with 100 μg TNFα/kg body weight; the others received sterile saline and were pair-fed (PF) with the TNFα group. After 24 h the fractional rate of protein synthesis (FSR) was measured in liver, kidney and lungs (by the flooding-dose method using [^{3}H]phenylalanine), as was tissue protein content. Changes in food intake in response to TNFα were also examined.

Diets . . .	Chow		Maize		Coconut		Fish		Butter		Pooled
	Saline	TNFα	Saline	TNFα	Saline	TNFα	Saline	TNFα	Saline	TNFα	SEM
Appetite suppression (%)	PF	32	PF	42	PF	33	PF	10·5	PF	19	0·89
FSR (%/d):											
Liver	74	106***	73	127***	68	83**	71	98***	68	74	2·1
Lung	47	67**	47	60***	45	71***	42	35*	33	41	1·7
Kidney	57	68	44	53**	45	71***	54	45*	60	64	0·78
Liver tissue protein (mg/g)	185	184	195	220	183	252**	259	216	199	176	6·89

Significantly different from own control: *P<0·05; **P<0·01; ***P<0·001.

Feeding the fats for 4 weeks was sufficient to produce the same modulatory pattern of TNFα on both appetite, and FSR in the liver, lungs and kidneys as seen at 8 weeks. While all fats low in linoleic acid blunted the anorexic effects, their influence on FSR was both organ-specific and determined by other fatty acid characteristics of the fats.

The authors are grateful to BASF/Knoll AG, Ludwigshaven, for the gift of recombinant TNFα. H.M. is supported by a grant from the Nutrition Consultative Panel of the UK Dairy Industry.

Modulation of the response of rats to endotoxin by butter, olive oil and corn oil. By H. T. BESLER and R. F. GRIMBLE, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

Fats are able to modulate the responses to inflammatory agents. We have previously shown that the suppressive effects of butter on the response to TNFα in rats may be due to its oleic acid content (Mulrooney & Grimble, 1992). Olive oil, like butter, is rich in oleic acid (690 and 220 g/kg respectively). We therefore compared the effect of feeding weanling rats for 4 weeks on synthetic diets containing 50, 100 or 200 g fat/kg or chow (27 g fat/kg). Each synthetic diet contained 10 g corn oil/kg to prevent essential fatty acid deficiency, and the remaining fat was either butter, corn oil or olive oil. All diets contained 180 g casein/kg plus 3 g DL-methionine/kg. Diets contained adequate vitamin and mineral content and included 50 mg vitamin E/kg.

Animals received a subcutaneous injection of 0·8 mg *Escherichia coli* endotoxin/kg (Difco strain 055:B9; END) or 0·2 ml (9 g sodium chloride/l) sterile saline/kg (SAL). Rectal temperatures were monitored thereafter. Animals were killed 24 h after endotoxin injection and tissues analysed. Saline-injected rats were killed after pair-feeding for 24 h.

Dietary oil . . .	Corn oil			Butter			Olive oil			Chow	Pooled
Fat (g/kg) . . .	50	100	200	50	100	200	50	100	200	27	SEM
Change in rectal temperature 2 h post-injection (°)											
Injection: END	−1·88*	−1·92*	−1·93	−1·18	−1·30	−1·73	−0·43	−0·50	−0·75	−1·17	0·43
SAL	+0·10	+0·19	+0·05	+0·22	+0·03	+0·02	+0·60	+0·42	+0·05	+0·05	
Liver reduced glutathione (mg/g)											
Injection: END	21·1***	19·4*	18·8	24·2**	19·0**	16·6	27·6	21·9	17·1	26·1	0·7
SAL	8·7***	8·1***	6·7***	9·8***	12·0***	10·4	19·4	18·5	11·3	14·6	
Food intake after END injection (g/d)	1·8***	1·4***	1·1***	3·7	2·8	2·3	3·9	2·7	2·3	2·0***	0·17

Values significantly different from corresponding olive oil END or SAL group fed on similar fat concentrations (ANOVA): *$P<0·5$; **$P<0·01$; ***$P<0·001$.

Mulrooney, H. M. & Grimble, R. F. (1992). *Proceedings of the Nutrition Society* (In the Press).

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Effects of glutamine feeding on plasma and muscle glutamine levels and on glutamine synthetase activity and glutamine release in the rat. By ELIZABETH OPARA, MARK PARRY-BILLINGS and ERIC A. NEWSHOLME, *Cellular Nutrition Research Group, Department of Biochemistry, University of Oxford, Oxford OX1 3QU*

Studies have shown that in patients with major burn injury and sepsis, plasma glutamine (GLN) levels and intracellular GLN stores in skeletal muscle fall markedly (Roth *et al.* 1982; Parry-Billings *et al.* 1990). These studies have also indicated that such a decrease may contribute to immunosuppression which occurs after injury. We therefore investigated the effect of GLN supplementation on plasma and skeletal muscle GLN levels and on glutamine synthetase (GS; *EC* 6.3.1.2) activity in skeletal muscle and glutamine release from skeletal muscle.

Male Wistar rats (100 g) were divided into three groups: controls (fed standard chow), alanine-fed (standard chow+alanine, 0·25 gN/kg per d) and glutamine-fed (standard chow+glutamine, 0·25 gN/kg per d). Rats consumed these diets for 2 weeks. GLN levels were measured in plasma and in soleus and extensor digitorum longus (EDL) muscles. Maximal GS activity was measured in soleus and EDL muscles. The rate of glutamine release was measured from soleus muscle in vitro.

GLN feeding increased plasma GLN levels (8%) and GLN content in EDL muscle (13%): there was also an increase in the GLN level in soleus muscle (10%), but this was not statistically significant. Feeding GLN had no effect on maximal GS activity in skeletal muscle or on the rate of glutamine release from skeletal muscle, although one might have expected that a long-term exogenous supply of glutamine would down-regulate skeletal muscle's capacity to synthesize and release glutamine.

Effect of GLN feeding on GLN levels in plasma (μmol/ml), soleus and EDL muscles (μmol/g) and on muscle GS activity (nmol/min per g) and the rate of glutamine release from soleus muscle (nmol/min per g)

	GLN level						GS activity				GLN release	
	Plasma		Muscle				Soleus		EDL		Soleus	
			Soleus		EDL							
Treatment	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	1·05	0·3	7·02	0·3	6·50	0·3	94	10	247	16	37	1
n	8		8		8		6		6		14	
Alanine-fed	1·04	0·3	7·43	0·2	6·92	0·2	104	6	238	15	36	1
n	8		8		7		6		6		13	
Glutamine-fed	1·13*	0·3	7·71	0·3	7·34*	0·4	95	4	250	16	38	1
n	7		7		7		8		8		24	

* Values significantly increased compared with controls.

These results suggest that long-term glutamine feeding is not detrimental to muscle's capacity to synthesize or release glutamine, which are both important processes for the provision of glutamine. They also suggest (although this study was performed on animals that were under no form of stress) that glutamine feeding may be beneficial in clinical conditions where decreases in plasma and muscle glutamine levels occur, by increasing both plasma and muscle glutamine levels and by doing so possibly improve immune function.

Parry-Billings, M., Evans, J., Calder, P. C. & Newsholme, E. A. (1990). *Lancet* **336,** 523–525.
Roth, E., Funovics, J., Muhlbacher, F., Schemper, M. & Mauritz, W. (1982). *Clinical Nutrition* **1,** 25–41.

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The effect of surgery and growth hormone suppression on plasma muscle and glutamine levels. By L. M. CASTELL[1], H. POWELL[2], M. PARRY-BILLINGS[1] and E. A. NEWSHOLME[1], [1]*Cellular Nutrition Research Group, University Department of Biochemistry, Oxford OX1 3QU* and [2]*Department of Anaesthetics, Hammersmith Hospital, London W12 0NN*

Decreased glutamine levels cause impaired function of the immune system both *in vitro* (Parry-Billings *et al.* 1990) and *in vivo* (Brambilla *et al.* 1970).

Pharmacological doses of growth hormone in humans and rats have effected changes including reversal of negative N balance and increased glutamine levels in plasma and muscle. We investigated whether the physiological surge of growth hormone following injury affected glutamine metabolism by administering somatostatin or a placebo to patients following cardiac bypass surgery.

Plasma glutamine levels were measured in eighteen patients undergoing surgery for cardiac bypass. This is the first report of such a detailed time-course of changes in plasma glutamine levels in major surgery. Blood samples were taken pre-, during and for 5 d post-operation. Plasma glutamine levels decreased by about 34% ($P<0{\cdot}001$) after surgery and remained decreased for 4 d (Fig.). Somatostatin effectively blocked the physiological surge of growth hormone after surgery but did not affect plasma glutamine levels. We conclude that the physiological surge in growth hormone after injury does not affect glutamine metabolism. Muscle glutamine concentration was also significantly decreased ($P<0{\cdot}001$) after surgery, and was not affected by somatostatin.

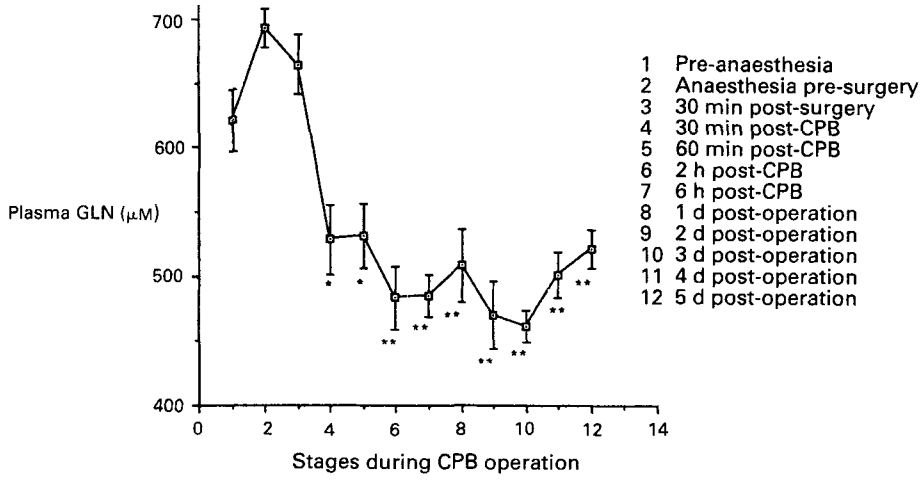

Fig. Plasma glutamine (GLN) levels in samples from cardiopulmonary bypass (CPB) patients (n 18) before, and at different stages after, operation. Significantly different from pre-surgical levels: $*P<0{\cdot}02$; $**P<0{\cdot}001$.

These results suggest that the surge in growth hormone concentration does not mediate changes in the concentration of glutamine in muscle or plasma after surgery.

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Brambilla, G., Parodi, S., Cavanna, M., Caraconi, C. E. & Baldini, L. (1970). *Cancer Research* **30**, 2665–2670.

Parry-Billings, M., Evans, J., Calder, P. C. & Newsholme, E. A. (1990). *Lancet* **366**, 523–525.

Increased TNF production does not correlate with cachexia in chronic inflammatory bowel disease. By M. A. O'CONNELL, D. G. WEIR and D. KELLEHER, *Department of Clinical Medicine, Trinity College and St James's Hospital, Dublin, Republic of Ireland*

Chronic exposure to tumour necrosis factor-α (TNF) in animal models leads to metabolic effects on the body, consequently affecting nutritional status. The nutritional disturbances associated with TNF are similar to those seen in chronic inflammatory bowel disease (CIBD). The aim of the present study was to investigate the role of TNF in mediating the systemic effects of malnutrition in CIBD.

Nutritional assessments were carried out on seventeen patients with CIBD (seven with ulcerative colitis (UC) and ten with Crohn's disease (CD)). Patients had significantly lower triceps skinfold (TSF) thickness values ($P<0{\cdot}05$) and mid-arm circumference (MAC) values ($P<0{\cdot}001$) when compared with age- and sex-matched controls. Serum TNF levels were measured by ELISA and by the L929 cytotoxicity assay. Serum was heat-treated for the bio-assay to eliminate the cytopathic effects of human serum.

There was no significant difference in TNF levels between UC and CD patients (UC 168·5 (SEM 45) pg/ml, CD 111·1 (SEM 28·1) pg/ml). However, serum TNF levels measured by the L929 assay were significantly higher in CIBD patients when compared with age- and sex-matched healthy controls (HC; 134·7 (SEM 27·9) pg/ml (n 24) v. 55·9 (SEM 30·5) pg/ml, (n 17); $P<0{\cdot}05$). Serum TNF levels did not correlate with anthropometric indices of malnutrition (TSF, r 0·252; MAC, r 0·120), or with disease activity, assessed by the Harvey-Bradshaw Index (r 0·170). Nine CD patients and eight UC patients were then studied to assess where the increased TNF levels may come from. Supernatants from unstimulated peripheral blood mononuclear cells (PBMC) showed significantly higher TNF levels on ELISA in CIBD patients than in controls (CIBD 159·5 (SEM 60·4) pg/ml, HC 36·0 (SEM 7·8) pg/ml; $P<0{\cdot}05$). TNF levels in supernatants stimulated with anti-CD3 for 48 h were not significantly different when patients were compared with controls (CIBD 626·5 (SEM 88·2) pg/ml, HC 447·1 (SEM 76·6) pg/ml). The increase in TNF levels of PBMC supernatants did not correlate with either anthropometric measurements or with disease activity, although again, these patients had significantly lower TSF and MAC levels ($P<0{\cdot}05$).

The results indicate that serum TNF levels and TNF levels in supernatants from unstimulated PBMC are significantly higher in CIBD patients than in controls, but there is no significant difference between UC and CD patients. TNF levels in serum and supernatants of unstimulated PBMC do not correlate with either disease activity or nutritional depletion. This suggests that the increase in TNF production in CIBD is not responsible for the malnutrition associated with the disease.

Dietary patterns in functional and organic dyspepsia. By A. MULLAN[1], F. GLEESON[2], P. O'MAHONEY[2], T. JOY[2], P. KAVANAGH[2] and M. J. GIBNEY[1], [1]*Department of Clinical Medicine, Trinity College* and [2]*Gastroenterology Unit, James Connolly Memorial Hospital, Dublin, Republic of Ireland*

Dyspepsia is defined as episodic or persistent symptoms that include abdominal pain or discomfort associated with the upper gastrointestinal tract. Organic dyspepsia is due to a disease process, while no focal lesion is identified in functional dyspepsia. Previous studies have reported food aversion in dyspeptic patients, but the present study provided quantitative data on their food and nutrient intakes. Forty organic dyspepsia (OD) patients and forty functional dyspepsia (FD) patients, endoscopically diagnosed, and eighty age- and sex-matched patients were recruited. Control subjects were in hospital for reasons unconnected with bowel function. A 7 d dietary history, using questionnaire and photographic food atlas of average food portions, was used to assess current dietary intake. Dietary habits were also studied.

Macronutrient intakes were similar in male patients and controls, with the exception of alcohol, which was significantly lower in OD males ($P<0{\cdot}05$). Males with OD also had significantly lower intakes of vitamin C and Cu ($P<0{\cdot}05$). Compared with the controls female patients, however, had significantly lower intakes of vitamin C and macronutrients, with the exception of protein. Females with OD had significantly lower intakes of fibre, alcohol, Fe, Zn, thiamine, pyridoxine, vitamin E (all $P<0{\cdot}05$) and folic acid ($P<0{\cdot}01$).

Daily nutrient intakes of female subjects

	Organic dyspepsia				Functional dyspepsia			
	Patients (*n* 21)		Controls (*n* 21)		Patients (*n* 26)		Controls (*n* 26)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy (MJ)	7·2***	2·2	9·3	2·5	6·9****	2·0	9·2	2·4
Protein (g)	67	16	74	17	68	22	74	16
CHO (g)	206*	82	271	98	195***	59	270	106
Total fat (g)	73*	28	93	32	67**	27	89	30
Fibre (g)	12·8**	5·2	17·6	5·6	16·7	6·0	18·5	6·7
Alcohol (g)	2·8**	3·4	10·8	12·8	6·0	5·0	10·8	11·8

Significantly different from controls: *$P<0{\cdot}05$; **$P<0{\cdot}01$; ***$P<0{\cdot}005$; ****$P<0{\cdot}001$.

In conclusion, female patients in the present study were found to have significantly lower intakes of nutrients when compared with controls. Lower micronutrient intakes in the OD females were associated with significantly lower intakes of fresh fruit and fruit juices. This suggests that female dyspepsia patients may be a vulnerable group.

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Serum antioxidants and malondialdehyde in homocysteinaemics with coronary heart disease, non-homocysteinaemics with coronary heart disease, and normal control subjects. By C. M. GOSS, K. M. YOUNGER and P. MATHIAS, *Department of Biological Sciences, College of Technology, Kevin Street, Dublin 8, Republic of Ireland* and I. GRAHAM, *Department of Cardiology, Adelaide Hospital, Dublin 8, Republic of Ireland*

Increased free radical activity has been implicated in the pathogenesis of atherosclerosis, to which homocysteinaemics are particularly susceptible. It is possible that homocysteine may play a role in the production of superoxide (Heinecke *et al.* 1987), and may be responsible for damage to the vascular endothelium, or it may cause oxidation of low-density lipoprotein (LDL), leading to its being scavenged subendothelially by monocyte macrophages, and hence to the accumulation of lipid in the arterial wall. The aim of the present study was to investigate free radical activity in homocysteinaemics, non-homocysteinaemics with coronary heart disease (CHD), and normal controls, by measuring serum malondialdehyde (MDA), a product of lipid peroxidation. Also, as compromised antioxidant status could increase susceptibility to free radical damage, serum vitamins A (total retinol) and E (α-tocopherol) were measured by HPLC. Validation studies showed that in pro-oxidant conditions *in vitro* (storage at 4°, the presence of iron) serum MDA increased markedly in normal serum, whereas storage at −20° for 4 weeks had little effect.

	Homocysteinaemia with CHD		CHD		Control	
Males (*n*) . . .	10		14		11	
Females (*n*) . . .	6		6		8	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	44	4·6	47	4·9	45	6·1
Serum MDA (nmol/ml)	1·32	0·38	1·7*	0·6	1·34	0·31
Vitamin A (μg/dl total retinol)	65	16	64	13	62	17
Vitamin E (mg/dl α-tocopherol)	1·21	0·3	1·34	0·5	1·16	0·2
Vitamin E/cholesterol (mg/g)	4·85	1·2	4·95	1·5	4·87	1·0

Significantly different from both other groups (Mann-Whitney U test): *$P<0·05$.

There was no difference in serum vitamin E or vitamin A between the three groups of subjects. However, there was evidence for increased lipid peroxidation in the group with CHD but without homocysteinaemia, with no correlation between serum MDA and serum vitamin E (or LDL or total cholesterol) in any of the groups, nor between plasma homocysteine and MDA in the homocysteinaemics. This limited sample also showed no relationship between age, sex or cigarette smoking and serum MDA. Thus these findings support the hypothesis that free radicals may be implicated in the pathobiology of CHD. However, this did not seem to be the case for CHD patients with homocysteinaemia, suggesting that the pathogenicity of homocysteine in atherosclerosis is not mediated via effects on lipid peroxidation.

Heinecke, J. W., Rosen, H., Suzuki, L. A. & Chait, A. (1987). *Journal of Biological Chemistry* **262**, 10098–100103.

Effects of polymer feeds on ileal integrity as assessed by diamine oxidase release. By J. L. ENGELMAN, Y. QURESHI, G. M. MURPHY and G. E. SLADEN, *Gastroenterology Unit, United Medical and Dental Schools of Guy's and St Thomas's Hospitals, Guy's Campus, London SE1 9RT*

Enteral feeding is often used in the critically ill and in patients with chronic gastrointestinal disease such as Crohn's disease (CD). However, the effects of different types of enteral feeding on intestinal structure and function have not been investigated fully in man (Silk, 1989). In the rat, elemental and polymer diets lead to ileal atrophy (Maxton *et al.* 1987). Diamine oxidase (DAO; *EC* 1.4.3.6 (histaminase)) is an enzyme found predominantly in the small bowel mucosa, particularly in the terminal ileum. DAO may be displaced from its intestinal endothelial binding sites into the peripheral circulation by intravenous heparin. The post-heparin plasma DAO (PHDAO) concentration is believed to provide a reliable index of small bowel mucosal mass and integrity.

We therefore studied the effects of a polymer feed on PHDAO release in six patients with normal gastrointestinal function who required nasogastric feeding (Fortison) as the sole source of nutritional support for a mean of 9·6 (range 8–10) d, and compared the values with (1) those obtained previously in this unit for control subjects fed normally with a normal diet (Rokkas *et al.* 1990), and (2) patients with active Crohn's disease, to determine whether intestinal mucosal hypoplasia occurs.

Intravenous heparin (5000 IU) was administered and blood samples were then collected at 0, 5 and 15 min and then at regular 15 min intervals for 2 h. DAO and the area under the curve (AUC) were calculated as described previously (Rokkas *et al.* 1990).

In the six patients the mean AUC was 10·2 (SEM 2·4) mU/l per 2 h. This result was significantly ($P<0·005$) lower than that of our normal range of 35·9 (SEM 5·0) mU/l per 2 h (n 17), but there was no significant difference from that of the patients with active CD, 14·3 (SEM 3·9) mU/l per 2 h (n 8).

Thus, polymer feeding in man is associated with a reduced post-heparin diamine oxidase response, a result consistent with ileal mucosal hypoplasia.

Maxton, D. G., Cynk, E. U. & Thompson, R. P. H. (1987). *Gut* **28,** 688–693.
Rokkas, T., Vaja, S., Murphy, G. M. & Dowling, R. H. (1990). *Gastroenterology* **98,** 1493–1501.
Silk, D. B. A. (1989). *Gut* **30,** 246–264.

Effects of clenbuterol on tissue mass after scald injury in the rat. By LUCIE MARTINEAU[1] and RODERICK A. LITTLE[2], [1]*Department of Physiological Sciences* and [2]*North Western Injury Research Centre, University of Manchester, Manchester M13 9PT*

Burn injury provokes some of the greatest increases in metabolic rate in mammals, including man, in spite of a reduction in food intake. This hypermetabolic response is associated with an increased protein catabolism, the persistent loss of body nitrogen occurring mainly from skeletal muscles (Bilmazes *et al.* 1978; Jahoor *et al.* 1988). Any treatment that could prevent or minimize this protein wasting would be of great therapeutic value. Numerous reports have shown that β_2-adrenergic agonists (e.g. clenbuterol) rapidly reduce body fat content and increase lean tissue mass in small mammals (e.g. Emery *et al.* 1984). The present study investigated the effects of clenbuterol on body weight, tissue mass, and protein and RNA content after scald injury in the rat.

Sixty-three male, Sprague-Dawley rats (230–250 g) were divided into seven groups of equal mean body weight. A 30% body surface area full-thickness scald of the clipped dorsum was produced in three groups of animals. They were allowed *ad lib.* access to either a powdered diet, or the same diet containing clenbuterol at a dose of 4 or 12 mg/kg diet. The remaining animals were clipped, and allowed either free access to the normal diet or pair-fed with one rat of similar body weight in one of the scalded groups. All animals were killed 3 d after injury or sham treatments.

Scald injury caused an overall reduction in food intake of 25% over 3 d, and a transient cessation of weight gain. Scald injury did not affect the masses of heart, liver, or epididymal fat pads, but caused significant ($P<0{\cdot}001$) weight loss of soleus (9 (SE 1)%), gastrocnemius (11 (SE 2)%), and plantaris muscles (11 (SE 1)%) compared with the pair-fed controls. This muscle wasting was accompanied by significant reductions in protein or RNA content, or both. Oral administration of clenbuterol (4 mg/kg diet) had no anabolic effects, either in the scalded animals or their pair-fed controls. While clenbuterol (12 mg/kg diet) did not affect the masses of heart and fat pads, significant increases in the masses (about 20%), and RNA (about 30%) and protein contents (about 20%) of all muscles were observed in both groups which received clenbuterol; the magnitude of these effects was greater ($P<0{\cdot}05$) in the scalded animals than in their pair-fed controls. Clenbuterol had no effect on body weight or muscle water content, but increased ($P<0{\cdot}001$) carcass water content. These data indicate that there is a selective mobilization of muscle protein and sparing of fat in the early phase following burn injury, and that β_2-adrenergic agonists, such as clenbuterol, may be of therapeutic value in inhibiting or reversing muscle atrophy associated with such an injury.

Bilmazes, C., Kien, C. L. & Rohrbaugh, D. K. (1978). *Metabolism* **27**, 671–676.
Emery, P. W., Rothwell, N. J., Stock, M. J. & Winter, P. D. (1984). *Bioscience Reports* **4**, 83–91.
Jahoor, F., Desai, M., Herndon, D. N. & Wolfe, R. R. (1988). *Metabolism* **37**, 330–337.

Measurement of postprandial apolipoprotein B-48 using a novel specific antibody. By A. S. PEEL, D. BULSARA, C. M. WILLIAMS and B. J. GOULD, *Nutritional Metabolism Research Group, School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

The two isoforms of apolipoprotein (apo) B, apo B-48 and apo B-100, have distinct roles in human lipoprotein metabolism. Apo B-48 has an identical amino acid sequence to the N-terminal 48% of apo B-100 and this has prevented production of specific antibody for apo B-48 which does not cross-react with apo B-100. By exploiting the hydrophilic nature of the charged apo B-48 C-terminal, in a largely hydrophobic protein, we have raised an antibody which specifically recognizes apo B-48 (Peel *et al.* 1992). We have used this antibody to monitor postprandial levels of apo B-48 following a high-fat meal.

Following an overnight fast, a healthy adult male was given a meal containing fat 96 g, protein 52 g and carbohydrate 137 g. Blood was collected at hourly intervals for 5 h postprandially. Chylomicrons were prepared from plasma following the method of Bochenek *et al.* (1987). The chylomicrons were run under denaturing conditions on an SDS-polyacrylamide linear gradient (5–20%) gel. The gel was blotted onto a nitrocellulose membrane and incubated with anti-apo B-48 antibody. The Western blot was visualized using a streptavidin–biotin system, with a horseradish peroxidase label and enhanced chemiluminescence with luminol substrate.

The staining intensity of the apo B-48 band was very low in the fasting sample, increased to a maximum at 2 h and decreased steadily up to 5 h.

Densitometry of apo B-48 band

Time (h) . . .	0	1	2	3	4	5
Relative change (% of 0 h value)	100	1720	2163	1742	1096	308

Chylomicron remnants have been implicated in the pathogenesis of atherosclerosis (Zilversmit, 1979). Simons *et al.* (1987) examined apo B-48 on stained gels and found the apo B-48:apo B-100 ratio was increased in patients with coronary artery disease. We will use the apo B-48 antibody to develop an immunological-based assay that can be used for the routine detection of lipoproteins of dietary origin.

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

Bochenek, W. J., Kapuscinska, B., Slowinska, R. & Rodgers, J. B. (1987). *Atherosclerosis* **64**, 167–172.
Peel, A. S., Komanduri, P., Williams, C. M., Morgan, L. M. & Gould, B. J. (1992). *Biochemical Society Transactions* **20**, 165S.
Simons, L. A., Dwyer, T., Simons, J., Bernstein, L., Mock, P., Poonia, N. S., Balasubramaniam, S., Baron, D., Branson, J. & Morgan, J. (1987). *Atherosclerosis* **65**, 181–189.
Zilversmit, D. B. (1979). *Circulation* **60**, 473–485.

Plasma ascorbic acid and dehydroascorbic acid levels in very low-birth weight infants.

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The immature tissues of the very low-birth weight (VLBW) infant are prone to damage by oxidants which have been implicated in the pathogenesis of both bronchopulmonary dysplasia (BPD) and retinopathy of prematurity (ROP). There has been much published work on antioxidants such as vitamin E and Se-dependent glutathione peroxidase (*EC* 1.11.1.9) in VLBW infants. However, knowledge of ascorbic acid (AA) levels in VLBW infants is very limited, and there have been no published reports on levels of the oxidized form dehydroascorbic acid (DHAA). It has been suggested that the DHAA: total ascorbic acid (TAA) ratio may be a marker of increased oxidative stress. Our aim was to measure plasma AA and DHAA levels over the first 8 weeks of life of the VLBW infant at risk of BPD or ROP.

We studied fifteen babies of mean (SD) gestational age 27·5 (2·8) weeks, and birth weight 933 (240) g. Plasma was obtained on days 1, 14, 28, 42 and 56. AA and DHAA levels were measured by HPLC. Fourteen (93%) of the babies required supplemental oxygen for a mean (SD) duration of 58 (14) d. Twelve (80%) babies required mechanical ventilation for a mean (SD) duration of 22 (6) d. Four (27%) babies developed BPD and 4 (27%) ROP. Vitamin C intake was from supplementation of parenteral alimentation, that present in maternal breast milk or preterm formula, and from supplementation as multivitamin drops when the body attained full feeding by the enteral route.

Day . . .	1		14		28		42		56	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TAA (μmol/l)	32	5	62	20	69	16	74	10	64	13
DHAA:TAA (%)	20	4	21	8	15	4	13	5	16	4

The American Society of Clinical Nutrition suggests that acceptable levels of TAA in infants are those >34 μmol/l (Greene *et al.* 1988). Normal levels of DHAA:TAA % in adults are 6–12%. Our study shows that TAA levels are low at birth, but rise to acceptable levels during the 1st 2 months of life of the VLBW infant. The increased DHAA:TAA ratio suggests that oxidative stress may be increased in VLBW infants. There was no difference in either TAA or DHAA:TAA % levels between babies who developed BPD and ROP and those who did not. There was no relationship between TAA levels and vitamin C intake at any stage.

Greene, H. L., Hambidge, K. M., Schanler, R. & Tsang, R. C. (1988). *American Journal of Clinical Nutrition* **48,** 1324–1342.

Tyrosine metabolism in premature babies: effects of vitamin C intake. By HILARY J. POWERS, *Department of Paediatrics, University of Sheffield S10 2TH*, ALAN T. GIBSON, *Neonatal Intensive Care Unit, Jessop Hospital for Women, Sheffield S13 7RE* and CHRISTOPHER J. BATES, *Dunn Nutrition Unit, Cambridge CB4 1XJ*

Prematurity increases the risk of transient neonatal tyrosinaemia. There have been claims that supplements of vitamin C can help alleviate this condition.

A study is being conducted first to investigate the relationship, if any, between vitamin C intake and the rate of tyrosine metabolism in premature babies, and second, to determine the daily intake of vitamin C sufficient to maximize the rate of tyrosine metabolism.

A feasibility study was conducted to examine the potential of a [^{13}C]tyrosine breath test for the measurement of tyrosine metabolism in premature babies. A dose of 10 mg/kg body weight, administered nasogastrically, was sufficient to produce a maximum ^{13}C enrichment of exhaled air of 9·01‰; 9·30% of the dose was metabolized within 240 min.

An intervention study is now under way. Well babies are recruited if less than 1800 g at birth. Pre-dose breath test measurements are made within 14 d of birth. A second measurement is made after 5 full days of receiving daily supplements of 0, 12, 42 or 92 mg vitamin C/kg body weight.

Results have been analysed for thirteen babies. The percentage dose metabolized in 300 min showed a significant increase over 6 d from a mean of 6·0 (SE 0·28) to 7·6 (SE 0·71) regardless of the vitamin C intake (P=0·024, paired t test). The control babies (n 5) showed a small fall in the dose metabolized over 6 d of 0·04 (SE 0·703) compared with an increase of 2·65 (SE 0·712) in the treatment babies (n 8). The values were significantly different (P=0·028, t test). When all babies were considered, the maximum rate of tyrosine metabolism increased from 21·2 (SE 2·86) nmol/min to 32·0 (SE 3·27) over the 6 d (P<0·001, paired t test). The magnitude of the increase over 7 d in the control babies was 6·0 (SE 1·25) compared with 14·3 (SE 2·70) in the treatment babies. This difference was significant (P=0·039, t test).

A [^{13}C]tyrosine breath test offers a useful non-invasive means of measuring tyrosine metabolism in the premature baby. There is preliminary evidence that supplementary vitamin C may be effective in increasing the rate of tyrosine metabolism. This has implications for neonatal care.

Vitamin C deficiency is associated with pressure sores in elderly patients with femoral neck fractures. By HELEN F. GOODE[1] and EILEEN BURNS[2], *Departments of* [1]*Medicine* and [2]*Medicine for the Elderly, St James's University Hospital, Leeds LS9 7TF*

In developed countries, 30% of all elderly patients suffer from pressure sores, and as many as 60% of patients with femoral neck fractures may develop sores, mostly within the 1st 5 d of their hospital stay. A number of contributory factors have been identified, including time spent immobile post-injury, reduced sensation, blood pressure, continence and nutritional status. The contribution of pre-existing specific nutritional deficiencies to pressure sore risk has not been studied.

We measured biochemical indices of nutritional state, including total leucocyte (WBC) vitamin C, Zn contents in polymorphonuclear cells (PMNC), plasma and muscle, plasma vitamins A and E, and plasma albumin, pre-operatively, within 48 h of admission, in twenty-one elderly patients (>75 years) with a fractured neck of femur. Results were compared with those from twelve healthy people aged 75–86 years. Ten patients (48%) subsequently developed pressure sores.

Indices of Zn status were similar in patients both with and without sores, although in some patients plasma Zn was reduced and was associated with low plasma albumin levels. Vitamins A and E were also similar in patients with and without sores. Leucocyte vitamin C, however, was significantly lower in patients who developed sores compared with those who did not. Differential leucocyte counts were normal in all patients.

	Plasma Zn (μmol/l)		PMNC Zn (nmol/mg protein)		Muscle Zn (nmol/mg protein)		Vitamin A (μg/dl)		Vitamin E (mg/dl)		Vitamin C (μg/10^8 WBC)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pressure sore	9·4	2·6	1·02	0·13	4·7	0·6	38·3†	9·5	10·4	2·5	6·3*	2·2
No sore	8·1†	1·6	1·02	0·28	4·5	0·6	42·9†	17·2	9·6	4·8	12·8	4·6
Well elderly	10·8	1·1	1·11	0·20	–	–	62·5	16·5	10·4	2·3	–	–

Significantly lower than no sore group: *$P<0·01$.
Significantly lower than well elderly group: †$P<0·01$.

Vitamin C intakes below the dietary reference value may be associated with an increased incidence of pressure sores (Brown & Seabrook, 1992) and vitamin C is known to have a role in wound healing and immunological regulation. Our data suggest that pre-existing vitamin C depletion may contribute to pressure sore development in elderly patients with femoral neck fracture, but Zn and vitamins A and E appear not to be involved.

Brown, K. M. & Seabrook, N. A. (1992). *Proceedings of the Nutrition Society* (In the Press).

Effect of vitamin C supplementation on the peroxyl radical trapping ability (TRAP) of plasma from female smokers. By C. W. MULHOLLAND and T. R. TRINICK, *Ulster Hospital, Dundonald, Belfast BT16 0RH* and J. J. STRAIN, *Nutrition Research Group, University of Ulster, Coleraine BT52 1SA*

Cigarette smoke contains a variety of free radical species and there is increasing evidence that these reactive compounds are involved in a number of radical-mediated processes associated with smoking (Church & Pryor, 1985). Vitamin C is a potent nutritional antioxidant; smokers have been reported to have lower plasma levels of vitamin C than non-smokers (Murata *et al.* 1988). Thus, smokers may have a higher requirement for this vitamin due to the oxidative stress generated by the free radical species present in cigarette smoke. The present study was designed to examine the effect of vitamin C supplementation on the radical trapping ability of plasma from female smokers.

Sixteen subjects (mean age 39 years, range 26–50 years) were randomized into two groups of eight. Group 1 received 1 g ascorbic acid/d for 14 d. Group 2 received a placebo tablet each day for 14 d. Blood samples were collected on day 0, day 15 and 6–8 weeks after supplementation, and analysed for vitamin C and radical trapping ability using the TRAP technique (Wayner *et al.* 1987).

Group . . .		Vitamin C (*n* 8)		Placebo (*n* 6)	
	Week	Mean	SE	Mean	SE
TRAP (μmol/l)	0	772	50·9	728	41·6
	2	788	54·0	689	28·0
	8	806	36·9	765	59·5
Vitamin C (μmol/l)	0	36·6	9·7	43·4	11·3
	2	111·3*†	12·2	41·4	10·2
	8	52·3	6·6	49·4	10·9

Significantly different from weeks 0 and 8 (Newman Kuels test): *$P<0·01$.
Significantly different from placebo group at week 2 (ANOVA): †$P<0·01$.

Despite significant increases in vitamin C levels in the treatment group following the 2-week supplementation period, there was no significant difference in radical trapping ability as assessed by the TRAP technique.

Church, D. F. & Pryor, W. A. (1985). *Environmental Health* **64,** 111–126.
Murata, A., Shiraiski, T., Fukuzaki, K., Kitahara, T. & Harvada, Y. (1988). *International Journal of Vitamin and Nutrition Research* **59,** 184–189.
Wayner, D. D. M., Burton, G. W., Ingold, K. U., Barclay, L. R. C. & Locke, S. J. (1987). *Biochimica et Biophysica Acta* **92,** 408–419.

Indices of lipid peroxidation in the heart of vitamin E-deficient pigs with or without supplementation with polyunsaturated fatty acids. By M. NOLAN, D. G. KENNEDY, W. J. BLANCHFLOWER and S. KENNEDY, *Veterinary Sciences Division, Department of Agriculture for Northern Ireland, Stormont, Belfast BT4 3SD*

Vitamin E and Se deficiency in pigs may present as Hepatosis Dietetica, Nutritional Degenerative Myopathy and Mulberry Heart Disease. Experimental reproduction of these diseases has frequently relied on the inclusion of supplements of polyunsaturated fatty acids (PUFA) for animals deficient in vitamin E or Se, or both, as a peroxidative challenge. The aim of the present study was to examine the effects of PUFA supplementation on two indices of lipid peroxidation in the tissues of vitamin E-deficient pigs.

Twenty-two 5-week-old pigs were randomly allocated to four groups. Groups A (5 pigs) and B (6 pigs) were fed on a wheat–barley-based diet deficient in vitamin E (2·0 (SEM 0·2) mg/kg), while groups C (5 pigs) and D (6 pigs) were fed on the same diet supplemented with 60 mg α-tocopherol/kg for 34 weeks. Pigs in groups A and C were then necropsied and tissues collected for analysis. Pigs in groups B and D were maintained on their respective diets, supplemented (125 g/kg) with vitamin E-stripped corn oil as a source of PUFA, for a further 18 d, after which they were necropsied. Concentrations of α-tocopherol, malondialdehyde and 4-hydroxynonenal (4HNE) in heart were measured using the HPLC–fluorescence detection (McMurray & Blanchflower, 1979), TBARS test (Ohkawa *et al.* 1979) and by thermospray liquid chromatography–mass spectrometry (Blanchflower *et al.* 1992), respectively.

	α-Tocopherol (nmol/g tissue)		Malondialdehyde (nmol/g protein)		4-Hydroxynonenal (nmol/g tissue)	
Group	Mean	SEM	Mean	SEM	Mean	SEM
A	7·7*	0·9	0·67	0·10	14·15*	1·53
B	4·9*	0·2	0·63	0·05	24·68*†	0·93
C	39·9	1·2	0·94	0·16	1·28	0·13
D	32·5	2·6	0·77	0·11	1·82	0·18

Significant effect of vitamin E deficiency: *$P<0·001$. Significant effect of PUFA supplementation: †$P<0·001$.

Vitamin E deficiency increased heart concentrations of 4HNE. Supplementation of vitamin E-sufficient animals with PUFA did not affect 4HNE concentrations. However, supplementation of vitamin E-deficient animals with PUFA increased 4HNE concentrations to levels above those found in animals deficient in vitamin E. The TBARS test revealed no effects of either vitamin E deficiency or PUFA supplementation.

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Blanchflower, W. J., Walsh, D. M., Kennedy, S. & Kennedy, D. G. (1992). *Lipids* (In the Press).
McMurray, C. H. & Blanchflower, W. J. (1979). *Journal of Chromatography* **176,** 488–492.
Ohkawa, H., Ohishi, N. & Yagi, K. (1979). *Analytical Biochemistry* **95,** 351–358.

Stability of vitamin E-deficient muscle plasma membrane. By S. PAGE, A. MCARDLE, N. J. PRESCOTT, R. H. T. EDWARDS and M. J. JACKSON, *Muscle Research Centre, Department of Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX*

Previous studies have indicated that some of the cytoprotective effects of α-tocopherol against skeletal muscle damage can be mimicked by other hydrocarbon chain analogues of tocopherol which have little antioxidant ability (Phoenix *et al.* 1991). Diplock (1982) has indicated the potential membrane stabilizing role of the tocopherols complementary to their antioxidant functions. We have, therefore, utilized a novel technique of producing muscle plasma membrane vesicles in order to examine plasma membrane stability in vitamin E-deficient and supplemented muscles.

Groups of 20-d-old female Balb C mice were fed on either a vitamin E-deficient diet (Dyets Inc., Pennsylvania, USA) or the same diet supplemented with 100 μg α-tocopherol acetate/g for 6 weeks. On sacrifice, muscles from the two groups of animals were incubated in a stretched position in 140 mM KCl–Hepes, pH 7·6, containing 100 U collagenase (type IVA, *EC* 3.4.24.3)/ml (Zubrzycka-Gaarn *et al.* 1991). Vesicles of plasma membrane produced by this treatment were collected by centrifugation and purified from erythrocytes and muscle fragments by centrifugation through Histopague-1077 (Sigma Chemical Co).

Purified vesicles were then incubated in phosphate-buffered saline (9 g NaCl/l; pH 7·3, 1 h 25°). Stability of vesicles was assessed at various times by measurement of creatine kinase (*EC* 3.7.3.2) activity in the supernatant following centrifugation and by counting the number of intact vesicles.

Vitamin E-deficient animals had a significantly lower plasma α-tocopherol content (0·9 (SEM 0·3) μg/ml) than supplemented animals (5·0 (SEM 1·4) μg/ml).

Monitoring creatine kinase release from isolated vesicles was found to be an unsatisfactory method of assessing vesicle stability, but counting vesicles by microscopy gave reproducible results. Vitamin E-deficient vesicles showed a significantly decreased stability in vitro compared with vesicles from supplemented animals (% remaining intact after 60 min, E− 56·0 (SEM 9·2) *v.* E+ 74·3 (SEM 1·2), $P<0·05$).

These results therefore support the hypothesis that purified vitamin E-deficient muscle membranes are relatively unstable and that vitamin E exerts a stabilizing role on the muscle plasma membrane.

Support from The Muscular Dystrophy Group of Great Britain and Northern Ireland is gratefully acknowledged.

Diplock, A. T. (1982). *Acta Vitaminology Enzymology* **4**, 303–309.
Phoenix, J., Edwards, R. H. T. & Jackson, M. J. (1991). *Biochimica et Biophysica Acta* **1097**, 212–218.
Zubrzycka-Gaarn, E. E., Hutter, O. F., Karpati, G., Klamut, H. J., Bulman, E. E., Hodges, R. S., Worton, R. S. & Ray, P. N. (1991). *Experimental Cellular Research* **192**, 278–288.

Calcium homeostasis during contractile activity of vitamin E-deficient skeletal muscle. By A. MCARDLE, R. H. T. EDWARDS and M. J. JACKSON, *Muscle Research Centre, Department of Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX*

Damage to normal skeletal muscle induced by excess contractile activity appears to be exacerbated by vitamin E deficiency (Jackson *et al.* 1983) and may be mediated by increased oxidizing free radical activity. In recent work we have demonstrated that excess contractile activity of normal muscle leads to an influx of extracellular calcium which may mediate further degradative pathways (McArdle *et al.* 1992.) Since free radical species may modify membrane Ca fluxes in muscle, we have examined the possibility that vitamin E deficiency leads to a modification in muscle Ca homeostasis during contractile activity.

A group of 20-d-old female Balb C mice were fed a vitamin E-deficient diet (Dyets Inc., Pennsylvania, USA) for 6 weeks. Control mice were identical except that the diet was supplemented with 100 μg α-tocopherol acetate/g. When the mice were killed, extensor digitorum longus muscles were carefully and rapidly removed, mounted on special holders and incubated as previously described (McArdle *et al.* 1992). Muscle creatine kinase (*EC* 3.7.3.2) efflux and ^{45}Ca accumulation was measured during incubation of muscles with or without stimulation via platinum electrodes (100 Hz for 0·5 sec every 2 sec, at 30V for 30 min).

Muscle Ca accumulation (μmol/g per 120 min) was unchanged between unstimulated vitamin E-supplemented (E+) and deficient (E−) animals (E+, 0·62 (SEM 0·5) *v.* E−, 0·79 (SEM 0·05)).

Vitamin E-deficient muscles were found to be damaged to a greater extent following contractile activity as indicated by creatine kinase efflux (E+, 32·5 (SEM 13·6) mU/mg per 180 min *v.* E−, 72·1 (SEM 13·7) mU/mg per 180 min; *n* 5) but Ca accumulation by both groups was similar: E+, 1·97 (SEM 0·21) μmol/g per 120 min *v.* E−, 1·93 (SEM 0·27) μmol/g per 120 min.

These results do not support the hypothesis that vitamin E deficiency leads to an exacerbated (free radical-mediated) failure of Ca homeostasis in muscle during contractile activity but rather suggest that vitamin E-deficient muscle is more susceptible to Ca-mediated degradative pathways.

Financial support from The Muscular Dystrophy Group of Great Britain and Northern Ireland is gratefully acknowledged.

Jackson, M. J., Jones, D. A. & Edwards, R. H. T. (1983). In *Biology of Vitamin E.* Foundation Symposium 101, pp. 224–239. London: Pitman.

McArdle, A., Edwards, R. H. T. & Jackson, M. J. (1992). *Clinical Science* **82,** 455–459.

Vitamin E status modulates the inflammatory response to endotoxin in rats. By KERRY TROUGHTON and R. F. GRIMBLE, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

Free radicals and cytokines are elicited from macrophages by inflammatory stimuli. Chaudhri & Clark (1989) showed that alloxan (a free radical generator) and butylated hydroxyanisole (an antioxidant) enhanced and suppressed tumour necrosis factor production respectively in mice given endotoxin. We therefore examined whether vitamin E status modified the response to endotoxin.

Thirty-six male weanling Wistar rats were randomly divided into three diet groups. Diets varied only in vitamin E content (g/kg, casein 180, solka floc 100, sugar 283·5, starch 283·5, L-methionine 3, vitamin E-free corn oil 100, vitamin E-free mineral mix 50 and either 0, 50 or 250 mg vitamin E/kg). Animals were fed *ad lib.* for 20 d.

On day 19 half of each group received 800 μg endotoxin (ENDO)/kg intraperitoneally. On day 20 these animals were killed. The remaining rats received sterile saline (9 g NaCl/l; SAL), were pair-fed with the corresponding endotoxin injected group and killed 24 h later.

Vitamin E content (mg/kg) . . .	0 (deficient)		50 (normal)		250 (supplemented)		Pooled
	SAL	ENDO	SAL	ENDO	SAL	ENDO	SEM
Erythrocyte vitamin E (mg/ml cells)	0·41		0·73*		1·12*†		0·02
Orosomucoid (μg/ml)	95	326*	76	255†§	86	260‡§	26
Plasma albumin (mg/ml)	34·5	30·9*	34·4	28·5†	33·4	31·5§‖	0·35
Glutathione (mg/g liver)	19·4	22·8	16·1	21·1†	15·6	25·3‡	0·4
Caeruloplasmin (Um/ml plasma)	125	145	96	115	71*	130‡	5
Lung PMN (% cells)	9·26	10·55	7·87	10·85†	6·97*	9·20‡	0·29

Significantly different ($P<0·05$; ANOVA) from: * deficient saline; † normal saline; ‡ supplemented saline; § deficient endotoxin; ‖ normal endotoxin.

Vitamin E status had complex modulatory effects on the response to endotoxin. While the increase in orosomucoid was enhanced by deficiency, supplementation reduced the fall in plasma albumin. Status also influenced values observed in saline-treated animals. Deficient rats exhibited increased plasma caeruloplasmin and infiltration of polymorphonuclear cells (PMN) into the lung. Vitamin E deficiency may therefore exacerbate the effects of inflammatory stimuli.

Chaudhri, G. & Clark, I. A. (1989). *Journal of Immunology* **143,** 1290–1294.

The effect of pregnancy on folate catabolism in the rat. By H. MCNULTY[1], J. M. MCPARTLIN[2], D. G. WEIR[2] and J. M. SCOTT[3], [1]*Biomedical Sciences Research Centre, University of Ulster at Coleraine BT52 1SA* and *Departments of* [2]*Clinical Medicine and* [3]*Biochemistry, Trinity College, Dublin 2, Republic of Ireland*

The incidence of folate-responsive megaloblastic anaemia of pregnancy is not uncommon, even in developed countries. Subclinical folate deficiency of pregnancy is more prevalent and can be detected in an estimated 25% of unsupplemented women in developed countries (Chanarin, 1985). The precise mechanism of maternal depletion of the vitamin is not known. The hypothesis examined in the present study was that folate deficiency of pregnancy might be the result of altered folate catabolism.

Mammalian folate catabolism involves cleavage of the molecule at the C9-N10 position to produce biologically inactive folate catabolites which are subsequently excreted in urine (Murphy *et al.* 1976). We have developed an HPLC method to determine urinary levels of the major catabolite acetamidobenzoylglutamate (apABGlu) in the rat, allowing endogenous folate catabolism to be measured for the first time. Three groups of female Wistar rats, two of which were pregnant, were investigated. One pregnant group was allowed access to food *ad lib.* (P:AL, *n* 6), while the other (P:PF, *n* 6) was restricted by pair-feeding against a third non-pregnant, *ad lib.*-fed group (NP:AL, *n* 6).

Acetamidobenzoylglutamate excretion (μg/rat per d)

Group . . .	NP:AL		P:PF		P:AL	
Day of pregnancy	Mean	SEM	Mean	SEM	Mean	SEM
−2	5·5	0·45	4·1	0·24	6·4	0·41
0	4·5	0·25	4·0	0·30	5·2	0·62
2	3·8	0·45	5·0	0·51	4·7	0·38
4	4·9	0·47	5·5	0·32	6·1	0·24
6	4·2	0·59	5·0	0·26	7·4**	0·54
8	5·0	0·63	5·0	0·45	9·3**	0·69
10	6·1	0·41	6·0	0·19	8·5*	0·77
12	4·5	0·43	6·5*	0·64	8·7**	0·76
14	4·4	0·71	8·2**	0·78	10·7***	0·90
16	4·5	0·47	13·6***	0·79	15·1***	0·69
18	5·5	0·91	13·9***	0·63	16·5***	0·60
20	4·5	0·61	9·4*	1·56	13·4***	0·50
22	4·8	0·91	8·1*	0·96	8·5*	0·93
24	5·0	0·74	7·1	0·57	7·7	0·53
28	6·3	0·55	4·8	0·35	5·7	0·53

Significantly different from NP:AL (Student's *t* test for unpaired data): *$P<0·05$, **$P<0·01$, ***$P<0·001$.

The results indicate that the rate of folate catabolism increases with progression of gestation in the rat, peaking at day 18 at levels of up to three times those of non-pregnant animals and falling to prepregnancy levels post-partum (day 22 onwards). These elevations were apparent even in the face of low levels of dietary folate and suggest that increased catabolism may represent a major mechanism whereby human pregnancy induces folate deficiency by imposing a drain on available folates.

Chanarin, I. (1985). *Clinical Haematology* **14,** 629–641.

Murphy, M., Keating, M., Boyle, P., Weir, D. G. & Scott, J. M. (1976). *Biochemical and Biophysical Research Communications* **71,** 1017–1024.

The effect of biotin deficiency on the fatty acid composition of porcine tissues. By K. M. COOPER, D. G. KENNEDY and S. KENNEDY, *Veterinary Sciences Division, Department of Agriculture for Northern Ireland, Stormont, Belfast BT4 3SD*

Biotin deficiency in pigs is characterized by skin and hoof lesions, reduced growth rate and impaired reproductive performance. The aim of the present study was to investigate the effects of biotin deficiency on tissue fatty acid composition in the pig. Nine 4-week-old pigs were randomly divided into a principal group of five pigs and a control group of four pigs. The control group was fed on a wheat-based diet which was supplemented with vitamins, minerals and 500 μg biotin/kg. The principal group was fed on the same diet, supplemented with 100 g egg white/kg, but no biotin supplement. The animals were maintained on a wire floor to minimize coprophagy. All pigs were necropsied on day 52 and tissues collected for subsequent analysis by capillary GC for fatty acid composition. Clinical signs characteristic of biotin deficiency were observed in the principal group from about day 35. Mean live weights of the principal group were lower than those of the control group from day 28 onwards ($P<0{\cdot}001$).

	Fatty acid (% of identified fatty acids)									
	C16:0		C18:0		C18:1		C18:2		C20:4	
Tissue	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver: +Biotin	18·9	1·2	33·6	1·1	15·1	1·3	16·5	0·5	14·6	0·7
−Biotin	16·6*	0·7	23·5*	0·9	15·6	1·3	17·3	0·9	23·4*	1·3
Kidney: +Biotin	25·5	0·5	19·8	0·1	21·0	0·9	19·0	0·5	12·6	0·3
−Biotin	21·6*	0·6	16·0*	0·5	18·1*	1·5	20·6*	0·8	19·6*	0·7
Heart: +Biotin	21·6	0·2	18·3	0·5	18·3	1·7	29·3	1·9	10·8	1·0
−Biotin	19·9	0·3	14·6*	0·5	13·9*	2·1	33·1*	2·4	15·7*	1·6
L. Dorsi: +Biotin	24·9	0·6	14·5	0·4	36·4	1·5	14·5	1·7	3·9	0·8
−Biotin	22·6	1·2	10·7*	0·6	36·4	2·9	16·5	2·3	5·5	1·7

Significant effect of biotin (ANOVA): *$P<0{\cdot}05$.

There were no significant effects of biotin deficiency on the concentrations of C14:0, C14:1, C16:1, C18:3 or C20:0 in any tissue. In the present study biotin deficiency increased the proportion of polyunsaturated fatty acids in the tissues examined. This finding suggests that tissues of biotin-deficient pigs may be more susceptible to lipid peroxidation, with possible adverse effects on animal health and keeping quality of meat.

This study was supported by F. Hoffmann-La Roche & Co., Basel, Switzerland.

The non-invasive measurement of protein turnover in man: comparison of end product methods with [^{15}N]glycine. By G. GROVE and A. A. JACKSON, *Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

The measurement of protein turnover represents an important aspect of the definition of protein status. A number of methods have been developed for exploring different aspects of protein turnover, but there is still the need to have a clearly characterized approach to the measurement of whole-body protein turnover by a non-invasive technique, suitable for application to free-living individuals. The method of choice is the single dose–end product method ([^{15}N]glycine and urinary ammonia) introduced by Waterlow *et al.* (1978). One of the reasons why this method has not received more widespread acceptance is the limited comparisons with other approaches. In the present study protein turnover has been measured in the same five individuals by four different methods for varying periods of time. [^{15}N]glycine has been used as the tracer, with urea and ammonia as the end products. The prime–intermittent dose method was carried out over 18 h and the single dose method was extended to 48 h.

Synthesis of end products (mgN/kg per h)

Duration of study (h) . . .	Single dose								Intermittent dose
	9	12	15	18	21	24	36	48	18
Ammonia	29	27	25	20	26	24	22	20	14
SD	13	7	6	4	9	9	8	9	4
Urea	33*	66	53	42	40	35	27	25	22
SD	13	15	11	11	7	7	4	5	4

* Value includes a correction for the labelled urea retained in the urea pool.

The results show that for the single dose method the absolute value obtained for synthesis depends upon the duration of urine collection and the end product. Relative to the value for synthesis obtained with the prime–intermittent method using the plateau enrichment in urinary urea between 9 and 18 h, comparable results were obtained with the single dose method based upon the excretion of label in ammonia for times between 12 and 48 h, or the excretion in urea between 36 and 48 h. There are theoretical reasons to expect the results obtained under these three sets of circumstances to be similar, based upon our understanding of the kinetics of the urea and ammonia pools. When the precursor method with labelled leucine has been used in normal adults, values for protein synthesis of similar magnitude have been obtained: 40 mgN/kg per h over 4 h (Rennie *et al.* 1982) and 20 mgN/kg per h over 24 h (Garlick *et al.* 1980).

These data imply that the single dose–end product method, in which [^{15}N]glycine is given and the amount of label excreted in the urine as ammonia over 12 h is measured, gives representative values for protein turnover in normal adults.

Garlick, P. J., Clugston, G. A., Swick, R. W. & Waterlow, J. C. (1980). *American Journal of Clinical Nutrition* **33**, 1983–1986.

Rennie, M. J., Edwards, R. H. T., Halliday, D., Matthews, D. E., Wolman, S. L. & Millward, D. J. (1982). *Clinical Science* **63**, 519–523.

Waterlow, J. C., Golden, M. H. N. & Garlick, P. J. (1978). *American Journal of Physiology* **235**, E165–E174.

Urea kinetics in healthy young women. By I. MCCLELLAND, C. PERSAUD and A. A. JACKSON, *Department of Human Nutrition, Southampton University Medical School, Southampton SO9 3TU*

Metabolic studies carried out in women show that there are important metabolic changes during the menstrual cycle. Changes in energy (Bisdee *et al.* 1988) and N balance (Calloway & Kurzer, 1982) have been described. We measured urea kinetics during the menstrual cycle to determine the effects of the level of N intake, the use of an oral contraceptive pill or the method of measurement.

Fourteen healthy women aged 21–37 years participated in one or more of the studies. Urea kinetics were measured on seven occasions by the prime–intermittent dose method (ID; Jackson *et al.* 1984) and the single dose method (SD; McClelland *et al.* 1992). There were six variations in the study design controlling for level of N intake, day of cycle, use of an oral contraceptive pill and method of measurement of urea kinetics.

Study	N intake	Method of measure-ment	Day of cycle	Pill user	N intake (mgN/kg per d)	Urea pro-duction (mgN/kg per d)	Pro-duction intake (%)	Urinary excretion (mgN/kg per d)	Excretion/intake (%)	Urea salvage (mgN/kg per d)	Excretion/pro-duction (%)
1 (*n* 7)	RDA-55 g protein	ID	No control	3 × Yes 3 × No	151	179	119	105	70	74	60
2 (*n* 7)	Habitual	ID	12	No	191	194	103	132	70	62	69
3 (*n* 7)	Habitual	ID	22	No	190	210	112	135	73	74	65
4 (*n* 6)	Habitual	ID	12	Yes	169	204	120	107	62	97	53*
5 (*n* 6)	Habitual	ID	22	Yes	168	193	115	119	71	74	62
6 (*n* 6)	Free-living habitual	SD	1,8,15, 22	3 × Yes 3 × No	207	248	122	143	70	105	59

Significantly different from study 2 (one-way ANOVA): *$P<0.05$.

In all studies urinary urea was 70% N intake, and urea production was 100–120% intake. With wide inter-individual differences for all the variables, the only statistically significant difference identified was the disposal of urea production to excretion or salvage on day 12 of the cycle in women taking the pill compared with women not on the pill. Women on the pill excreted proportionately less and salvaged proportionately more urea-N.

Bisdee, J. T., James, W. P. T. & Shaw, M. A. (1988). *British Journal of Nutrition* **61,** 187–199.
Calloway, D. H. & Kurzer, M. (1982). *Journal of Nutrition* **112,** 356–366.
Jackson, A. A., Picou, C. & Landman, J. (1984). *Human Nutrition: Clinical Nutrition* **38C,** 339–354.
McClelland, I., Danielsen, M. & Jackson, A. A. (1992). *Proceedings of the Nutrition Society* (In the Press).

Whole-body protein turnover and basal metabolism in chronic undernutrition. By M. J. SOARES[1], L. S. PIERS[2], P. S. SHETTY[2], A. A. JACKSON[1] and J. C. WATERLOW[3], [1]*Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*, [2]*Department of Physiology, St. John's Medical College, Bangalore, India* and [3]*Division of Clinical Sciences, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

We have shown that adaptation during chronic energy deficiency is associated with changes in the rate of whole-body protein turnover (WBPT). In chronically undernourished (UN) adults, protein synthesis tended to be higher, expressed per kg body weight (WT) and per kg fat-free mass (FFM; Soares *et al.* 1991). It was suggested that the relatively greater intake of energy of the UN on the study day may have contributed to these results. The present study was therefore conducted to control for this variable.

Five subjects body mass index (BMI) 20·8 (SD 1·0), with access to *ad lib.* energy and protein intake and five UN subjects (BMI 16·7 (SD 0·4)), were selected as detailed earlier (Soares *et al.* 1991). WBPT was measured using a single oral dose (100 mg) of [^{15}N]glycine. Protein turnover rates were calculated over 12 h (i.e. fed state) from the end product average flux of the cumulative excretion of isotope, as ammonia (Q_a) and urea (Q_u).

	Normal wt subjects (NW)		Undernourished subjects (UN)	
	Mean	SD	Mean	SD
Energy intake (kJ/kg WT per d)	193·0	8·9	198·0	6·8
Protein intake (g/kg WT per d)	1·0	0·02	1·02	0·01
BMR (kJ/kg WT per d)	99·0	9·2	120·3*	5·2
BMR (kJ/kg FFM per d)	120·5	9·5	134·9*	7·0
Protein synthesis (g/kg WT per d)	5·01	0·50	5·47	0·63
Protein synthesis (g/kg FFM per d)	6·11	0·56	6·14	0·84
Non-muscle:Muscle ratio	0·90	0·11	1·41*	0·35
Q_u:Q_a ratio	1·20	0·38	1·65	0·38

Significantly different from NW (*t* test): *P<0·05.

The results were similar to those obtained in the earlier study. The higher Q_u:Q_a ratio paralleled the change in body composition in the UN (r 0·81; P<0·005), who demonstrated a preponderance of non-muscle over muscle mass. BMR and protein synthesis rates in the UN were either higher or similar to the NW when expressed per kg WT or kg FFM. A close relationship between BMR and WBPT (r 0·80; P<0·005) was observed, as in the previous study. The UN were in a greater positive apparent N balance (53 (SD 14) *v.* 27 (SD 14) mg/kg per d; P<0·02), due to a significantly lower total urinary-N excretion (110 (SD 14) *v.* 133 (SD 14) mg/kg per d; P<0·05). The results substantiate our earlier conclusions that WBPT is conserved in chronic undernutrition. The findings cannot be attributed to the intake of food over the study period itself as acute changes in energy intake at a constant protein intake were not associated with equivalent changes in BMR or WBPT, in normal or undernourished subjects.

Soares, M. J., Piers, L. S., Shetty, P. S., Robinson, S., Jackson, A. A. & Waterlow, J. C. (1991). *Clinical Science* **81**, 419–425.

Effects of glycine and arginine supplementation on the metabolic response to tumour necrosis factor in rats fed low-protein diets. By EMMA A. L. HUNTER, TRACEY MEAKINS and R. F. GRIMBLE, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

Arginine and glycine may improve recovery from trauma (Pui & Fisher, 1979) and are closely related to sulphur amino acid metabolism. Cysteine, a product of methionine metabolism, improves the impaired metabolic response to tumour necrosis factor-α (TNF) in rats fed low-protein diets (Grimble *et al.* 1991). We examined the effect of glycine and arginine supplementation in response to TNF in rats fed low-protein diets (80 g casein/kg).

Male Wistar rats in metabolism cages received either 200 g casein/kg containing 12 g alanine/kg and 8 g cysteine/kg (diet A) or 80 g casein/kg supplemented with 18 g alanine/kg (diet B) or 5 g glycine/kg and 11·6 g arginine/kg (diet C). After 8 d, half the animals from each group received 100 μg TNF/kg body weight intraperitoneally. The remaining animals were injected with sterile non-pyrogenic saline (9 g NaCl/l) on day 9 and were pair-fed. Animals were killed 24 h after injection. A 24 h urine collection was made before and after injection.

	Diet A		Diet B		Diet C		Pooled
	Saline	TNF	Saline	TNF	Saline	TNF	SEM
Liver GSH (mg/g)	13·0†	27·1*†	5·6	10·8*	6·1	8·3	0·5
Lung GSH (mg/g)	7·7†	8·4†	6·4	5·9	6·0	7·9*†	0·2
Liver protein (g)	1·6†	1·6†	0·8	0·7	0·6	0·9†	0·05
Urinary SO_4 post-injection (mmol/24 h)	7·7†	1·9*†	0·4	0·4	0·8	0·2*	0·1

GSH, glutathione; SO_4, inorganic sulphate, significantly different (ANOVA) from: control, *$P<0·05$; or corresponding group receiving diet B, †$P<0·05$.

While diet A rats grew by 64 g during the study, those on diets B and C grew 8 g and 4 g respectively. Glycine and arginine were ineffective in restoring to normal the metabolic response to TNF with the exception of an increased lung glutathione (GSH) concentration and liver protein content. Changes in urinary inorganic sulphate excretion may indicate diversion of cysteine into GSH synthesis.

Grimble, R. F., Jackson, A. A., Persaud, C., Wride, M. A., Delers, F. & Engler, R. (1991). *Proceedings of the Nutrition Society* **50**, 169A.

Pui, Y. M. L. & Fisher, H. (1979). *Journal of Nutrition* **109**, 204–246.

Increased albumin synthesis in healthy volunteers with feeding. By K. A. HUNTER[1], P. E. BALLMER[2], S. E. ANDERSON[1], A. G. CALDER[1], P. J. GARLICK[1] and M. A. MCNURLAN[1], [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and [2]*Department of Internal Medicine, University of Bern, Bern, Switzerland*

Animal studies and in vitro work have shown that the rate of albumin synthesis can be influenced by nutrition (Kirsch *et al.* 1968; Pain *et al.* 1978) but few studies have been undertaken in man due, primarily, to limitations of methodology. The increased use of stable isotopes has facilitated human work, in particular studies where sequential measurements are necessary. Moreover, by giving the label with a flooding-dose of amino acid, even acute changes such as those after feeding can be investigated. The aim of the present study, therefore, was to determine if hepatic albumin output would respond acutely to feeding in human subjects. A flooding technique similar to that of Ballmer *et al.* (1990) but modified to use L-[d_5]phenylalanine as the tracer was employed.

Six healthy subjects (four male, two female, mean age 34 (SEM 3·3) years, mean body mass index 20·6 (SEM 0·9)) were studied. Two measurements were made per subject, once after a 12 h fast and once during the final 90 min of a feeding regimen consisting of five small hourly meals (energy and protein contents standardized to predicted energy expenditure). L-[d_5]Phenylalanine (43 mg/kg) enriched to 10 atoms % was administered as a 2% (w/w) solution in 0·45% (w/w) saline and small blood samples taken at intervals up to 90 min. Albumin fractional synthesis rates (FSR; %/d) were calculated from the increase in enrichment of albumin-bound phenylalanine and the free phenylalanine enrichment in the plasma determined by gas chromatography–mass spectrometry. Results are shown in the Table.

Subject	Fasted FSR (%/d)	Fed FSR (%/d)	Difference
1	4·10	5·63	+1·53
2	7·13	7·74	+0·63
3	5·55	6·08	+0·53
4	4·88	7·89	+3·01
5	6·68	8·11	+1·43
6	6·49	7·13	+0·64
Mean	5·81	7·10	+1.30
SEM	0·48	0·42	+0·39

In all subjects, feeding resulted in a positive response in albumin synthesis, the mean albumin FSR rising from 5·81 (SEM 0·48) %/d in the fasted state to 7·10 (SEM 0·42) %/d in the fed state. This difference in synthesis rates during fasting and feeding is statistically significant when analysed by paired *t* test (P=0·02).

We conclude that albumin synthesis is acutely sensitive to nutrient intake, a small but significant increase being apparent after feeding. Whether this response is specific to albumin or reflects an overall response in all liver proteins remains to be determined.

Ballmer, P. E., McNurlan, M. A., Milne, E., Heys, S. D., Buchan, V. & Garlick, P. J. (1990). *American Journal of Physiology* **259**, E797–E803.

Kirsch, R., Frith, L., Black, E. & Hoffenberg, R. (1968). *Nature* **217**, 578–579.

Pain, V. M., Clemens, M. J. & Garlick, P. J. (1978). *Biochemical Journal* **172**, 129–135.

Responses of chronically energy deficient tropical subjects to brief cold exposure. By M. VAZ, A. V. KURPAD, SARAL THANGAM, M. V. SHAILESH, J. F. ANDREWS* and P. S. SHETTY, *Nutrition Research Centre, St. John's Medical College, Bangalore 560034, India*

Thermogenic and cardiovascular responses to cold exposure were investigated in thirteen healthy male subjects of whom six were chronically energy deficient (CED). The CED subjects were of similar age, but had significantly lower body mass index, lower stature and lower body-weight than well-fed controls. CED subjects also had lower percentage body fat and fat-free mass. At an ambient air temperature of 28–29·5°, all subjects were exposed to a cooling stimulus of the chest and trunk using a continuous-flow jacket in which 40 m of polyethylene tubing was embedded. The 13° cold water stimulus was delivered at a flow-rate of 1·5 l/min for 40 min.

Cardiovascular variables monitored continuously before and during cold exposure included heart rate, stroke volume and cardiac output by impedance cardiography and blood pressure (BP) using an automated BP apparatus. Forearm blood flow was monitored by venous occlusion plethysmography using a mercury in silastic strain-gauge. Core body temperature was measured using an aural probe whilst three skin temperature probes were placed on the chest, under the jacket (to monitor the effectiveness of cooling), and on the exposed ventral forearm and a finger tip (to assess vasoconstriction). Oxygen consumption was continuously monitored using a ventilated hood.

There was a small thermogenic response (+3·4% in $\dot{V}_{O_2}$) in CED subjects during cold exposure ($P<0·05$) which was not observed in the controls. The CED subjects also demonstrated a significantly greater peripheral vasoconstriction as evinced by a lower finger-tip temperature, a marked reduction in forearm blood flow and a small but significant rise in diastolic blood pressure. All other cardiovascular parameters were similar.

	Baseline measurements				Responses to cold			
	Controls		CED		Controls		CED	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$\dot{V}_{O_2}$ (ml/min)	218·4	16·5	175·6**	16·0	215·1	15·3	181·5*†	15·9
Forearm blood flow (ml/min per 100 ml)	4·3	3·0	3·0	0·8	2·6†	1·6	1·0*†	0·6
Forearm vascular resistance	24·1	11·9	27·5	5·9	39·4†	19·2	105·4*†	61·7
Diastotic blood pressure (mm Hg)	71·6	3·9	68·7	8·5	74·5	5·9	72·8†	9·6

Significant differences between CED *v.* control subjects: *$P<0·05$; **$P<0·01$.
Significant differences between baseline *v.* cold exposure: †$P<0·05$.

A brief and mild cold exposure in tropical CED subjects therefore produced only a small but significant thermogenic response while the cardiovascular responses suggest a greater effort by the CED subjects to reduce body heat loss.

Study supported by The Nestlé Foundation, Switzerland.

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Measurement of free-living energy expenditure by continuous heart rate monitoring and doubly-labelled water. By L. DAVIDSON[1], G. MCNEILL[1,2], P. HAGGARTY[1] and J. S. SMITH[1], [1]*The Rowett Research Institute, Aberdeen AB2 9SB* and [2]*Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen AB9 2ZD*

Recent studies comparing heart rate (HR) monitoring with doubly-labelled water (DLW) measurements of free-living energy expenditure (EE) (Schulz *et al.* 1989; Livingstone *et al.* 1990) have found that differences between the two methods in individuals may be up to 50%. Possible reasons for this may include (1) monitoring EE by HR over a shorter period than by DLW, (2) problems with defining individual HR–EE relationships in individuals from a small number of activities, and (3) artefacts in the HR record due to either loss of electrode contact or electrical interference. To assess whether the agreement between HR monitoring and DLW could be improved by attention to these aspects, we carried out simultaneous HR monitoring and DLW studies in ten men over 9 consecutive days. The men had a mean age of 37 (range 25–54) years and body mass index (BMI) of 21·8 (range 19·5–23·7) kg/m^2. All had sedentary jobs but the amount and type of leisure-time activity varied widely between the subjects. HR was measured with a Polar Sports Tester 4000 HR monitor. The calibration of HR and EE was determined for each subject from 30 min values of EE and HR obtained during 24 h whole-body indirect calorimetry with a standard exercise protocol, and additional data points for individual leisure activities (e.g. running, gardening, aerobics) measured with an Oxylog portable oxygen consumption meter. A second-order polynomial was fitted to the individual HR–EE relationships. Where HR data were missing because of loss of contact of the electrodes, HR values from an equivalent activity period (recorded in an activity diary kept for the 9 d by each subject) were inserted. HR data containing artefacts which were likely to be due to electrical interference were removed by two computerized procedures. The first procedure took out HR values greater than 200 beats per min (bpm) and the second removed isolated high values 50 bpm above the previous value.

On average, EE from 9 d HR monitoring was +5·5 (SD 14·4)% higher than that by DLW. This agreement was better than that obtained using only 3 d HR monitoring (2 week days and 1 weekend day; +9·4 (SD 21·5)%) or that obtained from 9 d without insertion and filtering of HR data (+14·7 (SD 18·3)%). EE estimated from 9 d using the diary–respirometer technique was 12·3 (SD 12·7)% lower than that by DLW. This suggests that HR monitoring may be capable of providing better estimates of free-living EE than earlier work suggests, but that further effort is required to improve the performance of the HR method in individual subjects.

Livingstone, M. B. E., Prentice, A. M., Coward, W. A., Ceesay, S. M., Strain, J. J., McKenna, P. G., Nevin, G. B., Barker, M. E. & Hickey, R. J. (1990). *American Journal of Clinical Nutrition* **52**, 59–65.

Schulz, S., Westerterp, K. R. & Bruck, K. (1989). *American Journal of Clinical Nutrition* **49**, 1146–1154.

The effect of season and leisure activity on the energy expenditure of adult males. By P. HAGGARTY[1], G. MCNEILL[1,2], M. K. ABU MANNEH[1], L. DAVIDSON[1], E. MILNE[1], G. DUNCAN[1] and J. ASHTON[1], [1]*Rowett Research Institute, Aberdeen AB2 9SB* and [2]*Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen AB9 2ZD*

Ten adult males (age 37 (SD 9) years; weight 67·2 (SD 6·3) kg; body mass index (BMI) 21·8 (SD 1·6)) were selected for a study of the effect of season and leisure-time activity on energy expenditure. All subjects had sedentary occupations but leisure activities ranging from non-active to very active. Total energy expenditure (TEE) was measured over 10 d using doubly-labelled water (DLW). The two-point method of calculation was employed with the modification that both the ^{18}O and ^{2}H pool sizes were utilized. The mean proportional fractionated water loss was estimated at 0·26 and the RQ at 0·85. Basal metabolic rate (BMR) was measured by ventilated-hood indirect calorimetry. BMR and TEE were measured both in summer (June–August) and winter (February) in Aberdeen. The Table shows the BMR and TEE as a multiple of BMR at the two seasons.

	Summer		Winter		Difference
Subject	BMR (kJ/d)	TEE (×BMR)	BMR (kJ/d)	TEE (×BMR)	Summer–winter
1	7135	1·7	6679	1·5	–0·1
2	7493	1·9	7355	2·0	+0·1
3	6648	1·5	6116	1·6	+0·1
4	6986	1·8	7157	1·4	–0·4
5	7642	2·0	7648	2·2	+0·2
6	7040	2·2	6811	1·7	–0·5
7	7528	2·0	8302	2·0	–
8	6917	2·4	6630	1·7	–0·7
9	8117	2·4	8255	1·9	–0·5
10	6132	2·3	6030	2·3	–

The mean TEE of the group in summer was 2·0 (SD 0·3) × BMR, with a range from 1·5 to 2·4. All but one of the group exceeded the highest Department of Health (1991) estimate of 1·6 × BMR for individuals with sedentary occupations but high leisure activity levels. Winter values were slightly lower at 1·8 (SD 0·3) × BMR, but not significantly so (t=1·91; P>0·05 by paired t test). There was a tendency for the decrease in activity level to be greater in the subjects with the higher TEE in the summer (r −0·57; P<0·05). These results are in line with another study showing a large contribution of leisure activity to TEE (Livingstone *et al.* 1991), and suggest that present Department of Health estimates of requirements may underestimate the potential of leisure-time activity to influence TEE in individuals with sedentary occupations. The influence of season on TEE (probably via its effect on behaviour) suggests that seasonal effects should be considered when estimating habitual energy requirements from single DLW estimates of TEE, particularly in very active subjects.

Department of Health (1991). *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*. London: H.M. Stationery Office.

Livingstone, M. B. E., Strain, J. J., Prentice, A. M., Coward, W. A., Nevin, G. B., Barker, M. E., Hickey, R. J., McKenna, P. G. & Whitehead, R. G. (1991). *British Journal of Nutrition* **65**, 145–155.

The naturally-occurring dietary flavonoid quercetin: *in vitro* investigation of its effects on glycolytic flux. By S. M. CRONIN, N. M. O'BRIEN and P. A. MORRISSEY, *Department of Nutrition, University College, Cork, Republic of Ireland*

Quercetin, a naturally-occurring mutagen, is a normal constituent of many plant foods. It has been reported to induce mutations, chromosomal aberration, sister chromatid exchanges, DNA strand breaks and cellular transformation in *in vitro* test systems. For the most part, the data pertaining to the carcinogenicity of quercetin are contradictory. A biochemical characteristic of many tumour cells is their high rate of aerobic glycolysis. Many xenobiotics have been shown to stimulate glycolysis. Thus, the ability of certain xenobiotics to promote tumour cell proliferation may be related to this stimulation of glycolysis. The flux through the glycolytic pathway is controlled, in part, at the level of phosphofructokinase (PFK-1; *EC* 2.7.1.11). The most potent endogenous stimulator of PFK-1, one of the key enzymes of glycolysis, is fructose-2, 6-bisphosphate (Fru-2, 6-P_2). Recent experimental evidence indicates that Fru-2, 6-P_2 plays a key role in the high rate of glycolysis observed in tumour cells under aerobic conditions and of normal cells in response to growth factors and tumour promoters (e.g., phorbol esters). The objective of the present study is to investigate and compare the effects on glycolytic flux of the natural flavonoid quercetin and the known toxic xenobiotic phorbol 12-myristate 13-acetate (PMA) in a cell culture model.

Rat kidney cells (NRK-49F) were cultured in an atmosphere of air containing 5% CO_2 at 37° in Dulbecco's Modified Eagles medium. The cells were exposed to either PMA or quercetin. The glycolytic metabolites, fructose-6-phosphate (F6P), glucose-6-phosphate (G6P), Fru-2, 6-P_2 and lactate and the glycolytic enzymes lactate dehydrogenase (LDH; *EC* 1.1.1.27), pyruvate kinase (PK; *EC* 2.7.1.40) and 6-phospho-2-fructokinase (PFK-2; *EC* 2.7.1.105), the enzyme that catalyses the synthesis of Fru-2, 6-P_2, were measured.

Fru-2, 6-P_2 was detectable in these cells where its concentration was approximately 160 pmol/mg protein under steady-state conditions. From dose–response studies it was determined that the maximal effects on the glycolytic flux indicators, lactate and Fru-2, 6-P_2, were obtained at doses of quercetin equal to 29 nM and doses of PMA equal to 16 nM. It was decided, therefore, to use these concentrations of the test chemicals in all subsequent studies. PMA treatment led to the expected increase in Fru-2, 6-P_2 (210 (SE 9) pmol/mg protein) compared with the control value (165 (SE 6) pmol/mg protein) after 5 h incubation. In addition, there was an increase in the metabolite after the addition of quercetin from 165 (SE 15) to 276 (SE 8) pmol/mg protein. Lactate release increased from 2·2 (SE 0·1) to 3·6 (SE 0·1) μmol/mg protein after exposure to PMA, and to 3·0 (SE 0·1) μmol/mg protein after exposure to quercetin. There was no change in the concentration of G6P or F6P, nor in the activities of LDH or PK after 5 h incubation with either PMA or quercetin. In addition, no significant additivity of the effect of PMA together with quercetin was observed. This suggests that both agents share a common pathway in the mechanism by which they stimulate glycolysis. It was found that the activity of PFK-2 was increased 5 h after exposure to 16 nM PMA and after exposure to 29 nM quercetin. The increase in Fru-2, 6-P_2 and PFK-2 brought about by these two agents was prevented when the cells were incubated in the presence of cycloheximide (1 mM).

The overall findings suggest that quercetin stimulates glycolysis in a similar fashion to PMA.

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Weight-for-height influences outcome in childhood leukaemia. By J. J. REILLY[1], I. ODAME[2], J. MCCOLL[3], P. MCALLISTER[3], B. GIBSON[2] and B. A. WHARTON[1], *Departments of* [1]*Human Nutrition*, [2]*Haematology* and [3]*Statistics, University of Glasgow, Yorkhill Hospitals, Glasgow G3 8SJ*

Acute lymphoblastic leukaemia (ALL) is the most common form of childhood cancer affecting approximately 1 in 3000 children with the highest incidence between 2 and 6 years of age. Recent advances in treatment have improved 5-year disease-free survival to approximately 75%, but further improvements are likely to require the identification of prognostic factors which are as yet unknown. The aim of the present study was to test the hypothesis that relative weight is a prognostic factor in childhood ALL. The present study included all cases of childhood ALL treated at the specialist Scottish treatment centre in Glasgow on the fixed UKALL-X treatment protocol (n 66, mean age 4 years 11 months). Weight-for-height was expressed as a standard deviation score (Z score). Clinical outcome was defined as: no relapse to present, or time to first relapse. The influence of the following known prognostic factors on outcome was tested (log ranks test, P values in parentheses): age at diagnosis (P=0·607); gender (P=0·547); risk status (a function of age, gender, leucocyte count at diagnosis and the morphological and immunological phenotype of ALL, P=0·078); randomization to one of four alternative intensification chemotherapy protocols (P=0·963). None of the known prognostic factors had a significant influence on outcome. However, weight-for-height Z score at diagnosis did have a significant influence on probability of relapse (log ranks test, P=0·005). The sample was divided into thirds of the distribution with respect to weight-for-height: group A, Z score $<-0{\cdot}5$; group B, Z score $-0{\cdot}5$ to $+0{\cdot}5$; group C, Z score $>0{\cdot}5$. Incidence of relapse was nine out of nineteen cases in group A (47%) but only six out of forty-seven (13%) in groups B and C. We conclude that weight-for-height does influence outcome in childhood ALL. Children at the lower end of the distribution of weight-for-height are at significantly increased risk of relapse. The mechanism by which weight-for-height influences outcome was not investigated: undernutrition has been shown to impair immune function and haematopoiesis; it has also been suggested that variation in body fatness might influence the pharmaco-kinetics of the agents used in chemotherapy.

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Reported energy intakes and basal metabolic rates of normal healthy children aged 5–15 years. By S. A. BOND and S. A. WOOTTON, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

In establishing the estimated average requirement (EAR) for energy in growing children, the panel on dietary reference values noted the shortage of information on simultaneous measurements of both energy intake and expenditure in children aged 3–10 years and 11–18 years (Department of Health, 1991). The aim of the present study was to characterize both energy intake and the metabolic demand for energy at rest in eighty-five boys and sixty-one girls growing normally (i.e. ±2 SD for height) aged between 5–15 years.

Metabolizable energy intake (EIN) was estimated from 7 d records of weighed food intake. Basal metabolic rate (BMR) was determined by indirect calorimetry (Datex Deltatrac) and predicted from age, gender, weight and height (Schofield *et al.* 1985).

		EIN (MJ/d)		BMR (MJ/d)		% Predicted BMR		EIN:BMR	
Group	*n*	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Boys:									
5–10 years	34	8·14	0·20	5·04	0·13	104	2	1·65	0·05
11–15 years	51	9·92	0·31	7·12	0·13	105	1	1·43*	0·04
Girls:									
5–10 years	38	7·03	0·18	4·47	0·08	104	1	1·60	0·04
11–15 years	23	7·71	0·37	5·53	0·22	100	3	1·45	0·09

* Value is significantly different from boys 5–10 years ($P<0·001$).

The mean energy intakes reported in this study were similar to the EAR for energy for each group (boys 7–10 years 8·24 MJ/d; boys 11–14 years 9·27 MJ/d; girls 7–10 years 7·28 MJ/d; girls 11–14 years 7·92 MJ/d). Although the mean BMR was close to that predicted for each group, the percentage difference between actual and predicted values for individuals ranged from −35% to +26%. The ratios of reported energy intake:BMR (EIN:BMR) for the older children was less than that of the younger children ($P<0·001$). The mean EIN:BMR for the older boys was also less than the physical activity level (PAL) of 1·56×BMR used to set the EAR for boys aged 10–18 years. The older girls reported a mean EIN:BMR which was in closer agreement with the PAL used to set the EAR (1·48×BMR) for girls aged 10–18 years.

These observations suggest that the EIN:BMR ratio may be a useful guide to assess whether the reported energy intake reflects habitual energy intake and energy requirements. The apparently lower reported EIN:BMR ratios seen with the older age group may reflect lowered physical activity or alternatively under-reporting or energy restriction during the period of measurement.

Department of Health (1991). *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Reports on Health and Social Subjects* no. 41. London: H.M. Stationery Office.

Schofield, W. N., Schofield, C. & James, W. P. T. (1985). *Human Nutrition: Clinical Nutrition* **39c**, Suppl. 1, 1–96.

Energy intakes and resting energy expenditure in children of short stature and those receiving growth hormone. By C. M. SMITH, *Department of Nutrition and Dietetics, Southampton General Hospital,* E. S. MCCAUGHEY and P. R. BETTS, *Department of Paediatrics, Southampton General Hospital* and S. A. BOND, S. A. WOOTTON and A. A. JACKSON, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

We have previously shown in studies of short normal children before receiving treatment with growth hormone (rhGH) that, relative to their size, the metabolic demands for energy at rest of short children were greater than those of taller children (Walker *et al.* 1990). The extent to which changes in energy expenditure in short normal children might be constitutive or reflect their immediate past nutritional intake remains unclear. The aim of this study was to determine whether children of short stature aged 9–10 years consumed less food than children of normal stature and whether accelerated growth following rhGH treatment was associated with changes in basal metabolic rate (BMR) and energy intake.

Energy intake (from weighed food intake over 7 d), BMR (by indirect calorimetry; Datex Deltatrac) and fat-free mass (FFM; from skinfold thickness) were determined in (1) short children who were less than −2·0 SD for height (*n* 15), (2) short children who were in their second year of rhGH treatment (Genotropin 30IU/m^2 per week, *n* 15) and (3) children of normal stature matched for age and gender (*n* 16).

	Short		Short treated		Normal	
	Mean	SD	Mean	SD	Mean	SD
Height (SDS)	−2·45^a	0·39	−1·51^b	0·34	0·24^c	0·70
Weight (kg)	21·4^a	3·2	24·4^a	3·0	31·3^b	6·40
FFM (kg)	17·8^a	2·0	21·3^b	2·5	24·6^c	2·9
Energy intake (kJ/kg FFM per d)	353^{ab}	59	387^d	60	319^b	64
BMR (kJ/kg FFM per d)	232^{ab}	29	233^d	19	212^b	23
Energy intake:BMR	1·54	0·31	1·66	0·23	1·50	0·24

Values within the same row with unlike superscript letters were significantly different: $P<0·05$ (ANOVA).
SDS, standard deviation score.

Short untreated children expended less energy in absolute terms than either the short treated or controls When corrected for differences in FFM, both short stature groups exhibited greater BMR values than the control group although only the difference between short treated and normal groups attained statistical significance. A similar pattern was observed for the energy intakes of the three groups. The short treated children exhibited energy intake and BMR values which were comparable to those of the short children despite being more like the normal stature controls in terms of height and weight. Similar energy intake:BMR ratios were observed.

These results show that for short children the apparently increased metabolic demands for energy when BMR is expressed per unit FFM are matched by an increased intake of food, and this demand may be constitutive and not influenced by rhGH treatment.

Walker, J. M., Bond, S. A., Voss, L. D., Betts, P. R., Wootton, S. A. & Jackson, A. A. (1990). *Lancet* **360,** 1331–1334.

Supplementing meals with leaf concentrate in Sri Lankan nursery schools: impact on anthropometric status. By J. GLADWIN[1], S. V. RAJASURIYA[2], P. E. SOYSA[3], D. N. COX[1], J. FRASER[1] and A. ASHWORTH[1], [1]*Centre for Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT,* [2]*Sarvodaya Women's Movement, 32 Rawattewatte Road, Moratuwa, Sri Lanka* and [3]*Faculty of Medicine, University of Colombo, Colombo 8, Sri Lanka*

Sri Lanka has a traditional food known as Kola Kenda (KK) which has leaf juice, rice and coconut as the main ingredients. An enriched form of KK is being promoted in nursery schools in the school-meal programme. This involves processing leaf juice to make a leaf concentrate (LC) which is then substituted for leaf juice in the traditional KK recipe. The impact of LC on the growth and morbidity of children aged 3–5 years is being evaluated. Only the anthropometric data are reported here.

Sixty-four nursery schools were randomly selected in rural and peri-urban areas within 2 h travelling of Colombo. From June 1990, following a baseline period with traditional KK, the children received either: (a) traditional KK (control group), (b) KK enriched with leaf concentrate (LC group), (c) traditional KK and 2·4 g whey protein, equivalent to the protein in LC (protein group) or (d) traditional KK with 60 mg vitamin A 4-monthly (vitamin A group). The rationale for including the protein and vitamin A groups was that, in the event of a beneficial impact of LC, the responses in these groups in which nutrients were given singly might help elucidate the LC response.

Weights and heights of approximately 1800 children were measured twice pre-intervention (rounds 1 and 2, beginning January and April 1990 respectively) and twice post-intervention (rounds 3 and 4, beginning August and November 1990 respectively).

Standardized Z scores were calculated for weight-for-age, height-for-age and weight-for-height. The change in Z score during the baseline period and during the intervention period were calculated and compared using MANOVA for repeated measures.

The children initially were marginally stunted and wasted (mean height-for-age and weight-for-height Z scores at round 1 being −1·07 and −1·44 respectively). The change in Z score was not significantly different among the groups for any of the indices when compared (a) pre-intervention, (b) post-intervention or (c) post-intervention minus pre-intervention.

Changes in Z score pre- and post-intervention

	Weight-for-age		Height-for-age		Weight-for-height	
	Change pre	Change post	Change pre	Change post	Change pre	Change post
Control	–0·029	0·033	0·056	–0·014	–0·099	0·041
LC	–0·034	0·016	0·053	–0·018	–0·100	0·018
Protein	–0·022	0·042	0·025	–0·027	–0·071	0·060
Vitamin A	–0·022	0·016	0·092	–0·045	–0·120	0·041

Change pre, Z score in round 2 minus Z score in round 1; Change post, Z score in round 4 minus Z score in round 3.

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Effect of diet and stage of lactation on milk composition in rats. By A. P. PINE, L. A. EMSLEY, G. F. ALLAN and N. S. JESSOP, *Institute of Energy and Resource Management, The University of Edinburgh, Edinburgh EH9 3JG* and J. D. OLDHAM, *Scottish Agricultural College Edinburgh, West Mains Road, Edinburgh EH9 3JG*

Severe protein undernutrition significantly impairs lactational performance in rats even though females attempt to sustain performance by mobilizing body protein (Pine *et al.* 1992). Although the major determinant of performance is yield, it remains unclear as to whether there are also changes in milk composition.

Forty-eight multiparous Sprague-Dawley rats were mated, caged individually at 22° with a 12 h light–dark cycle and offered a diet high in protein (H; 215 g crude protein (CP)/kg dry matter (DM)) *ad lib.* until parturition. Subsequently, half the females continued to receive diet H whilst the remainder were offered a diet low in protein (L; 90 g CP/kg DM) *ad lib.* Litters were adjusted to twelve pups on day 1 of lactation. On days 2, 4, 8 and 12 of lactation females (*n* 6) from both dietary groups were separated from their litter for the first 2 h of the light cycle, after which they were lightly anaesthetized (diethyl ether) and injected subcutaneously with 5 IU oxytocin. Milk samples (0·5–0·75 ml) were obtained by gently stripping the left thoracic and abdominal teats. Milk was analysed for lactose enzymatically, for protein with Coomassie Blue using casein as a standard, and for lipid gravimetrically following a three times extraction with chloroform-methanol (2:1, v/v).

	Day 2		Day 4		Day 8		Day 12		
Diet . . .	H	L	H	L	H	L	H	L	SED
Lactose (mg/g)	$11·6^{a}$	$10·8^{a}$	$14·0^{a}$	$14·8^{ab}$	$18·6^{b}$	$10·4^{a}$	$26·5^{c}$	$19·7^{b}$	2·0
Protein (mg/g)	$90·5^{ab}$	$79·8^{bc}$	$89·4^{ab}$	$76·4^{c}$	$91·1^{ab}$	$95·4^{a}$	$91·6^{ab}$	$81·2^{bc}$	5·0
Lipid (mg/g)	$162·1^{a}$	$180·2^{a}$	$148·7^{a}$	$182·8^{a}$	$180·2^{a}$	$255·4^{b}$	$177·8^{a}$	$201·2^{a}$	20·2

^{a,b,c} Means with unlike superscripts differed significantly: $P<0·05$.

For group H, stage of lactation influenced milk lactose content (r^2 0·85, $P<0·001$) but not protein or lipid. For group L, lactose remained unchanged until day 8 then rose by day 12 with lactose content being lower than for group H on days 8 and 12. Milk protein tended to be lower in group L than group H although on day 8 showed a transient increase. Milk lipid also rose in group L on day 8 but otherwise was unaffected by diet or stage of lactation.

It is interesting to note that maternal tissue protein reserves will have been substantially depleted by day 6 (Pine *et al.* 1992). It would appear that mobilization of these reserves could not sustain milk protein levels at those of group H and that the most marked changes in milk composition were coincident with their depletion.

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Pine, A. P., Jessop, N. S. & Oldham, J. D. (1992). *Animal Production* (In the Press).

Lactose digestion in breast-fed Gambian infants. By C. A. NORTHROP-CLEWES and P. G. LUNN, *Dunn Nutrition Centre, Milton Road, Cambridge CB4 1XJ*

We have recently demonstrated that a major part of growth faltering in Gambian village infants can be attributed to persistent damage to the mucosa of the small intestine (Lunn *et al.* 1991). Which feature of this enteropathy is responsible for the poor growth of the children remains unclear, but one possibility is that the capacity of the damaged intestinal mucosa to hydrolyse lactose may be compromised. Lactose provides up to 50% of the energy intake of breast-fed infants but is dependent on the enzyme lactase (*EC* 3.2.1.108). This enzyme is located on the tips of the villi and is particularly vulnerable to mucosal damage.

Gambian infants receive about 700 ml breast milk/d (containing 49 g lactose), in eight to twelve breast-feeding episodes. This results in a steady-state intake of about 2 g lactose/h. Normally lactose is cleaved by lactase to glucose and galactose which are immediately absorbed. During lactose maldigestion most intact lactose will pass into the colon where it may cause flatulence and diarrhoea. However, a small proportion of lactose is absorbed from the small bowel by a paracellular route and excreted quantitatively in the urine. This proportion can be measured using lactulose, an isomer of lactose, which is resistant to lactase and is absorbed by the same route. Following an oral dose of lactulose, the urinary lactose:lactulose (L:LL) ratio gives a measure of intestinal lactase activity.

Using this method, serial estimates of lactose maldigestion have been obtained as part of a longitudinal investigation into intestinal status and growth of infants in a rural area of The Gambia. Children (*n* 119), aged between 2–15 months, were seen monthly when anthropometric measurements were recorded and an intestinal permeability test was carried out. As part of this test, infants were given 400 mg lactulose/kg following which urine was collected for 5 h. The urinary L:LL ratios are shown in the Table.

Age (months) . . .	0–3	3–6	6–9	9–12	12–15
L:LL ratio	0·304	0·424	0·633	0·653	0·708
SEM	0·02	0·03	0·05	0·04	0·06
n	183	216	183	172	112

An L:LL ratio of 0·4 represents the upper limit of normal; values higher than this indicate hypolactasia. Lactose digestion showed a progressive deterioration with age ($P<0·001$) and was significantly related to poor growth in both weight and length ($P<0·001$) giving regression coefficients of –0·30 and –0·35 respectively. When corrected for age, more highly significant relationships were obtained between the L:LL ratio and weight growth r –0·402, and the L:LL ratio and length growth r –0·378 ($P<0·0001$). The squares of these coefficients suggest that lactose maldigestion might account for up to 13·6% and 15·4% of growth faltering in length and weight respectively.

Lunn, P. G., Northrop-Clewes, C. A. & Downes, R. M. (1991). *Lancet* **338**, 907–910.

Evidence of energy-sparing adaptations in Gambian women during pregnancy, assessed by whole-body calorimetry. By S. D. POPPITT[1], A. M. PRENTICE[1], E. JEQUIER[2], Y. SCHUTZ[2] and R. G. WHITEHEAD[1], [1]*MRC Dunn Nutrition Unit, Keneba, The Gambia* and *Milton Road, Cambridge CB4 1XJ* and [2]*Department of Physiology, University of Lausanne, Switzerland*

Many women in developing countries have to maintain pregnancy on dietary intakes which may not provide the extra 335 MJ during pregnancy recommended by FAO/WHO/UNU (1985). This abstract addresses the hypothesis that in marginally-nourished women an energy-sparing suppression of metabolism may act via reductions in sleeping energy expenditure (sleeping EE) and dietary-induced thermogenesis (DIT) and an increase in work efficiency, in addition to a suppression of basal metabolic rate (BMR).

Components of daily energy expenditure were measured serially by whole-body 24 h calorimetry in twenty-one Gambian women when non-pregnant, non-lactating (NPNL) and at 6, 12, 18, 24, 30 and 36 weeks of gestation. Weight gain was 6·8 (SD 2·8) kg, fat deposition 2·0 (SD 2·5) kg and growth of lean tissue 5·0 (SD 2·5) kg. BMR was depressed during the first 18 weeks of gestation and the total cumulative maintenance cost of pregnancy was only 8·4 MJ. Individual responses to pregnancy correlated with changes in body mass (36 weeks: $\triangle$ BMR *v.* $\triangle$ weight; r 0·60, $P<0·01$: $\triangle$ BMR *v.* $\triangle$ lean body mass (LBM); r 0·62, $P<0·01$). There was no significant increase in (1) the cost of weight-dependent treadmill exercise (0% slope; F 0·71, $P=0·64$: 5% slope; F 1·97, $P=0·10$), (2) 24 h energy expenditure (24 h EE; F 0·72, $P=0·64$) or (3) activity and DIT, (F 1·02, $P=0·43$, computed as 24 h EE − (BMR + exercise)) during pregnancy.

Changes in energy expenditure during pregnancy, compared with NPNL

Stage of gestation (weeks) . . .	*n*	6	12	18	24	30	36
BMR (MJ/d)	21	−0·27	−0·18	0·01	0·08	0·20	0·46
Exercise (kJ/30 min): (0% slope)	9	−4·33	−20·9	−12·0	−30·5	−16·8	−20·7
(5% slope)	9	−20·4	−34·0	4·40	−33·6	−37·7	−6·8
24 h EE (MJ/d)	9	−0·34	−0·28	−0·16	−0·25	−0·04	0·33
Activity: DIT	9	−0·12	−0·11	−0·11	−0·27	−0·18	0·06

Total metabolic costs over 36 weeks were 143·7 MJ, which comprised the fetus (estimated as 43 MJ), the deposition of maternal fat (92 MJ) and the cumulative maintenance costs (8·7 MJ). These were far lower than in well-nourished Western populations where studies in England (Prentice *et al.* 1989), Scotland (Durnin *et al.* 1987), the Netherlands (van Raaij *et al.* 1987) and Sweden (Forsum *et al.* 1988) have reported total costs of pregnancy to be 334 MJ, 281 MJ, 286 MJ and 489 MJ respectively.

This study was supported by the Nestlé Foundation.

Durnin, J. V. G. A., McKillop, F. M., Grant, S. & Fitzgerald, G. (1987). *Lancet* **ii,** 897–900.

Food and Agriculture Organization/World Health Organization/United Nations University Expert Consultation (1985). WHO Technical Report Series 724, pp. 1–206. Geneva: WHO.

Forsum, E., Sadurskis, A. & Wager, J. (1988). *American Journal of Clinical Nutrition* **47,** 942–947.

Prentice, A. M., Goldberg, G. R., Davies, H. L., Murgatroyd, P. R. & Scott, W. (1989). *British Journal of Nutrition* **62,** 5–22.

van Raaij, J. M. A., Vermaat-Miedma, S. H., Schonk, C. M., Peck, M. E. M. & Hautvast, J. G. A. J. (1987). *Lancet* **ii,** 953–955.

Iron deficiency in Asian toddlers and the failure of progression of the weaning process. By M. B. DUGGAN and L. HARBOTTLE, *Department of Paediatrics and Centre for Human Nutrition, University of Sheffield, Sheffield S5 7AU*

Fe deficiency in British Asian children had been linked to delayed introduction of solids, i.e. a delayed start to the process of weaning from a milk-based infant diet to adult food. The observation of Fe deficiency in older Asian toddlers was explained by avoidance of commercial baby foods containing meat which had not been slaughtered according to Islamic ritual. More detailed analysis of the composition of the weaning diet of Asian toddlers with and without biochemical Fe deficiency suggests that neither explanation is satisfactory.

Combined data on the weighed dietary intake and the nutritional and biochemical Fe status had been collected on ninety-six Asian toddlers living in Sheffield (Duggan *et al.* 1991). There was considerable variation in Fe status. Fe deficiency was defined by the following criteria: serum ferritin <10·0 μg/l; haemoglobin <11·0 g/dl; mean corpuscular volume <70 fl; mean corpuscular haemoglobin <27 pg/l; and zinc protoporphyrin >80 μmol/per mol haemoglobin. Twelve children all aged >12 months fulfilled the criteria for Fe deficiency and (excluding infants) twenty children for normal Fe status. The dietary characteristics of these two groups of children, briefly reported previously (Duggan, 1991), have been examined in detail, with special attention to the sources of dietary energy and Fe.

	Fe deficient		Normal Fe status	
	Mean	SD	Mean	SD
Age (months)	19·5	6·3	20·6	6·4
Wt (kg)	11·48	2·02	10·49	1·83
Energy (kJ/d)	3470	739	3706	1308
Total milk (kJ/d)	2095	829	1725	811
Baby food* (kJ/d)	136	296	303	553·7
Family food† (kJ/d)	1238	760·7	1689	1411·25
Fe (mg/d)	2·82	1·49	4·14	2·42
Fe (%): from milk	14·1		18·8	
from cereals	38·7		38·3	

* Baby foods are foods manufactured commercially, specifically for babies; † family foods are those prepared for consumption by all family members.

The following differences were significant ($P<0·02$): in Fe-deficient children dietary energy in kJ/kg per d was lower, the % total energy due to cow's milk and the % energy due to commercial sweets and puddings was higher. However, in both groups the contribution to total energy from both family foods and commercial baby foods was lower than had been anticipated. It appears that, despite an acceptably early start to weaning, the process is protracted with prolonged reliance on milk. This results in a paradoxically high contribution of milk, a poor vehicle for Fe, to the total Fe intake. Dietary advice should be amended to encourage more rapid progression of the weaning process.

The study was supported by the Health Promotion Research Trust.

Duggan, M. B. (1991). *Proceedings of the Nutrition Society* **50,** 235A.
Duggan, M. B., Steel, G., Elwisa, J., Harbottle, L. & Noble, C. (1991). *Archives of Disease in Childhood* **66,** 1386–1389.

Perirenal adipose tissue development in artificially- and ewe-reared lambs over the first month of life. By C. J. DARBY, L. CLARKE, M. A. LOMAX and M. E. SYMONDS, *Department of Biochemistry and Physiology, University of Reading, Whiteknights, PO Box 228, Reading RG6 2AJ*

The control of thermoregulation during neonatal development is significantly influenced by the rate at which brown adipose tissue (BAT) adopts the characteristics of white adipose tissue (Symonds *et al.* 1992), a process that can be delayed by cold-rearing lambs (Darby *et al.* 1992). The present study investigates the extent to which perirenal adipose tissue development can be influenced by the level and type of nutrient intake.

Fifteen new-born lambs (mean (SEM) birth weight 5·22 (0·55) kg) were entered into this study and were either reared artificially (AR; *n* 7) on a 2 l volume of milk containing 200 g milk replacer (VOLAC LAMLAC, Royston, Herts) at an ambient temperature of 10–15°, or reared with the ewe (ER; *n* 8), having unrestricted access to their mother's milk at an ambient temperature of 5–20°. Perirenal adipose tissue was sampled at 30 d of age and analysed as described by Symonds *et al.* (1992).

Analysis of perirenal adipose tissue

	Wt (g)		Lipid content (g)		Protein content (mg)		Mitochondrial protein (mg)		GDP-binding (pmol/mg protein)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
AR	10·2***	1·0	1·8***	0·4	708	98	277*	114	61***	14
ER	156·9	18·1	87·2	15·5	1333	294	889	255	5	1

Significantly different from ewe-reared: *$P<0·05$; ***$P<0·001$.

ER lambs weighed 53% more at 30 d of age (ER 15·50 (SEM 0·80), AR 10.20 (SEM 0·25) kg; $P<0·001$) and possessed fifteen times more perirenal adipose tissue which contained forty-three times more lipid than AR lambs. This tissue also contained three times the level of mitochondrial protein, but in the ER group very little GDP-binding was found, indicating the virtual absence of uncoupling protein. In contrast, the level of GDP-binding to mitochondrial uncoupling protein was twelve times greater in AR lambs, demonstrating it still possessed some characteristics of BAT.

It is concluded that when lambs are ewe-reared and have unlimited access to feed this is associated with an enhancement of the rate at which BAT adopts the characteristics of white adipose tissue.

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Darby, C. J., Clarke, L., Lomax, M. A. & Symonds, M. E. (1992). *Proceedings of the Nutrition Society* **51,** 82A.

Symonds, M. E., Bryant, M. J., Clarke, L., Darby, C. J. & Lomax, M. A. (1992). *Journal of Physiology* **455,** 487–502.

Nitrogen metabolism across the liver of growing steers fed a high nitrogen grass pellet diet.

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Forage diets fed to growing cattle result in a high rumen ammonia production, which may be responsible for the lower growth rates seen on these diets, due to an increase in amino acid (AA) deamination to urea, (Fitch *et al.* 1989; Reynolds *et al.* 1991). The present study endeavours to construct a N balance of ammonia AA and urea across the liver of cattle fed high N grass pellets, and examines the effect of supplementing the diet with urea to increase rumen ammonia production.

Four Friesian steers (live weight (LW) 240 (SD 3) kg) were surgically implanted with catheters to measure net hepatic metabolism (Reynolds *et al.* 1991) and fed on a diet of 27 g DM/kg LW per d high protein grass pellets (G; 31·5 gN/kg) at hourly intervals. Urea (U) was mixed with grass pellets at 2·14 g urea/kg$^{0.75}$ per d in a cross-over design. Samples were taken during 4 h of para-amino hippuric acid infusion in order to measure blood flow.

The Table shows ammonia, urea, and amino acid N (AAN) fluxes (mmol/min), across the liver.

		G	GU	SED
Ammonia-N	Hepatic uptake	2·13	3·61	1·56
Total AAN	Hepatic uptake	6·21	4·62	1·61
Urea-N	Hepatic production	3·77	5·69	2·56
Hepatic N balance		4·58	2·54	1·00

Addition of urea to the diet resulted in a significant ($P<0·05$) increase in the plasma concentrations of ammonia and urea in the portal vein and urea in the artery. Hepatic uptake of ammonia and production of urea-N were both increased when urea was fed, but these differences were not significant due to large variations in blood flow rates. Ammonia-N uptake overall accounted for 60% of the urea-N production, indicating that the remainder of the N for urea production comes from another source, probably AA. AAN uptake by the liver was more than sufficient to account for the extra N required and a greater proportion of the hepatic AAN was required to balance urea-N output during urea supplementation, (G, 26%; GU, 45%).

It is concluded that in cattle fed on high-N grass pellets the hepatic uptake of AAN is substantial relative to urea-N flux and may provide an explanation for the lower levels of protein retention observed in cattle fed on high forage diets.

The support of the AFRC is gratefully acknowledged.

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

Reynolds, C. K., Tyrrell, H. F. & Reynolds, P. J. (1991). *Journal of Nutrition* **121,** 1004–1015.

The place of nutrition in the general practice consultation: a pilot study. By M. K. PATEL, D. H. RADIA, S. KEIR, A. GORAYA and J. POWELL-TUCK, *Department of Human Nutrition, The London Hospital Medical College, Turner Street, Whitechapel, London E1 2AD*

A pilot study has been performed to begin to gain insight into how often General Practitioners (GP) or their patients raise nutrition-related subjects during consultations. Four medical students attending three practices kept records during 519 GP consultations. For each consultation the problem for which the patient was attending was recorded and whether or not it was directly nutrition-related. Secondary nutritional problems were also noted and records made as to whether discussion of diet was doctor- or patient-initiated. Nutrition or diet were specifically discussed on fifty-five occasions (10·6% consultations) of which thirty-nine were doctor-led and sixteen patient-led. During the fifty-five consultations, sixty-five nutrition-related topics were discussed of which hypertension (12), constipation (8), obesity (6) and hyperlipidaemia (5) were the most common, with separate records for angina (4) and oesophagitis (4) closely following. The age group at which nutrition was most commonly discussed was 41–65 years (15·6% of 140 consultations), while it was surprisingly seldom discussed with patients bringing children aged 0–5 years (3·4% of 88 consultations). These figures underestimate the frequency at which nutrition is raised in general practice because they exclude those patients attending special dietetic, health promotion and diabetic clinics organized in the practices; nor can the practices be regarded as being necessarily representative. Nevertheless, the data strengthen the argument that nutrition training in medical schools is under-represented, at least as far as those destined to enter general practice are concerned, where at least 10% of patients may need nutritional advice.

Does chronic undernutrition in adult Masai influence morbidity and survival? By M. J. MURRAY, A. B. MURRAY and N. J. MURRAY, *Department of Medicine, University of Minnesota, Minneapolis, Minnesota, 55455, USA*

Much attention has been paid to the mortality and morbidity of chronically-undernourished children but little to the effects of chronic undernutrition on the health and survival of the parents who must provide for the children. We have studied the impact of chronic undernutrition over 12 years on health and survival of 403 Masai parents aged 18–35 years and compared the results with those observed in 386 normally-nourished African parents of similar age and sex distribution. All were exposed to the same hazards and diseases. None received anti-malarial prophylaxis or preventive inoculations. Normally-nourished Africans lived nearer market towns and had easier access to food. We chose a body mass index (BMI) of <18·6 for men and <16·8 for women to represent undernutrition. Each was examined initially for clinical and laboratory evidence of overt and occult disease and those with disease were excluded (6% of the undernourished and 14% of the nourished) from the study. All were re-examined at 4, 8 and 12 years and a history obtained of health events and times of incapacity in the intervening years.

	Undernourished (*n* 403)		Nourished (*n* 386)		
Male:female ratio . . .	0·93		0·91		*P* value
	Mean	SD	Mean	SD	NS
Age (years)	27·4	2·13	28·1	1·97	NS
Initial BMI: Male	18·34	0·13	20·48	0·16	<0·001
Female	16·49	0·09	18·13	0·14	<0·001
Final BMI: Male	18·71	0·15	20·39	0·12	<0·001
Female	16·81	0·11	18·18	0·11	<0·001
	n	%	*n*	%	
Deaths	0	0	5†	1·29	NS
Malarial attacks/year	11*	2·73	43*	11·13	<0·001
Other infections and parasites/year	17	4·22	36	9·33	<0·01
Malignancy/year	0	0	5	1·29	NS
Major trauma/year	12	2·97	15	3·88	NS
Miscellaneous/year	6	1·44	5	1·29	NS
Mean days of incapacity per person per year	0·27		0·65		<0·001

* None used nets; † automobile accidents.

Chronic undernutrition had no adverse effect on mortality and morbidity. Infections, especially malaria, were significantly less in the undernourished and the mean annual time of incapacity per individual was also reduced. Chronic undernutrition in those adults living intimately with their environment may be an advantage for survival and reduced morbidity.

Anthropometric measurements in elderly orthopaedic patients: 6-month follow-up of high- and low-nutritional risk groups. By M. LUMBERS, *NESCOT, Ewell, Surrey* and L. DRIVER, R. J. H. HOWLAND, M. J. OLDER and C. M. WILLIAMS, *The Nutritional Metabolism Research Group, University of Surrey, Guildford GU2 5XH*

Sixty female elderly orthopaedic patients (elective and emergency) were categorized into high- and low-nutritional risk groups according to whether they had three or more of the following objective measurements below the 5th percentile on admission to hospital: (1) body-weight (BW), (2) triceps skinfold thickness (TSF), (3) mid-upper arm muscle circumference (MUAMC), (4) serum albumin and (5) haemoglobin (Lumbers *et al.* 1992). Patients were visited at intervals (4, 8 and 24 weeks after discharge) and repeat anthropometric measurements (BW, TSF, MUAMC) and assessments of mobility and activities of daily living were carried out. Changes in anthropometric measurements from admission to each visit were calculated and analysed for all patients (high-risk (HRA) and low-risk (LRA)) and for patients admitted for emergency surgery only (high-risk (HRE) and low-risk (LRE)).

Median values with 25th and 75th percentiles

Group . . .	HRA (*n* 14)		LRA (*n* 46)		HRE (*n* 13)		LRE (*n* 19)	
	Median	(25th:75th percentiles)	Median	(25th:75th percentiles)	Median	(25th:75th percentiles)	Median	(25th:75th percentiles)
Admission measurements for:								
Age (years)	84·5	(79·3:90·3)	77·0*	(70·0:82·0)	86·0	(81·5:90·5)	80·0†	(75·0:85·0)
BW (kg)	43·7	(38·1:47·9)	63·5***	(58·3:70·9)	41·5	(37·8:47·5)	61·4††	(54·6:68·6)
Change from admission to 24 weeks:								
BW (kg)	0·4	(−1·5:3·5)	1·3	(−1·6:2·2)	0·0	(−2·2:4·3)	−0·8	(−1·8:1·8)
TSF (mm)	−1·5	(−1·9:0·6)	2·4**	(−0·2:3·8)	−1·4	(−1·9:−0·4)	2·4†	(0·0:2·7)
MUAMC (cm)	−0·1	(−1·3:0·5)	−1·8*	(−2·8:−0·9)	0·0	(−2·0:0·4)	−1·4†	(−2·7:−1·0)

Significantly different from HRA: *$P<0·01$, **$P<0·001$, ***$P<0·0001$.
Significantly different from HRE: †$P<0·05$, ††$P<0·0002$.

In both high-risk groups there were significant losses in TSF from admission to 24 weeks post-discharge; over the same period the low-risk groups showed significant gains in TSF. High-risk patients showed no significant changes in MUAMC over the period of measurement whereas there were significant losses in this measurement in both low-risk groups. These data suggest that body fat loss is more marked, but muscle mass is conserved, in patients assessed as at nutritional risk. It is speculated that the differences found could reflect long-term adaptation to inadequate nutrition in the high-risk group. Approximately half of the high-risk patients were still using frames, and only 27% of the HRA group and 20% of the HRE group were using no aid at all, at 6 months after surgery. These differences in walking aids used (sticks *v.* frames) and their duration of use could also influence arm anthropometry in this clinical group.

Lumbers, M., Driver, L., Howland, R. J. H. & Williams, C. M. (1992). *Proceedings of the Nutrition Society* **51,** 131A.

Factors associated with undernutrition in pre-school Tanzanian children. By A. M. GRAY, J. EATON-EVANS, P. G. MCKENNA and J. J. STRAIN, *Human Nutrition Research Group, University of Ulster at Coleraine BT52 1SA*, N. C. ROLLINS, *Department of Child Health, The Queen's University, Belfast BT12 6BJ* and P. KATEGILE, T. MOSHA and K. MTEBE, *Sokoine University of Agriculture, Morogoro, PO Box 3006, Tanzania*

It is important to identify the socio-economic factors and feeding practices of families with undernourished children in order to plan appropriate nutritional intervention programmes. In the present study, the nutritional status of all 730 children aged 2–7 years was assessed in three villages in the Morogoro region of Tanzania during January to March 1991 (immediately before the rainy season). Children were considered as undernourished if (1) height-for-age (HA) or weight-for-age (WA) or weight-for-height (WH) were less than the 3rd centile of the reference values (World Health Organization, 1983), or (2) blood haemoglobin (Hb) was less than 110 g/l, (3) packed cell volume (PCV) was less than 34%, (4) signs of vitamin A deficiency were found by conjunctival impression cytology (Amedee-Manesme *et al.* 1988), or (5) goitre was detected (Perez *et al.* 1960). A questionnaire was used to obtain information on the children's past medical history, infant feeding and family socio-economic background.

Results showed that 52·9% of children were stunted (low HA), 41·3% were underweight (low WA), 5·2% were wasted (low WH), 28·1% had signs of vitamin A deficiency, 29·2% had goitre, 10·7% had low Hb and 14·7% had low PCV. For all variables the sample size was 730 except for Hb (n 609) and PCV (n 416).

Stepwise multiple regression analysis suggested that for the study population there were positive associations between HA and family land-holding, children's age, reported presence of pyrexia and presence of goitre:

$$\text{HA} = 2{\cdot}49 + 0{\cdot}91 \text{ land } (P<0{\cdot}001) + 0.10 \text{ age } (P<0{\cdot}01) + 5{\cdot}52 \text{ pyrexia } (P<0{\cdot}05) + 3{\cdot}13 \text{ goitre } (P<0{\cdot}05)$$

where land is the number of acres owned per family; age is the age of child in months; pyrexia is reported presence of pyrexia; and goitre is the presence of goitre. The following factors did not reach significance: signs of vitamin A deficiency; number of children per family; number of live births per family; number of child deaths per family; age of weaning; age at which breast feeding stopped; reported presence of diarrhoea, upper respiratory tract infections and conjunctivitis.

Between villages, the incidence of children with symptoms of undernutrition and the socio-economic status of children with low HA differed from that of the whole group even though the three villages were ethnically homogeneous. The villages also differed in land-holding, crop and animal husbandry, other employment opportunities, water supply and health care facilities. It is suggested, therefore, that any nutrition intervention programme in this area should target the particular problems of individual villages.

Amedee-Manesme, O., Luzeau, R., Wittenpen, J. R., Hanck, A. & Sommer, A. (1988). *American Journal of Clinical Nutrition* **47**, 875–885.

Perez, C., Schrimshaw, N. S. & Munoz, J. A. (1960). *Endemic Goitre*, WHO Monograph Series no. 44, pp. 369–384. Geneva: WHO.

World Health Organization (1983). *Measuring Change in Nutritional Status. Guidelines for Assessing the Nutritional Impact of Supplementary Feeding Programmes for Vulnerable Groups*. Geneva: WHO.

Nutrient intakes in a group of self-reported milk allergy subjects. By M. MCGOWAN and M. J. GIBNEY, *Division of Nutritional Sciences, Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, Dublin 8, Republic of Ireland*

Once a diagnosis of food allergy has been established the only proven form of therapy is strict avoidance of the offending food. This may prove difficult if there is widespread distribution of the food or if multiple foods are avoided. Nutritional problems may occur if the food(s) avoided are important sources of nutrients or if foods are avoided inappropriately due to misdiagnosis. The aim of the present study was to assess the dietary adequacy of a group of self-reported milk allergy subjects. Subjects were recruited through media advertisements. Questionnaire information was collected on 323 respondents with respect to the foods implicated, symptoms experienced and method and source of diagnosis of food allergy. A group of thirty-eight milk allergy subjects (eight male, thirty female) were selected for detailed study. Dietary assessments, using a 7 d dietary history method, with a photographic food atlas to estimate portion sizes, were carried out on this subgroup and on age-, sex- and occupation-matched controls.

The results of the dietary assessments revealed that the milk allergy group had significantly higher intakes of fibre, vitamin C, vitamin E, Fe, total folic acid and carotene and a significantly lower intake of Ca in comparison with controls ($P<0{\cdot}05$). There were no differences in protein, fat or carbohydrate intakes. One-third of the milk allergy group took Ca supplements.

Mean daily nutrient intakes are given in the Table.

	Subjects (*n* 38)		Controls (*n* 38)	
	Mean	SD	Mean	SD
Energy (MJ)	8·43	2·77	8·66	2·61
Fibre (g)	31	12	23*	8
Vitamin C (mg)	135	79	94*	69
Vitamin E (mg)	8·5	5	5·1*	2·2
Fe (mg)	14·8	6·5	12*	3·6
Total folic acid (μg)	297	113	238*	96
β-Carotene (μg)	7942	8169	4486*	3773
Ca (mg) in diet	568	244	832*	344
Ca (mg) dietary and supplemental	698	321	833	245

*$P<0{\cdot}05$.

The milk allergy group, thus, had higher intakes of many nutrients and lower intakes of Ca in comparison to controls. The milk allergy group had higher intakes of fruit, vegetables, whole grain cereals and fresh meat.

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Dietary knowledge and practice of nutritional supplement users. By M. KEARNEY and M. J. GIBNEY, *Division of Nutritional Sciences, Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, Dublin 8, Republic of Ireland*

From a sample of 175 nutritional supplement users, recruited in Health Food shops around Dublin and Greystones, forty healthy subjects aged 22–66 (mean 38) years were selected (thirty-four female, six male), who used on average more than 1·5 types of supplement per day. These were matched with forty controls for age, sex and education. Twenty-five per cent of each group smoked. Nutrient intakes of both groups were measured using a 7 d dietary history method, with the aid of a photographic food atlas. Nutritional knowledge was assessed using an interview assisted questionnaire (twenty-seven questions). Perceived knowledge (total number of questions −don't knows/total number of questions), accuracy of knowledge (number of correct responses/total number of questions −don't knows) and correct knowledge (perceived × accuracy) were calculated for both groups.

There were no significant differences between subjects and controls (mean (SD)) in intake of energy (8·06 (3·41) *v.* 7·78 (2·23) MJ/d); % protein (15·4 (3·2) *v.* 15·9 (2·4/d)); % fat (39·3 (8·0) *v.* 38·6 (5·7)/d); % carbohydrate (42·2 (7·4) *v.* 43·2 (6·2)/d) and % alcohol (5·0 (5·9) *v.* 3·0 (2·9)/d). Data below are exclusive of nutrient supplements.

	Subjects (*n* 40)		Controls (*n* 40)		
	Mean	SD	Mean	SD	*P* value
Fibre (g/d)	24·3	7·9	19·7	7·2	0·008
Vitamin C (mg/d)	101	49	73	32	0·003
Vitamin D (μg/d)	4·5	5·5	2·5	1·5	0·020
Vitamin E (mg/d)	8·5	5·4	4·8	6·4	0·000
β-Carotene (μg/d)	5327	3946	3368	2097	0·007
Total folate (μg/d)	267	76	214	87	0·005

There were no significant differences between the subjects and controls (mean (SD)) in perceived knowledge % (86 (11) *v.* 88 (8)); accuracy of knowledge % (73 (8) *v.* 74 (8)) and correct knowledge % (63 (11) *v.* 64 (10)).

These results show that regular users of supplements have a similar level of nutritional knowledge compared with controls. However, their choice of foods leads to an increased intake of many key nutrients independent of their intake of supplements.

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Comparison of energy, ascorbic acid and fibre estimates by a food-frequency questionnaire and a 10 d weighed record in smokers. By R. L. THOMPSON[1], B. M. MARGETTS[2] and D. A. WOOD[1], [1]*Preventive Cardiology, (Medicine I), University of Southampton, Royal South Hants Hospital, Southampton SO9 4PE* and [2]*Institute of Human Nutrition, University of Southampton, Southampton SO9 3TU*

We have used a food-frequency questionnaire (FFQ) developed by the Medical Research Council Unit in Cardiff in a study of dietary change consequent upon giving up smoking. We assessed the relative validity of the FFQ by comparison with a 10 d weighed record (WR) in 122 men and 179 women cigarette smokers aged 40–59 years, living in Southampton.

Method . . .	WR		FFQ				Spearman R	
Nutrient	Mean	SE	Mean	SE	Bias*	SE	U	A
			Men					
Energy (MJ/d)	10·1	0·2	9·7	0·2	−0·4	0·2	0·38	—
Ascorbic acid (mg/d)	57·8	3·0	62·0	2·4	4·2	2·9	0·51	0·54
Fibre (g/d)	19·1	0·6	21·1	0·6	2·0	0·6	0·49	0·64
			Women					
Energy (MJ/d)	7·0	0·1	7·2	0·1	−0·2	0·2	0·36	—
Ascorbic acid (mg/d)	54·9	2·5	68·3	2·3	13·5	2·4	0·52	0·54
Fibre (g/d)	16·0	0·4	20·4	0·6	4·5	0·5	0·56	0·63

Spearman R, rank order correlation coefficient (FFQ *v.* WR; $P<0·001$ for all results shown).
U, Unadjusted; A, energy adjusted (Willett & Stampfer, 1986).
* Mean daily difference FFQ–WR (Bland & Altman, 1986).

The Table shows that the mean difference (bias) between the two methods was small for energy but larger for ascorbic acid and fibre, particularly in women. Spearman rank order correlation coefficients were statistically significant for all nutrients, and adjusting for energy intake increased the correlation. The main source of these differences was over-reporting frequency of consumption of selected food items: fruit for ascorbic acid; and fruit, baked beans, peas, bread and high-fibre cereals for fibre. Using the Bland & Altman (1986) method the difference between the dietary methods was plotted against their mean for each subject. Bias for energy was small and there appeared to be a trend suggesting that as intake increased the mean difference also increased. The same was true for fibre in women and ascorbic acid in men. For ascorbic acid in women and fibre in men, even though the bias was large, it appeared to be consistent across the range of intakes. These results suggest that for these nutrients the FFQ may be useful for dietary assessment of groups but caution should be taken in the assessment of individuals.

Bland, J. M. & Altman, D. G. (1986). *Lancet* **i**, 307–310.
Willett, W. C. & Stampfer, M. J. (1986). *American Journal of Epidemiology* **124**, 17–27.

Cigarette smoking and intake of antioxidant vitamins: a dose-type response effect. By C. BOLTON-SMITH, M. WOODWARD (Honorary Research Fellow), C. A. BROWN and H. TUNSTALL-PEDOE, *Cardiovascular Epidemiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY*

Cigarette smokers are at higher risk of heart disease, lung cancer and poorer general health than non-smokers. Smokers inhale a high number of free radicals in cigarette smoke and have both an increased requirement for, and a generally lower intake of, antioxidant vitamins (Bolton-Smith *et al.* 1991*a*). Since heavy smokers are at higher risk of disease than light smokers, it was investigated whether a dose–response effect exists between antioxidant vitamin intake (vitamin C, carotene and vitamin E) and the number of cigarettes smoked per d (Cigs/d) in cross-sectional data from 1874 men and 1818 women (aged 40–59 years) in the Scottish Heart Health Study.

Diet was assessed by food-frequency questionnaire (Bolton-Smith *et al.* 1991*b*) and cigarette number by self-reported consumption, which was confirmed from serum cotinine and thiocyanate concentrations and expired-air carbon monoxide (Woodward *et al.* 1991). Vitamins were adjusted for total energy intake (inclusive of energy from alcohol) expressed as nutrient densities (ND), amounts/4·18 MJ. The results were very similar when alcohol was excluded. No relationship was found between smoking and retinol intake.

		Men				Women			
Cigs/d . . .		<14	15–24	25–34	≥35	<14	15–24	25–34	≥35
n . . .		421	872	381	200	580	987	215	36
Vitamin C (mg ND):	Mean	22	20	19	17***†	29	25	24	19***†
	SD	10	9	8	8	17	15	13	10
Vitamin E (mg ND):	Mean	2·8	2·7	2·5	2·2***†	3·5	3·1	3·0	2·6*†
	SD	2·0	1·8	1·4	1·6	2·5	2·4	2·1	1·1
Carotene (mg ND):	Mean	1·33	1·16	1·13	0·92***†	1·89	1·72	1·60	1·18*†
	SD	1·05	0·93	0·89	0·75	1·68	1·44	1·35	0·91
Energy (MJ):	Mean	9·85	10·55	10·88	11·37***†	7·48	7·51	8·10	8·88***†
	SD	2·48	2·64	2·72	2·95	2·21	2·10	2·05	3·49

Significant difference between groups by analysis of covariance (on the appropriately transformed data) adjusted for age and social class (occupation): *$P<0·05$, ***$P<0·001$.

Significant linear effect: †$P<0·01$.

Clear dose–response effects are observed for each antioxidant vitamin and for both sexes. Although these data are cross-sectional it seems possible that a lower antioxidant vitamin status in heavy smokers compared with light smokers could be contributing to their greater risk of disease.

Bolton-Smith, C., Casey, C. E., Gey, K. F., Smith, W. C. S. & Tunstall-Pedoe, H. (1991*a*). *British Journal of Nutrition* **65**, 337–345.

Bolton-Smith, C., Smith, W. C. S., Woodward, M. & Tunstall-Pedoe, H. (1991*b*). *British Journal of Nutrition* **65**, 321–335.

Woodward, M., Tunstall-Pedoe, H., Smith, W. C. S. & Tavendale, R. (1991). *Journal of Clinical Epidemiology* **44**, 1405–1410.