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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Fifty-fifth Meeting of the Nutrition Society for the presentation of oral communications was held as parallel sessions in Lecture Theatres 2 and 4 of the Medical and Biological Sciences Building, Southampton University, on Thursday and Friday, 8/9 September 1988.

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Nutrient intakes of high- and low-sugar consumers during pregnancy. By WENDY DOYLE[1], MARGARET SANDERSON[2] and A. H. A. WYNN[1], [1]*Nuffield Laboratories of Comparative Medicine, Institute of Zoology, London NW1 4RY* and [2]*Department of Applied Chemistry and Life Sciences, Polytechnic of North London, London N7 8DB*

Four hundred and nineteen pregnant women of mixed ethnic origin living in Hackney, East London, recorded their food intakes for seven consecutive days using household measurements. The data were analysed using the Nuffield Laboratories of Comparative Medicine database and food tables (Paul & Southgate, 1978). 39% were from social class manual, unskilled and 28% were unemployed.

Distribution of nutritional data are generally skewed towards the higher values so that the mean values are larger than medians. Medians are therefore used as being more representative of a group. The added sugar intake ranged from 9·6 to 199 g/d, with a median intake of 50·8 g/d. The low- and high-sugar consumers were defined as those whose intake fell within the lowest quartile (35·3 g/d) and the highest quartile (70·2 g/d) respectively.

The median nutrient intakes were compared per total energy intake and per 4184 kJ (1000 kcal). Using the Mann-Whitney U test, there were few significant differences ($P<0·001$) in the median intake of micronutrients between the high- and low- added sugar consumers, based on the total energy content of the diet. There were, however, significant differences ($P<0·001$) on a nutrient density basis, in starch, fibre, protein, total fat, polyunsaturated fats, ω3 fats and cholesterol as well as most micronutrients.

Median vitamin and mineral intakes of pregnant women (/4184 kJ (/1000 kcal) intake) selected for significance of difference between low- and high-sugar consumers at P<0·001 using Mann-Whitney U test

Nutrient	Low sugar (<35 g/d) (*n* 104)	High sugar (>70 g/d) (*n* 107)	Nutrient	Low sugar (<35 g/d) (*n* 104)	High sugar (>70 g/d) (*n* 107)
Sodium (mg)	1410	1237	Potassium (mg)	1604	1391
Calcium (mg)	483	418	Magnesium (mg)	148	118
Phosphorus (mg)	713	573	Iron (mg)	6·5	5·4
Copper (mg)	0·84	0·68	Zinc (mg)	5·47	4·36
Chloride (mg)	2207	1928	Carotene (μg)	1066	844
Thiamin (mg)	0·65	0·51	Riboflavin (mg)	0·98	0·80
Niacin (mg)	8·59	6·71	Vitamin C (mg)	49·5	35·7
Vitamin E (mg)	3·85	3·10	Pyridoxine (mg)	0·78	0·65
Vitamin B_{12} (μg)	2·52	1·81	Pantothenic acid (mg)	2·40	1·86
Sulphur (mg)	369	288	Folic acid (μg)	117	87·9
Biotin (μg)	14·0	10·9			

These results show that a high-sugar intake has a nutrient dilutant effect on many nutrients. The nutrient densities reduced in this way include those considered to be important during pregnancy (Crawford *et al.* 1986).

Crawford, M. A., Doyle, W., Craft, I. L. & Laurance, B. M. (1986). *Progress in Lipid Research* **25**, 249–254.

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

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Effects on volatile fatty acid production and gut epithelial proliferation of adding haricot beans to a wholemeal bread diet. By FIONA B. KEY and J. C. MATHERS, *Department of Agricultural Biochemistry and Nutrition, The University, Newcastle upon Tyne NE1 7RU*

Sakata (1987) infused volatile fatty acids (VFA) into the ileum of rats, observed increased epithelial proliferation along the gut and suggested that VFA are physiological lumen trophic factors mediated by a systemic mechanism. We have stimulated large bowel (LB) VFA production by giving haricot beans (*Phaseolus vulgaris*) to rats and investigated the effects on epithelial renewal.

Four groups of six male Wistar rats (initial weight 249 g) were housed individually in metabolism cages and offered 20 g/d of diets containing 500 g freeze-dried wholemeal bread, 2 g Cr_2O_3 and 0, 150, 300 or 450 g freeze-dried, cooked haricot beans/kg. After 21 d, rats were injected peritoneally with the metaphase arrest agent vincristine sulphate (1 mg/kg body-weight) and killed 2 h later. Samples were taken of duodenal, caecal and colonic tissue for histology; digesta from the terminal sixth the small intestine and faeces for Cr determination; and caecal contents for VFA measurement. Organic matter (OM) disappearance in the LB was calculated as ileal flow minus faecal output (Goodlad & Mathers, 1987) and VFA absorption estimated from this, knowledge of caecal VFA proportions and using conventional stoichiometric assumptions. The number of crypt cells/1000 crypt cells arrested in metaphase per 2 h was used as a measure of epithelial proliferation.

	Dietary haricot beans (g/kg diet)				SEM	Significance of dietary effect		
	0	150	300	450	(*n* 6)	Lin	Quad	Dev
OM disappearance (g/d)	0·5	1·2	1·9	2·6	0·13	***	NS	NS
Estimated VFA absorption (mmol/d)								
Acetic acid	3·8	9·1	14·7	19·8	1·01	***	NS	NS
Propionic acid	1·2	3·7	5·2	7·5	0·39	***	NS	NS
Butyric acid	0·6	1·2	2·0	2·1	0·08	***	NS	NS
Arrested cells/1000 crypt cells per 2 h								
Duodenum	87(6)	83(6)	99(5)	85(5)	5·2	NS	NS	NS
Caecum	19(6)	19(5)	23(4)	18(3)	3·0	NS	NS	NS
Colon	16(4)	13(4)	13(6)	17(4)	3·4	NS	NS	NS

Lin, Quad and Dev are linear, quadratic and deviations from linear and quadratic effects of diet respectively. NS, not significant.

***$P<0·001$.

Figures in parentheses are number of observations per mean.

Inclusion of haricot beans in the diet produced large linear increases in OM disappearance in the LB with proportional increases in estimated VFA absorption. However, there were no effects of diet on epithelial renewal rates in any part of the intestine, possibly because VFA absorption on the basal diet was greater than the doses used by Sakata (1987) and maximized any stimulatory effect.

The authors thank D. S. Parker for a gift of vincristine sulphate, the Anatomy Department for histological preparations and the AFRC for a Food Research Studentship (FBK).

Goodlad, J. S. & Mathers, J. C. (1987). *Proceedings of the Nutrition Society* **46**, 149A.
Sakata, T. (1987). *British Journal of Nutrition* **58**, 95–103.

Acute central effects of interleukin-6 on body temperature, thermogenesis and food intake in the rat. By N. J. BUSBRIDGE[1], M. J. DASCOMBE[1], S. HOPKINS[2] and N. J. ROTHWELL[1], [1]*Department of Physiological Sciences, University of Manchester, Manchester M13 9PT* and [2]*Department of Rheumatology, Hope Hospital, Salford M60 8HD*

Many forms of infectious illness are associated with fever which is often accompanied by a reduction in food intake and a rise in metabolic rate. These responses, together with other facets of the acute-phase response, have been ascribed to the actions of interleukin-1 (IL-1). We have shown that IL-1β stimulates metabolic rate and brown adipose tissue activity in the rat (Dascombe *et al.* 1987). Recently another cytokine, interleukin-6 (IL-6), has been claimed to share some actions with IL-1 (Wong & Clark, 1988), so we have tested its effects on body temperature, food intake and oxygen consumption ($\dot{V}_{O_2}$).

Recombinant human IL-6 (1–40 ng/2 μl) or vehicle (saline, 9 g sodium chloride/l) was injected into the third ventricle of the brain (icv) of conscious rats via previously implanted guide cannulae. Significant increases in colonic temperature (0·4±0·1°) occurred 30 min to 3 h after injection of IL-6 (20 ng). Resting $\dot{V}_{O_2}$ (measured at 24°) was also stimulated by injections of 20 or 40 ng IL-6 (19 (SE 5)%), but not by 1 ng IL-6 or by saline (0–3%). Peak responses occurred approximately 60 min after injection.

Administration of IL-6 (20 ng icv) at the start of the dark phase (19.00 hours) suppressed food intake over the following 3 h (saline 9·3 (SE 0·7) g, IL-6 6·1 (SE 0·7) g; $P<0·05$), but intakes were almost identical (23 (SE 3) g) over the next 9 h.

In separate rats, killed 1 h after injection of IL-6 (40 ng), the thermogenic activity of brown adipose tissue, assessed from in vitro GDP binding to mitochondria, was increased (by 31%) compared with saline-injected controls.

These results demonstrate that central injection of IL-6 elicits changes in food intake, metabolic rate and body temperature which are comparable to those of interleukin-1. IL-6 may therefore be an important endogenous mediator of fever.

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Wong, G. G. & Clark, S. C. (1988). *Immunology Today* **9,** 137.

Whole-body protein turnover and skeletal muscle protein synthesis in small-cell lung cancer patients of normal weight. By C. J. H. WOODWARD* and P. W. EMERY, *Department of Food and Nutritional Sciences, King's College (KQC), London W8 7AH* and R. L. SOUHAMI, *Department of Radiotherapy and Oncology, University College Hospital, London WC1E 6AU*

There is evidence that cancer cachexia is associated with decreased muscle protein synthesis (Emery *et al.* 1984) and increased whole-body protein turnover (Jeevanandam *et al.* 1984). It is unclear whether similar changes are found in patients of normal weight. We have therefore measured protein turnover in six newly diagnosed male patients with small-cell lung cancer and in four control subjects. A constant infusion of [1−^{13}C]leucine was given in the fed state, and muscle protein synthesis was measured using a single quadriceps biopsy taken at the end of the infusion (Emery *et al.* 1984).

	Patients		Controls	
	Mean	SE	Mean	SE
Body-wt (kg)	72	3	73	4
Age (years)	52	2	39	8
Leucine turnover (μmol/kg per h):				
Flux	143	10	135	8
Oxidation	54	6	36	3
Synthesis	89	5	99	5
Breakdown	79	6	74	4
Muscle protein synthesis rate (%/h)	0·023	0·003	0·033*	0·004

n 3.

The patients showed increased rates of leucine oxidation compared with controls (+50 %; P=0·05). However, this was probably an artefact caused by hyperventilation when using the Douglas bag, as carbon dioxide production values were also significantly higher in patients than in controls (306 (SE 14·7) *v.* 237 (SE 8·3) ml/min; P<0·01): hyperventilation may in turn be attributable to bronchial congestion present in most of the patients. No other significant changes in protein turnover were found. Muscle protein synthesis rates in both groups were lower than those reported by others for normal subjects (Halliday *et al.* 1988).

Small-cell lung cancer patients of normal weight therefore showed no major abnormalities of protein turnover. Further studies with larger and more closely matched groups would be needed to detect more subtle changes. Changes in protein turnover previously observed in cachectic patients may be associated with the weight-losing syndrome rather than cancer *per se*.

This study was supported by the Cancer Research Campaign. The authors thank Dr D. Halliday, Clinical Research Centre, Harrow HA1 3UJ, for analytical facilities.

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Jeevanandam, M., Horowitz, G. D., Lowry, S. F. & Brennan, M. F. (1984). *Lancet* **i**, 1423–1426.

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The effect of recombinant human tumour necrosis factor and lymphotoxin on the sympathetic firing rate of efferent nerves to brown adipose tissue. By S. J. HOLT, R. F. GRIMBLE and D. A. YORK, *Department of Human Nutrition, School of Biochemical and Physiological Sciences, Southampton SO9 3TU*

Recombinant human tumour necrosis factor α (rTNFα) administered peripherally to rats produces a rapid onset monophasic fever (Bibby & Grimble, 1989) at low doses and a 'shock' response, involving hypothermia, at higher doses (Kettlehut *et al.* 1987). The importance of the hypothalamus in the development of fever has been demonstrated and recently it has been indicated that the pyrogenicity of rTNFα is due to its central action. The primary function of brown adipose tissue (BAT) is heat production via sympathetic activation and therefore we have examined the effect of injection of human rTNFα and lymphotoxin (rTNFβ) into the third cerebral ventricle on the sympathetic firing rate of the efferent nerves to interscapular BAT.

Female lean (*Fa/?*) Zucker rats were anaesthetized with urethane, 1·2 g/kg intraperitoneally, and placed in a stereotaxic frame. Interscapular BAT was isolated and a small thermocouple inserted into the right-hand lobe. The nerves to the lobe on the left-hand side were exposed, one was isolated, cut transectionally, dissected into several filaments and one filament placed on a pair of silver hook electrodes. The nerve activity was amplified, monitored by an oscilloscope and action potentials converted to pulses per second through a window discriminator. For intracerebroventricular (icv) injections, a syringe fitted with a fine (28 gauge) needle was stereotaxically implanted into the third ventricle at the level of the ventromedial hypothalamus. rTNFα or rTNFβ (100 or 200 ng in 0·5 μl sterile saline (9 g sodium chloride/l)) or vehicle were injected over a 30 s period. BAT and rectal temperature were continuously monitored.

		Efferent discharge rate (spikes/s)		Δ BAT temperature (°)		Δ Rectal temperature (°)	
		Mean	SEM	Mean	SEM	Mean	SEM
rTNFα	0	2·40	0·26	No change		No change	
(ng injected)	100	1·90	0·10	−0·21	0·04	−0·15	0·03
	200	1·16	0·20	−0·51	0·04	−0·50	0·05
rTNFβ	0	2·26	0·20	No change		No change	
(ng injected)	100	3·02	0·22	+0·40	0·04	+0·25	0·03
	200	3·94	0·30	+0·52	0·07	+0·36	0·06

Injection (icv) of rTNFα gave a dose-dependent inhibition of nerve firing rate. The response was rapid and acute (firing rate returned to baseline levels within 12 min of injection). The fall in BAT temperature preceded the fall in rectal temperature. Lymphotoxin treatment stimulated sympathetic firing rate and this was accompanied by a rise in BAT and rectal temperatures. These results indicate that rTNFα and lymphotoxin act centrally and the pyrogenic or hypothermic effects involve modulation of the sympathetic outflow to BAT.

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Bibby, D. C. & Grimble, R. (1989). *Proceedings of the Nutrition Society* **48**, 69A.
Kettlehut, I. C., Fiers, W. & Goldberg, A. L. (1987). *Proceedings of the National Academy of Sciences, USA* **84**, 4273–4277.

Effect of insulin on whole-body leucine kinetics in post-absorptive, non-insulin dependent (type 2) diabetic patients. By P. J. PACY, G. C. FORD, H. MERRITT and D. HALLIDAY, *Nutrition Research Group, Clinical Research Centre, Harrow HA1 3UJ*

Over the last few years a number of groups world-wide have examined the effect of insulin on whole-body leucine kinetics. In non-diabetics, studied by means of the euglycaemic clamp technique, and insulin-dependent (type 1) diabetic individuals, insulin has been shown to significantly reduce leucine flux (Fukagawa *et al.* 1985; Nair *et al.* 1987). The response of other variables of leucine metabolism, oxidation and synthesis is more variable. In contrast it has been reported that in obese, non-insulin-dependent (type 2) diabetics, insulin did not influence any index of whole-body leucine kinetics, particularly flux (Staten *et al.* 1986). A major problem with the protocol of the latter study was that although average blood glucose levels during intensive insulin treatment were less than those with more conventional administration (8·1 (SE 2·0) *v.* 14·4 (SE 6·1) mM; $P<0·05$) this was not the case during leucine infusion (6·2 (SE 2·7) *v.* 9·9 (SE 4·2) mM; not significant).

The aim of the present study was to determine the effect of acute insulin infusion on whole-body leucine kinetics in post-absorptive, lean, type 2 diabetics. Their clinical details were: three male, one female; age 47·3 (SE 9·3) years; body-weight 68·0 (SE 7·7) kg; body mass index 22·6 (SE 1·3); glycosylated haemoglobin 12·0 (SE 1·9)%; duration of known diabetes 6·8 (SE 3·3) years. Each patient was studied after a 12–14 h overnight fast by means of an 8 h primed continuous infusion of L-[1−^{13}C]leucine (0·5 mg/kg per h). All oral hypoglycaemic agents were withheld for the preceding 24 h. Insulin infusion (1–2 units/h) was started after 4 h. Whole-body leucine kinetics were determined using the steady-state reciprocal pool model, i.e. plasma [^{13}C]ketoisocaproic acid enrichment (Schwenk *et al.* 1985).

	No insulin		Insulin infusion	
	Mean	SD	Mean	SD
Leucine:				
Flux (μmol/kg per h)	105	17	92*	18
Oxidation (μmol/kg per h)	25	7	18	4
Synthesis (μmol/kg per h)	80	12	74	15
Ketoisocaproic acid (μM)	35	6	26	7
Glucose (mM)	12	2	6*	2
Oxygen uptake (ml/min)	263	20	264	9
Carbon dioxide production (ml/min)	204	16	198	6

Significantly different (paired *t* test): *$P<0·05$.

These findings suggest that the principal effect of insulin on lean type 2 diabetics is inhibition of proteolysis. This is similar to that reported in controls and type 1 diabetics but contrasts with that in obese type 2 diabetics. These results might reflect differences in insulin resistance between the groups.

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Staten, M. A., Matthews, D. E. & Bier, D. M. (1986). *Diabetes* **35**, 1249–1253.

Diurnal variation in large bowel metabolism in rats given diets with and without wheat bran. By J. C. MATHERS and J.-M. FOTSO TAGNY*, *Department of Agricultural Biochemistry and Nutrition, The University, Newcastle upon Tyne NE1 7RU*

In many studies of large bowel function, animals have been fed once daily and sampling carried out at one time during the day. For interpretation of results from studies on fermentation pattern (Key & Mathers, 1987) or transit time (TT) (Goodlad & Mathers, 1987), it is important to know whether dietary effects persist throughout the day.

Two groups of sixteen male Wistar rats (initial weight 172 g) were housed in individual metabolism cages and offered daily 15 g iso-nitrogenous diets containing 600 g freeze-dried cooked maize, 2 g Cr_2O_3 and 0 (−WB) or 200 g wheat bran (+WB)/kg together with casein, egg albumin, methionine, tryptophan, sucrose, maize oil, minerals and vitamins. After a 10 d adaptation period, faeces were collected for 7 d and animals killed (four per diet) at 4, 10, 16 and 22 h after feeding. Volatile fatty acids (VFA) were measured in caecal contents and Cr in caecal and colonic contents. TT was calculated as the quantity of Cr found in the organ divided by daily intake (Goodlad & Mathers, 1987).

	Caecal VFA (mmol/mol)						Transit time (h)			
	Acetate		Propionate		Butyrate		Caecum		Colon	
Diet . . .	−WB	+WB	−WB	+WB	−WB	+WB	−WB	+WB	−WB	+WB
Period after feeding (h)										
4	632	636	217	137	102	184	11	8	13	13
10	648	654	191	128	118	183	15	8	18	13
16	641	632	208	142	112	182	12	8	11	10
22	617	583	170	143	157	220	11	7	12	12
SE of mean	18·2		13·6		12·8		1·3		2·1	

Faecal dry matter output was doubled by dietary inclusion of WB. Diet had no significant effect on caecal total VFA concentration but inclusion of WB was associated with significantly ($P<0·05$) increased butyrate and reduced propionate proportions. When considered over both diets, butyrate proportion increased linearly whilst acetate gave a quadratic response with time after feeding. There were no significant effects of time on caecal or colonic TT but WB inclusion reduced caecal TT by 37% without affecting colonic TT. No interactions between diet and time after feeding were detected.

Goodlad, J. S. & Mathers, J. C. (1987). *Proceedings of the Nutrition Society* **46**, 149A.
Key, F. B. & Mathers, J. C. (1987). *Proceedings of the Nutrition Society* **46**, 11A.

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Post-absorptive body protein turnover in surgical patients formerly maintained on a constant diet. By F. CARLI[1], J. GANDY[2], G. C. FORD[2], H. MERRITT[2], M. READ[2], V. RAMACHANDRA[1], M. PEARSON[2] and D. HALLIDAY[2], [1]*Division of Anaesthetics* and [2]*Nutrition Research Group, Clinical Research Centre, Harrow HA1 3UJ*

Changes in body protein mass associated with the metabolic response to injury produce a negative nitrogen balance related to the severity of the injury, the age of the patient, the pre-injury nutritional status and the post-injury nutritional intake. Several studies on body protein kinetics following surgery have been carried out with conflicting reports on changes in protein synthesis (O'Keefe *et al.* 1981; Clague *et al.* 1983). These observed differences may be due to the different methodologies used, the type and severity of injury or the nutritional intake throughout the study. The aim of this study was to investigate the sequential changes in protein turnover occurring in a well-defined surgical model receiving a constant nutritional intake before and after surgery.

Six healthy females (age 40 (SE 4, range 37–45) years, weight 59·6 (SE 7·6, range 50·0–71·4) kg) scheduled for elective total abdominal hysterectomy (TAH) were studied. The daily feeding regimen, consisting of 5–6 g nitrogen and 4184–5020 kJ (1000–1200 kcal), was started 7 d before surgery and continued for 1 week post-operatively. Leucine kinetics were measured after a 12 h overnight fast using a primed constant infusion of [1−^{13}C]leucine. Plasma [^{13}C]ketoisocaproic acid levels were determined to indicate precursor pool enrichment from which leucine flux and oxidation were calculated.

Flux/breakdown (μmol/kg per h, mean (SD)) increased from a pre-operative value of 108·1 (16·6) to 126·1 (17·4) on the 2nd post-operative day (P=0·01), to 125·3 (21·9) (not significant) on day 4 and to 119·6 (23·4) (not significant) on day 7 (significance determined by paired t test).

Corresponding mean whole-body protein synthesis values (μmol/kg per h) increased from 93·3 (11·1) pre-operatively to 107·1 (15·5) (P=0·03), 104·3 (15·9) (not significant) and 99·2 (18·7) (not significant) on days 2, 4 and 7 respectively. We conclude that whole-body protein breakdown and synthesis increased significantly 2 d after surgery (TAH) and thereafter tended to return to the pre-operative levels.

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O'Keefe, S. J. D., Moldawen, L. L., Young, V. R. & Blackburn, G. L. (1981). *Metabolism* **30**, 1150–1154.

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Effects of dietary wheat bran on activities of key enzymes of lipid and carbohydrate metabolism in rat liver. By LAURENTINA M. R. PEDROSO, HEATHER J. FINLAYSON and J. C. MATHERS, *Department of Agricultural Biochemistry and Nutrition, The University, Newcastle upon Tyne NE1 7RU*

Increasing intake of foods rich in non-starch polysaccharides (NSP) may stimulate fermentation in the large bowel, resulting in more fermentation end-products (principally volatile fatty acids) reaching the liver via the portal vein (Demigne & Remesy, 1985). We are investigating the effects of such manipulations on hepatic carbohydrate and lipid metabolism.

Livers were obtained from rats fed once daily on maize-based diets containing 0 (−WB) or 200 g wheat bran (+WB)/kg (Mathers & Fotso Tagny, 1989) and killed at 4, 10, 16 or 22 h after feeding. Specific activities of glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; G6PDH), malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$) (*EC* 1.1.1.40; ME), isocitrate dehydrogenase ($NADP^+$) (*EC* 1.1.1.42; IDH) and ATP-citrate (*pro*-3*S*)-lyase (*EC* 4.1.3.8; CL) were determined by standard methods. Results expressed in nmol substrate utilized/min per mg protein are shown in the Table.

Period after feeding (h)	G6PDH		ME		IDH		CL	
	−WB	+WB	−WB	+WB	−WB	+WB	−WB	+WB
4	24·1	15·3	19·8	16·7	27·7	26·3	25·3	13·8
10	22·4	16·9	34·4	23·3	37·3	36·6	22·1	14·8
16	22·6	14·0	20·9	20·0	41·9	35·9	23·3	12·6
22	26·5	17·0	25·9	21·4	26·4	24·6	19·0	16·3
SE of mean	2·19		2·74		4·46		2·25	

There were significant ($P<0·01$) effects of time after feeding on activities of ME and IDH. Inclusion of WB in the diet was associated with significant reductions in activities of the two principal NADPH-producing enzymes (G6PDH ($P<0·001$) and ME ($P<0·05$)) and of CL ($P<0·001$), which suggests a fall in demand for NADPH and of carbon from glycolysis for lipid synthesis with the higher NSP diet. Further studies are needed on the mechanisms by which these effects occur and of their implications for in vivo fatty acid and sterol biosynthesis.

Demigne, C. & Remesy, C. (1985). *Journal of Nutrition* **115**, 53–60.
Mathers, J. C. & Fotso Tagny, J.-M. (1989). *Proceedings of the Nutrition Society* **48**, 52A

The effect of the level of dietary protein and the quality of dietary energy on urea kinetics in young children recovering from severe malnutrition. By T. DOHERTY[1], M.-H. DE BENOIST[1], J. HIBBERT[1], C. PERSAUD[1,2] and A. A. JACKSON[1,2], [1]*Tropical Metabolism Research Unit, University of the West Indies, Kingston, Jamaica* and [2]*Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

When the metabolic demand for protein exceeds the dietary intake adaptive mechanisms are brought into play, whereby nitrogen losses from the body are minimized. By decreasing the rate of urea production dietary protein is used more efficiently, and instead of being excreted in urine, a greater proportion of the urea is hydrolysed in the bowel, making the N available for further metabolic interaction. The factors that control urea hydrolysis are unclear. In the present study we have looked at the interaction of the level of protein intake and the quality of dietary energy on urea kinetics in four groups of young children during recovery from severe malnutrition. Two groups received a diet with a protein:energy ratio of 10·6, enriched with either fat (FAT/10) or maize starch (CHO/10). Two groups received a diet with a protein:energy ratio of 8·8, enriched with either fat (FAT/8) or maize starch (CHO/8). All diets were given at 711 kJ (170 kcal)/kg per d and after 7 d a metabolic balance was conducted and urea kinetics measured with intermittent oral doses of [^{15}N]urea (Jackson *et al.* 1984).

			Urea production (P) (mg N/kg per d)		Urea excretion (mg N/kg per d)		N from urea available for synthesis (S) (mg/kg per d)		
Diet	*n*	N intake (mg/kg per d)	Mean	SE	Mean	SE	Mean	SE	S/P %
FAT/10	5	2·16	1·2	0·2	0·7	0·1	0·37	0·12	33
FAT/8	6	1·85	1·6	0·1	0·64	0·04	0·77	0·4	44
CHO/10	4	2·16	1·4	0·1	0·83	0·2	0·37	0·17	27
CHO/8	6	1·85	1·1	0·1	0·36	0·05	0·69	0·36	58

There was an interaction between the level of dietary protein and the quality of dietary energy. The dietary protein was utilized with least efficiency on the FAT/8 diet. Urea was retained for further metabolic interaction on all diets, but this was most efficient on the lower protein diets, and more efficient on the CHO than the FAT diets. There was no difference in S/P for the two fat diets, but S/P was significantly greater on CHO/8 than on CHO/10 ($P<0·005$).

These results provide support for the idea that the handling of urea in the bowel is modulated by both the energy and protein content of the diet.

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The effect of total parenteral nutrition on protein synthesis in endotoxaemic rats. By S. A. ASH and G. E. GRIFFIN, *Department of Communicable Diseases, St George's Hospital Medical School, London SW17 0RE*

The host's response to infection is characterized by anorexia, acute phase protein synthesis and skeletal muscle protein catabolism. The administration of endotoxin (lipopolysaccharide) to rats has been used to successfully mimic these features in an animal model (Jepson *et al.* 1986). We have developed a system for continuous intravenous infusion of endotoxin into small rats, body-weight 80–100 g. The aim of the present study was to examine the effect of total parenteral nutrition (TPN) on in vivo rates of protein synthesis in liver, and skeletal and cardiac muscles of endotoxaemic rats.

A jugular vein of each rat (n 6 for each group) was cannulated under general anaesthetic, and the cannula exteriorized to a rubber seal. After a 4 d recovery period, animals were infused with endotoxin (*Escherichia coli*. 0127:B8, 0·08 mg/kg per h) for 18 h before killing. Animals given TPN were infused with isoenergetic and isonitrogenous amounts equivalent to the normal oral daily intake of *ad lib.*-fed rats of this size. In vivo rates of protein synthesis were measured by the method of Garlick *et al.* (1980) using an intravenous flooding dose of [^{3}H]phenylalanine.

		Protein synthesis (% protein pool synthesized/d)					
Nutrition . . .		Oral		Starved		TPN	
Endotoxin . . .		−	+	−	+	−	+
Tissue:							
Heart	Mean	19·9^a	15·5^b	19·0^a	15·3^b	20·8^a	16·2^b
	SD	2·2	1·8	0·6	2·5	1·6	2·7
EDL	Mean	23·5	9·3^a	12·4^b	11·5^{ab}	17·3	11·2^{ab}
	SD	2·1	1·2	1·0	2·5	1·6	1·3
Soleus	Mean	20·0^a	11·8^b	17·3	12·3^b	22·2^a	13·9^b
	SD	1·6	2·4	1·9	2·9	3·3	1·9
Liver	Mean	107·9^a	129·4^b	90·0	132·5^b	109·2^a	145·7
	SD	6·7	6·6	7·5	6·8	8·3	7·8

EDL, extensor digitorum longus.

^{a,b}Mean values in a horizontal row sharing a common superscript letter are not significantly different from one another at $P<0·02$ (Gabriel's test for multiple comparisons).

The administration of endotoxin reduced protein synthesis rates in cardiac and skeletal muscles but increased synthesis in liver as expected, with liver being the major site of acute phase protein synthesis. As endotoxin caused a fall in food intake to 20% of normal, starved groups were compared. Protein synthesis in skeletal muscle fell after 18 h starvation in skeletal muscle, with a further fall produced by endotoxin in soleus, but not EDL muscle. Cardiac muscle protein synthesis appeared resistant to 18 h starvation. TPN was unable to reverse the fall in protein synthesis produced by endotoxin in cardiac and both types of skeletal muscle. However, TPN augmented the rise in protein synthesis seen in the liver of endotoxaemic rats.

We conclude that TPN is unable to reverse the catabolic effects of endotoxin on cardiac and skeletal muscle, but promotes hepatic protein synthesis in endotoxaemic rats.

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Faecal energy, fat and nitrogen losses in patients with cystic fibrosis using pancreatic replacement therapy. By S. A. WOOTTON, ALISON BEHN and JEANETTE ELLIS, *Department of Human Nutrition, Southampton University Medical School, Southampton SO9 3TU*

Restricted energy intake and malabsorption resulting from pancreatic insufficiency, abnormal bile salt metabolism or defects in transport across the gastrointestinal mucosa are well-recognized features of cystic fibrosis (CF) (Littlewood & MacDonald, 1987). The steatorrhoea and decreased metabolizable energy may even result in an energy deficit sufficient to restrict growth and weight gain despite supplementary enzyme therapy and consumption of more than the recommended amount of energy and protein. The aim of the present study was to determine (1) whether high faecal losses of energy or low energy intake, or both, could account for poor weight gain in CF patients receiving pancreatic supplements and (2) the origins of energy within the stool.

Seven CF patients (aged 9–24 years) who were comparatively well but underweight and growing poorly despite using pancreatic supplements (Creon, DUPHAR) and seven healthy matched controls (9–22 years) participated in the study. Stools were collected for the last 3 d (denoted by carmine markers) of a 5 d period of weighed food intake. The weighed stools were analysed for energy (bomb calorimetry), protein (Kjeldahl) and total lipid (Massion & McNeely, 1973). Nutrient intake was assessed using a computerized database of food composition tables. The results are shown in the Table.

		Energy intake (kJ/kg per d)		Faecal energy (kJ/d)		Faecal energy (% intake/d)		Faecal lipid (g/d)		Faecal protein (g/d)	
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CF	7	359*	89	834	656	7·8	3·7	4·6	5·6	8·5	6·1
Control	7	201	120	556	336	6·0	3·5	2·7	2·2	7·4	4·5

Significantly different from control value: $^*P<0{\cdot}02$.

Significant relations were observed between the amount of lipid consumed and lipid absorbed (r 0·995), the total weight of stool collected and faecal energy (r 0·959) as well as total weight and faecal lipid (r 0·725). Although there appeared to be some degree of association between faecal lipid and faecal energy (r 0·791), faecal lipid could only account for 5–39% of the energy within the stool.

From these results it would appear that the pancreatic supplementation used by these patients at the time of the study appeared to ensure that faecal losses remained comparable to those observed in healthy controls. The poor weight gain in these CF patients could not be simply explained on the basis of low energy intake or high faecal losses when they are comparatively well.

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Tumour necrosis factor α affects protein synthesis in liver and skeletal muscle but not skin of Wistar rats. By YVONNE M. CHARTERS and R. F. GRIMBLE, *Department of Human Nutrition, Southampton University Medical School, Southampton SO9 3TU*

Wan *et al.* (1985) showed that endotoxin altered protein synthetic rates in skin, muscle and liver of rats. Endotoxin is a potent stimulator of tumour necrosis factor α (TNFα) production. Beutler *et al.* (1985) showed that in mice, skin and liver removed 61% of an intravenous dose of TNFα. We therefore examined the manner in which TNFα influenced protein synthesis in these tissues and in a skeletal muscle (tibialis).

Male Wistar rats (151±1 g), from the Southampton University Medical School colony, were caged separately and injected intravenously with recombinant human TNFα (endotoxin content <0·137 ng/mg protein; BASF/Knoll AG, Ludwigshaven) at doses of 30 or 300 μg/kg body-weight, or sterile non-pyrogenic saline (9 g sodium chloride/l). Protein fractional synthetic rates (FSR) were measured 8 and 24 h after injection by the 'massive dose' method using [^{3}H]phenylalanine, together with tissue protein content (Garlick *et al.* 1980). No food was available for 8 h after injection but free access was allowed for the TNFα-treated animals thereafter (CRMX, Labsure, Manea, Cambs). Saline controls were pair-fed the intakes of the equivalent TNFα-treated rats. Intakes were 15 and 7 g/d for those receiving 30 and 300 μg TNFα/kg respectively (*ad lib.* intake 18 g/d). Free access to water was allowed throughout.

Protein synthesis

			Period after injection	Tibialis				Liver				Skin			
	Dose (μg/kg			(mg/d)‖		FSR (%/d)		(g/d)		FSR (%/d)		(g/d)		FSR (%/d)	
Treatment	body-wt)	*n*	(h)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Saline	–	6	8	8·9	0·5	15·9	1·1	0·86	0·1	87	4	1·68	0·2	43	2
TNFα	30	6	8	6·4†	0·6	13·7	1·0	1·13*	0·1	111†	5	2·09	0·2	45	4
TNFα	300	6	8	6·9†	0·4	13·4	1·0	1·15*	0·1	112†	3	1·73	0·1	44	1
Saline, pair-fed	–	6	24	8·8	0·7	15·0	0·5	1·00	0·1	93	6	1·69	0·3	41	4
TNFα	30	6	24	9·7	0·6	16·2	0·5	1·11	0·1	99	6	1·85	0·2	47	6
Saline, pair-fed	–	6	24	6·7	0·5	13·2	0·9	0·87	0·1	89	4	2·00	0·2	43	5
TNFα	300	6	24	7·6‡	0·8	14·8	1·2	1·11*§	0·1	111†§	3	2·01	0·2	49	4

Significantly different from corresponding control (analysis of variance): *P<0·05, †P<0·01 and from lower TNFα dosage, ‡P<0·05, §P<0·01.

‖Calculated from the protein FSR and total tissue content.

TNFα had stimulated hepatic protein synthesis 8 h after injection. The opposite occurred in muscle at this time. Evidence of enhanced liver protein synthesis was still present 24 h after injection of the highest dose, despite the inhibitory influence of reduced food intakes. Muscle protein synthesis returned to normal at this time in rats receiving the lowest dose of TNFα but remained suppressed at the higher dose due to anorexia. Protein synthesis appeared to be unaffected by TNFα in skin.

The authors are grateful to BASF/Knoll AG for the gift of TNFα.

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Effect of cigarette smoking and age on selenium status. By V. W. BUNKER[1], D. CAMPBELL[1], A. J. THOMAS[2] and L. J. HINKS[1], [1]*Chemical Pathology and Human Metabolism* and [2]*Geriatric Medicine, Southampton General Hospital, Southampton SO9 4XY*

Selenium, as a component of glutathione peroxidase (*EC* 1.11.1.9; GSH-Px), catalyses the reduction of lipid hydroperoxides and hydrogen peroxide and is involved in preventing free radical-induced tissue damage. GSH-Px may also have a less well-defined role in arachidonic acid metabolism in platelets.

The Se status of seventy-five people aged <70 years and twenty-eight people aged >70 years was determined by measuring Se levels in plasma, erythrocytes and platelets and the GSH-Px activity in erythrocytes and platelets. Nineteen subjects, only one of whom was aged over 70 years, smoked more than five cigarettes a day.

Se levels in plasma and erythrocytes were approximately 20% lower than previously reported by this laboratory (Lloyd *et al.* 1983). The probable cause of this is the decreased importation of North American wheat (which is high in Se) and its replacement with EEC and UK wheats of low-Se content (A. Mills, Ministry of Agriculture, Fisheries and Food, personal communication).

Platelet GSH-Px activity was significantly ($P<0.05$) reduced in the smokers (mean (95% confidence interval, CI)): 120 (104–136) U/g protein compared with non-smokers: 138 (131–145) U/g protein. Se levels in plasma and erythrocytes and GSH-Px activity in erythrocytes tended to be lower in smokers but the differences were not significant. Reduced activity of GSH-Px in platelets might lead to the accumulation of lipid hydroperoxides which can activate the cyclooxygenase pathway and promote aggregation.

Se status of apparently healthy non-smokers

	<70 years (*n* 57)		>70 years (*n* 27)	
	Mean	95% CI	Mean	95% CI
Se:				
Plasma (μmol/l)	1·22	1·17–1·27	1·04***	0·95–1·13
Erythrocyte (nmol/g Hb)	4·93	4·68–5·19	4·77	4·35–5·18
Platelet (nmol/g protein)	27·6	26·0–29·2	26·6	24·4–28·7
GSH-Px:				
Erythrocyte (U/g Hb)	24·2	22·6–25·8	24·4	21·6–27·1
Platelet (U/g protein)	138	131–145	131	114–148

Hb, haemoglobin. Significantly different from younger subjects: ***$P<0.001$.

The Table shows that subjects aged >70 years had lower plasma Se levels than subjects aged <70 years. Reduced dietary intake of Se in the elderly is a possible cause. However, since age had no effect on the other measurements we conclude that the Se status of healthy elderly people appears adequate.

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Inflammation associated with oxygen injury in the preterm guinea-pig. By F. J. KELLY and G. PHILLIPS (Introduced by R. F. GRIMBLE), *Department of Human Nutrition, The University of Southampton, Southampton SO9 3TU*

Exposure of neonatal and adult animals to elevated concentrations of oxygen results in extensive lung injury (Clark & Lambertsen, 1971). The mechanisms involved in the development and progression of pulmonary injury are, however, incompletely understood. In addition, it is not known if animals born prematurely respond in a similar manner to term or adult animals.

Hartley strain guinea-pig pups were delivered by Caesarian section at 65 d gestation (term 68 d). Following resuscitation, litters were randomly divided into two groups; control (21% O_2) and hyperoxic (95% O_2). Pups, along with a surrogate, lactating mother, were placed in 25-litre Perspex cages, flushed with either gas mixture. Pups (four to six at each time point) were removed at 24, 48, 72 and 96 h for detailed biochemical and morphological analysis. Bronchoalveolar lavage (BAL) was carried out on all animals and utilized to determine protein content, total and differential cell counts and morphological analysis of the inflammatory cells present in the airways.

	BAL composition			
	Control (21% O_2)		Hyperoxic (95% O_2)	
	Mean	SD	Mean	SD
Protein content (mg/ml)	0·39	0·14	1·63**	0·36
Total cell no. ($\times 10^6$)	4·5	2·9	11·6*	3·0
Macrophages ($\times 10^6$)	1·98	0·42	3·25	0·68
Neutrophils ($\times 10^6$)	0·032	0·01	2·10**	0·61
Eosinophils ($\times 10^6$)	2·45	0·71	5·56*	0·89

n 4–6. *$P<0·05$, **$P<0·01$ (Student's *t* test).

An increase in protein accumulation in the alveoli of 95% O_2-exposed pups was evident as early as 48 h. By 96 h there was a fourfold increase in alveolar protein. This change, which is indicative of an increase in alveolar capillary permeability, was accompanied by an increase in the number of inflammatory cells (macrophages, neutrophils and eosinophils) present in the airways. Following 96 h hyperoxic exposure, inflammatory cell numbers had increased threefold. Differential cell counts revealed that these increases were due largely to greater numbers of neutrophils and eosinophils in the alveoli of these animals. These results demonstrate that the immature lungs of preterm animals respond to hyperoxia by exhibiting signs of increased alveolar capillary permeability, which is coincidental with elevated numbers of inflammatory cells in the airways.

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Effect of tumour necrosis factor α (TNFα–cachectin) on lipid metabolism during litter-removal in the lactating rat. By R. D. EVANS and D. H. WILLIAMSON, *Metabolic Research Laboratory, Nuffield Department of Clinical Medicine. Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE*

Tumour necrosis factor α (TNFα; cachectin) is a macrophage cytokine which inhibits lipoprotein lipase (*EC* 3.1.1.34; LPL) activity in vitro (Beutler & Cerami, 1986) and in vivo (Semb *et al.* 1987); it may have a role in tumour-associated cachexia (Beutler & Cerami, 1986). We have described the effect of tumour burden on lipid metabolism during lactation and litter-removal (Evans & Williamson, 1988); transplantable tumour inhibits the 'switch-on' of LPL activity in white adipose tissue (WAT) on premature removal of the litter, and consequently of exogenous lipid accumulation in WAT. We have attempted to mimic this effect using TNFα.

Lactating rats of approximately 300 g were used. At 11–12 d lactation, the pups were removed; food intake for the subsequent 24 h was measured, and animals were injected intravenously with 0·5 ml saline (9 g sodium chloride/l) (controls) or 0·5 ml phosphate-buffered recombinant human TNFα ($1·5 \times 10^6$U; 185 μg) under ether anaesthesia. After 1 h [1−^{14}C]triolein was administered orally, and carbon dioxide collected for the next 5 h. The animals were then killed and tissues sampled. Tissue lipids were extracted and LPL assayed (Oller do Nascimento & Williamson, 1986).

Food intake was similar in both groups (38 (SE 2·5) g for controls (*n* 5), 43 (SE 1·9) g for TNFα-treated animals (*n* 5)). Absorption of lipid was significantly decreased in TNFα-treated rats (35 (SE 11)% administered dose/5 h against 75 (SE 3)% for controls, $P<0·05$) and $^{14}CO_2$ production was also decreased in TNFα-treated animals (1·06 (SE 0·39)% absorbed dose/h against 4·80 (SE 0·85)% in controls, $P<0·01$). Accumulation of exogenous labelled lipid in WAT was diminished compared with controls (0·40 (SE 0·20)% in TNFα-treated animals compared with 2·42 (SE 0·35)% in controls, $P<0·01$) and brown adipose tissue (2·25 (SE 1·2)% *v.* 8·8 (SE 2·3)%, $P<0·05$). Plasma [^{14}C]lipid accumulation was unchanged by the administration of TNFα, despite previous findings of an increase in tumour-bearing animals (Evans & Williamson, 1988). LPL activity was significantly decreased in WAT (0·487 (SE 0·092) nmol fatty acids released/min per mg acetone dried tissue *v.* 1·52 (SE 0·237) for control animals, $P<0·001$), but unchanged in heart.

These results indicate that TNFα is capable of inhibiting LPL in a tissue-specific manner: the activity was depressed in WAT with concomitant decrease in lipid accumulation despite the large physiological stimulus to lipid deposition in WAT during weaning, whereas there was no change in LPL in heart. The partial inhibition of lipid absorption in TNFα-treated rats is a novel finding, as is the decrease in exogenous lipid oxidation rate ($^{14}CO_2$ production).

The authors are grateful to BASF/Knoll AG, Ludwigshafen, FRG, for the supply of TNFα.

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Accelerated protein turnover during muscle wasting in the chronically vitamin-E-deficient rat. By A. OMER[1], P.C. BATES[1], P. DONACHIE[1], M. A. GOSS-SAMPSON[2] and D. J. MILLWARD[1], [1]*Nutrition Research Unit, London School of Hygiene and Tropical Medicine, St Pancras Hospital, 4 St Pancras Way, London NW1 2PE* and [2]*Institute of Child Health, 30 Guildford Street, London WC1*

Vitamin E deficiency results in a reduced ability to terminate lipid-phase free-radical chain reactions resulting in lipid peroxidation and tissue damage, which includes a myopathy. We have investigated the mechanisms of the muscle wasting with repeated in vivo measurements of protein synthesis in rats fed on vitamin-E-deficient and supplemented diets (at one and ten times recommended dietary allowance) for up to 62 weeks.

Weanling rats were fed on diets based on casein (200 g/kg) and linoleic acid (50 g/kg) containing 0 (−E), 100 (+E) or 1000 (+HD) mg vitamin E/kg + 40 μg Se/kg (as sodium selenite). Growth rates, food intake, vitamin E status, rate of protein synthesis (k_s), and RNA content in gastrocnemius muscle and liver were measured at 3 and 32 weeks and in separate animals maintained on commercial vitamin-E-deficient and supplemented diets at 62 weeks as previously described (Omer *et al.* 1986).

The first signs of faltering in the −E group occurred at 10 weeks, with a significant weight deficit at 18 weeks compared with the +E group. Growth ceased after 20 weeks and at 32 weeks weights were 571 (SD 45) g −E, and 681 (SD 41) g +E. At 62 weeks weights were 493 (SD 77) g −E compared with 745 (SD 103) g +E. There was an increased food intake in the −E group, first apparent at 18 weeks. Behavioural signs of the myopathy were apparent from 20 weeks. The HD group had a slower initial growth with smaller body-weights at 6 weeks and remained smaller than the +E group throughout, with a final weight similar to that of the −E group; food intakes were the same as the +E animals at 18 weeks but decreased at 32 weeks. This group was generally more aggressive than the other rats. At 3 weeks, notwithstanding the increased erythrocyte fragility, undectable plasma tocopherols, and hepatic lipid peroxidation and enlargement, no changes in muscle mass or k_s were detectable in the −E group. However, at 32 weeks muscle mass was reduced from 5·4 (SD 0·5) g/kg body-weight +E (n 7), and 5·8 (SD 0·6) g/kg body-weight HD (n 8), to 4·9 (SD 0·4) g/kg body-weight −E (n 9, $P<0·05$), although no difference was apparent at 62 weeks. At 32 weeks the muscle wasting in the −E group was associated with a 69% increase in RNA concentration, and a 54% increase in the synthesis rate per RNA giving an increase in k_s from 2·97 (SD 0·56)%/d +E, and 3·17 (SD 0·77)%/d HD, to 7·43 (SD 1·84)%/d −E. This increase in k_s implies a large increase in the rate of proteolysis. At 62 weeks, although the RNA concentration was still elevated, k_s was not different in −E or +E groups, although the falling muscle mass at this time implies that the accelerated proteolysis was maintained. Hepatic protein synthesis was not influenced by vitamin E status. The oxidative damage which induces the myopathy and muscle catabolism in vitamin E deficiency is associated with accelerated protein synthesis and proteolysis, possibly reflecting damage repair and regeneration, in contrast to the reduced protein turnover which usually accompanies muscle growth inhibition and wasting (Rennie *et al.* 1986).

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Effect of maintenance feeding and compensatory growth on hind-limb muscle protein metabolism of lambs during subsequent refeeding. By S. D. WHEATLEY* and M. J. BRYANT, *Department of Agriculture, University of Reading, Earley Gate, Reading RG6 2AT* and M. A. LOMAX, *Department of Physiology and Biochemistry, University of Reading, PO Box 228, Reading RG6 2AJ*

Compensatory growth is the ability of animals, previously restricted in feed intake, to grow at a faster rate than unrestricted counterparts of the same weight, during the subsequent refeeding period. The compensating animal may exhibit superior body protein gains and an enhanced carcass muscle protein content. Whether these responses are mediated by a change in the metabolism of the muscle mass has yet to be established.

We have measured muscle tyrosine metabolism using a model similar to that of Oddy & Lindsay (1986). Muscle protein synthetic and degradative rates are estimated from the net exchange and gross uptake of [^{3}H]tyrosine across the hind-limb muscle mass. Eight twin-born wether lambs, live weight range 26·5–31·0 kg, were offered a ration of a barley-based concentrate (857 g dry matter (DM)/kg, 30·6 g nitrogen/kg DM, 17·4 MJ/kg DM) and hay twice daily for a daily growth rate of 0 (RC) or 200 g (controls; C) live weight. After 10 weeks of maintenance feeding, RC lambs were offered the same ration in quantities to allow a daily growth rate of 200 g. Tyrosine metabolism was measured during weeks 1, 4 and 8 of both growth treatments and also during week 10 of feed restriction in treatment RC.

	Muscle protein turnover (%/d)			N intake
	Synthesis	Degradation	Gain	(g/d)
Maintenance	2·07	2·65	–0·58	5·2
SEM	0·28	0·31	0·05	0·2
Week 1:				
C	6·84	3·28	3·56	28·3
RC	3·22	0·44**	2·78	30·5
SED	1·74	0·90	1·02	1·8
Week 4:				
C	6·22	2·80	3·42	31·8
RC	8·32	4·85	3·47	30·1
SED	2·52	1·32	1·76	2·3
Week 8:				
C	4·64	2·57	2·07	36·5
RC	6·57	1·82	4·75*	37·0
SED	1·53	0·46	1·11	0·6

SED, standard error of the difference. *$P<0·05$, **$P<0·01$.

Ten weeks of maintenance feeding resulted in a reduction of 65% in synthetic rate, no increase in degradative rate, and a net loss of muscle protein, when compared with a general mean for control lambs. A net muscle protein gain was restored on refeeding, initially by a dramatic reduction in the degradative rate, with only a small increase in synthetic rate. During the later stages of refeeding, protein gain was increased above control values. These results demonstrate that compensatory growth may increase muscle protein deposition by altering the relative rates of synthesis and degradation.

Oddy, V. H. & Lindsay, D. B. (1986). *Biochemical Journal* **233**, 417–425.

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Tumour necrosis factor α enhances hepatic metallothionein-I content but reduces that of the kidney. By R. F. GRIMBLE, *Human Nutrition Department, Southampton University Medical School, Southampton SO9 3TU* and I. BREMNER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Rearrangements in zinc metabolism occur during infection. Plasma Zn falls and hepatic Zn content increases. Interleukin-1 (IL-1) and tumour necrosis factor α (TNFα) are among the cytokines released from macrophages in response to components of invading bacteria. Studies using bacterial endotoxin and IL-1 indicate that serum Zn changes are related to increased metallothionein (MT) production in liver and kidney (Di Silvestro & Cousins, 1984, 1985). TNFα also produces a fall in serum Zn, however, its effect on MT in liver and kidney is unknown. We examined the effect of recombinant human TNFα on serum Zn and hepatic and renal MT-I.

Male Wistar rats (154±1 g) were separately caged, and fed on laboratory chow. Animals received intravenous injections of TNFα (endotoxin content <0·137 ng/mg protein) or sterile, non-pyrogenic saline (9 g sodium chloride/l), via the lateral tail vein (*n* 4). Rats were stunned and decapitated 8 and 22 h after injections, blood was collected and liver and kidney rapidly frozen in liquid nitrogen. Pair-fed, saline-injected controls were included in the study since TNFα reduced appetite. Serum Zn was measured by atomic absorption spectrometry and tissue MT-I by radioimmunoassay (Mehra & Bremner, 1983). Mean 22 h food intakes were 19, 19, 15, 14 and 7 g for rats receiving 0, 20, 50, 100 and 300 μg TNFα/kg body-weight.

	Serum Zn (μg/ml)		MT-I (μg/organ)			
Period after injection (h) . . .	8	22	8		22	
Treatment (*n* 4/group)			Liver	Kidney	Liver	Kidney
Control, *ad lib.* fed	1·94	—	158	36·2	—	—
TNFα (20 μg/kg)	1·64*	1·48**	384**	26·4	214	—
Control, pair-fed	—	1·97	—	—	140	—
TNFα (50 μg/kg)	1·26**	1·27**	268*	—	163	—
Control, pair-fed	—	1·64	—	—	63	—
TNFα (100 μg/kg)	0·81**	1·74**	285*	18·9	122	17·3**
Control, pair-fed	—	2·06	—	—	81	34·8
TNFα (300 μg/kg)	0·86**	1·34**	276*	23·6	270	13·0**
Control, pair-fed	—	1·89	—	—	311	39·5
SEM	0·12	0·09	29	4·0	39	3·0

Value significantly different from control value by analysis of variance: *P<0·05, **P<0·01.

TNFα brought about a dose-dependent fall in serum Zn 8 h after injection. The MT-I of liver but not kidney was enhanced at this time by TNFα. Renal MT-I was significantly depressed 22 h after injection.

TNFα thus mimics the effects of endotoxin and IL-1 on serum Zn and liver MT content. It has an opposite effect to these inflammatory agents on renal MT but the same effect as turpentine (Morrison *et al.* 1988).

The authors are grateful to BASF/Knoll AG, Ludwigshafen, FRG, for the gift of TNFα.

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Estimates of iron status in the Northern Ireland population. By J. J. STRAIN, MARGO E. BARKER, KATE A. THOMPSON, P. G. MCKENNA, A. P. WILLIAMSON, MARION E. WRIGHT, SALLY I. MCCLEAN and NORMA G. REID, *Centre for Applied Health Studies, University of Ulster at Coleraine, Cromore Road, Coleraine BT52 1SA*

The iron status of a representative sample of the Northern Ireland population was assessed from blood samples taken from subjects (18–64 years of age) who participated in the Northern Ireland Diet and Health Study. Blood samples were obtained from 87% of the eligible participating sample.

A single non-fasted venous blood sample was taken from each subject in the evening and was analysed within 48 h. Measurements of Fe status included serum ferritin (SF), serum transferrin saturation (TS), haemoglobin (Hb) and mean corpuscular Hb concentration (MCHC). The total number of valid results were: 226 men, 290 women for SF; 209 men, 268 women for TS; 222 men, 286 women for Hb and MCHC. SF levels of the whole population were normalized by logarithmic transformation.

Analysis of variance showed that men had significantly higher ($P<0{\cdot}001$) SF (men, 105·6 (median 78·5) μg/l; women, 43·0 (median 33·5) μg/l), TS (men, 24·8 (SD 11·01)%; women, 20·7 (SD 8·37)%) and Hb (men, 149 (SD 11·3) g/l; women, 132 (SD 11·5) g/l) values than women. SF levels also increased significantly ($P<0{\cdot}001$) with age; the increase being particularly marked after 45 years of age in women.

Classification of subjects according to Fe status is given in the Table. Cut-off points have been taken from Bindra & Gibson (1986) and Cook *et al.* (1986). Multiple criteria were also used to determine Fe deficiency (subjects having abnormally low values of any two of SF, TS or MCHC) and Fe deficiency anaemia (subjects having abnormally low values of any two of SF, TS or MCHC and an abnormally low Hb).

	SF (μg/l)		TS (%)	Hb (g/l)	MCHC	Fe	Fe deficiency
	<12	>300	<16	<120 (<130)	(g/l) <320	deficiency	anaemia
% Men	1·8	3·5	10·5	0·5 (2·3)	6·8	1·4	0·5
% Women (18–44 years of age)	17·9	0	26·0	13·4	13·9	11·0	6·6
% Women (45–64 years of age)	4·3	0·3	31·0	9·8	8·7	5·7	4·6

Among men the prevalence of excessive Fe stores (SF>300 μg/l) was higher than the prevalence of deficient Fe stores (SF<12 μg/l). The prevalence of Fe deficiency was similar in the Northern Ireland population compared with a representative sample of the United States population (Cook *et al.* 1986). The prevalence of Fe deficiency anaemia, however, was higher in Northern Ireland women compared with US women. Chronic inflammation may have influenced Fe status measurements in men and the older women, resulting in disproportionately elevated SF relative to TS or MCHC.

This work was supported by the Health Promotion Research Trust.

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Estimation of energy expenditure by heart rate: a validation study. By SANA M. CEESAY, ANDREW M. PRENTICE, KENNETH C. DAY, GAIL R. GOLDBERG, PETER R. MURGATROYD and WENDY SCOTT, *MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL* and G. B. SPURR, *Department of Physiology, Medical College of Wisconsin, Milwaukee, WI, USA*

Most previous attempts to predict total energy expenditure (TEE) from heart rate (HR) have been based on accumulated or averaged HR over the entire period under investigation or over extended sub-periods of the total. The main problem with this approach is that it fails to allow for the fact that, although HR and energy expenditure (EE) are closely correlated during exercise, there is often no discernible relation during periods of light activity or rest.

In the present study, TEE was measured by a new minute-by-minute HR method using a modification of the technique of Spurr *et al.* (1988). This requires the definition of a 'FLEX' HR for each subject above which there is a strong relation between HR and oxygen consumption ($\dot{V}_{O_2}$) and below which the two variables are rather poorly correlated, due largely to the influence of posture on stroke volume.

Before calorimeter (CAL) measurements, twenty healthy subjects (eleven male, nine female) were individually calibrated using standard techniques while wearing the PE 3000 Sports Tester (Polar Electro, Finland) to establish the relation between HR and $\dot{V}_{O_2}$ for different postures at rest and during exercise. FLEX HR was defined as the mean of the highest HR during the standing and the lowest HR during stepping measurements. Sedentary EE was defined as the mean expenditure during periods of lying down, sitting and standing.

Simultaneous measurements of HR and EE were then made during 21 h of continuous whole-body calorimetry which included four × 30 min periods of different types of exercise. Mean TEE.CAL was 8063 (SD 1445) kJ. Minute-by-minute HR was converted to energy expenditure using the relation: TEE.HR = sleep EE + sedentary EE + activity EE. Sleep EE was calculated as basal metabolic rate predicted from standard equations (Schofield *et al.* 1985). Activity EE was estimated from each individual's calibration curve for all heart rates in excess of FLEX. Sedentary EE was assigned to all remaining periods. Statistical analysis was performed using paired *t* tests and linear regression.

The HR method yielded a mean non-significant underestimate in TEE of −1·2 (SD 6·2) % (range −11·4 to + 10·6%), paired *t* −1·28. Regression of TEE.HR (*Y*) on TEE.CAL (*X*) yielded $Y = 0{\cdot}868\,X + 927$ kJ, r 0·943, SE 458 kJ, n 20.

From the results of the present study, we conclude that the use of minute-by-minute HR monitoring in conjunction with a method such as we have proposed involving a cut-off threshold to discriminate between periods of activity and inactivity, can provide reasonable estimates of EE in epidemiological or large-scale community studies. The method is not as accurate as the doubly-labelled water technique, but has advantages in terms of cost and ease of use.

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The iron status of healthy, housebound, and hospitalized elderly women. By ANITA J. THOMAS, VALDA W. BUNKER, MARGARET LAWSON, MAUREEN STANSFIELD, NIDISH SODHA and BARBARA E. CLAYTON, *Faculty of Medicine of the University of Southampton, Southampton General Hospital, Southampton SO9 4XY*

The iron status of three groups of elderly women was examined by means of 5 d duplicate diet analysis, and haematological and biochemical indices. The groups comprised thirteen apparently healthy elderly women (mean age 77 years), thirteen housebound women (mean age 79 years) living in their own homes and eating a self-selected diet, and seventeen elderly female patients (mean age 82 years) from a long-stay geriatric unit who had been resident for more than 3 months, in a stable medical condition and eating their customary diet. Eleven of these patients had a chronic, significant healing problem, either a pressure sore or leg ulcer. Metabolic balance studies (5 d) were undertaken on all healthy and housebound and four hospitalized women.

	n		Fe intake (μmol/d)	Haemoglobin (g/l)	Serum Fe (μmol/l)	Total Fe-binding capacity (μmol/l)	Transferrin saturation (%)	Plasma ferritin (μg/l)
Healthy (H)	13	Mean	156*	139	20	60	34	62*
		95% CI	123–193	136–142	18–22	57–63	31–36	50–75
Housebound (HB)	13	Mean	167*	128	11	67	18	30*
		95% CI	130–215	119–138	9–14	63–71	14–21	19–48
Hospitalized (HP)	17	Mean	96*	121	10	50	18	126*
		95% CI	83–114	107–135	7–13	45–56	13–22	52–307
Statistical analysis:								
H *v.* HB			NS	†	†††	NS	†††	††
H *v.* HP			†††	†	†††	†	†††	NS

95% CI, 95% confidence interval; NS, not significant.

Arithmetic means except for * geometric means.

The recommended daily intake of Fe is 179 μmol (Department of Health and Social Security, 1979).

Paired *t* tests for parametric data, Mann Whitney for non-parametric data: †$P<0{\cdot}05$, ††$P<0{\cdot}01$, †††$P<0{\cdot}001$.

Mean daily Fe retention did not differ significantly from zero in the healthy and housebound groups, but negative balance was observed in the hospitalized subjects.

The Fe status of the healthy elderly women appeared satisfactory, with metabolic equilibrium on a mean dietary intake slightly less than that recommended (Department of Health and Social Security, 1979), with satisfactory haematological and biochemical indices, in contrast to the housebound women who appeared to be borderline Fe deficient, although in metabolic equilibrium on similar intakes. Evidence of biochemical Fe deficiency in the hospitalized women was accompanied by low dietary intake and negative metabolic balance.

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Comparison of heart rate monitoring with the doubly-labelled water [$^2H_2{}^{18}O$] method for measurement of energy expenditure in free living subjects. By M. B. E. LIVINGSTONE, J. J. STRAIN and P. G. MCKENNA, *Biomedical Sciences Research Centre, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB, N. Ireland* and W. A. COWARD and S. M. CEESAY, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

The doubly-labelled water (DLW) method is the most accurate method of providing an integrated and representative estimate of total energy expenditure (TEE) in the unencumbered individual. However, its application is cost prohibitive in large-scale population studies where the use of a suitably validated, less sophisticated and more cost-effective technique, such as heart rate (HR) monitoring, is needed.

TEE was measured simultaneously in fourteen (nine male, five female) free living adults over 15 d by the DLW method and between 2 and 4 separate days by HR monitoring. The calibration procedures described by Ceesay *et al.* (1989) were adopted, in which individual HR:oxygen consumption ($\dot{V}_{O_2}$) calibration curves were derived from five varying levels of activity, and a 'FLEX' HR which discriminated between rest and activity was identified. Daytime HR was monitored over 16-h periods. Individual calibration curves were used to assign an energy value to minute-by-minute recorded HR above FLEX. Below FLEX, energy expenditure was estimated from individually determined values for resting metabolic rate (RMR). Standardized measurements of basal metabolic rate (BMR) by indirect calorimetry were substituted for the sleeping metabolic rate.

		FLEX HR		Daytime HR		RMR:BMR		DLW TEE (MJ/d)		HR TEE (MJ/d)	
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Males	9	95	9	87	9	1·30	0·07	14·62	3·44	15·10^{NS}	3·05
Females	5	102	3	93	3	1·33	0·06	9·77	2·08	9·19^{NS}	0·94
Total	14	98	8	89	8	1·31	0·07	12·89	3·80	12·99^{NS}	3·83

^{NS}, not significantly different from TEE.DLW (paired *t* test).

On average the HR method resulted in a non-significant overestimate in TEE of +2·0 (SD 17·9)%. Correlation analysis yielded r +0·778, $P<0·01$. Individual HR TEE discrepancies ranged from −22·2% to +52·1% with nine values lying within ±10% of DLW TEE. However, since day-to-day variation in TEE resulted in a mean intra-individual coefficient of variation in HR TEE of 15 (SD 10)%, an increased number of sampling days would be expected to improve the precision of individual estimates of HR TEE. The HR method may provide more reliable estimates of sedentary TEE than previous methods of prediction since TEE will largely be derived from RMR values. Nevertheless in the range FLEX ±5 heart beats the method may lose precision due to an overlap in resting and active conditions. While individual estimates of TEE may be subject to unacceptable error the HR method provides not only a close estimation of the TEE of population groups but also objective indices of habitual physical activity patterns and cardiorespiratory function.

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Modification of the metabolic effects of recombinant human tumour necrosis factor α by diets rich in coconut oil. By D. C. BIBBY and R. F. GRIMBLE, *Human Nutrition Department, Southampton University Medical School, Southampton SO9 3TU*

We have previously shown that chronic feeding of a diet rich in coconut oil inhibits metabolic responses to *Escherichia coli* endotoxin (Wan & Grimble, 1987). The present study examines whether the same is true for responses to tumour necrosis factor α (TNFα).

Weanling male Wistar rats were fed for 9 weeks on diets containing either 200 g maize oil/kg or 190 g coconut oil and 10 g maize oil/kg, and 20% of energy as protein (Wan & Grimble, 1987). Rats from each dietary group were given either TNFα (30 or 300 μg/kg body-weight; BASF Knoll AG) or saline (9 g sodium chloride/l) intravenously. Rectal temperatures were measured before and at 0·5, 1, 2, 3 and 4 h after injection. TNFα-treated rats were killed 24 h after injection, and saline-injected rats 24 h later, after being pair-fed the intakes of the TNFα-treated groups. Intakes of the 30 and 300 μg TNFα/kg groups were 9 and 7 g/d for the maize-oil group and 11 and 4 g/d for the coconut-oil group. Blood, liver and tibialis muscle were rapidly removed. Serum albumin and tissue protein were measured as described previously (Wan & Grimble, 1987) and serum copper by atomic absorption spectroscopy.

	Liver protein (g)		Tibialis protein (mg)		Serum Cu (μg/l)		Serum albumin (mg/ml)	
Injection	Maize	Coconut	Maize	Coconut	Maize	Coconut	Maize	Coconut
Saline	2·29[a]	2·34[a]	150[a]	162[a]	1870[a]	1860[a]	45·4[a]	44·9[a]
TNFα (30 μg/kg)	2·45[b]	2·18[a]	136[b]	158[a]	3050[c]	2860[c]	44·4[a]	43.8[a]
Saline	2·41[a]	2·10[a]	149[a]	154[a]	1930[a]	1960[a]	45·9[a]	43·8[a]
TNFα (300 μg/kg)	2·72[c]	2·51[bc]	136[b]	141[b]	3120[c]	2710[c]	41·4[c]	40·5[c]
Within group SD	0·24	0·17	7	11	341	450	1·7	2·0

[a–c]Values not sharing a common superscript letter are significantly different (analysis of variance): $P<0·05$.

Six animals per group.

One hour after injection, rectal temperatures had fallen by 0·5° and 1·3° for the 30 and 300 μg/kg dose in maize-oil-fed rats, while in the coconut-oil-fed rats the falls were 0·7° and 0·9°.

Both doses of TNFα elicited characteristics of the acute-phase response in maize-oil-fed rats. The coconut oil diet, low in linoleic acid, abolished the effects on liver and tibialis protein and impaired the increase in serum Cu. Alterations in sensitivity of target tissue for TNFα may therefore have been brought about by chronic consumption of a low linoleic acid diet.

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Wan, J. M. & Grimble, R. F. (1987). *Clinical Science* **72**, 383–385.

The effect of a turpentine-induced inflammatory response or triple hormone infusion on energy expenditure in rats. By G. MCWILLIAMS, H. J. G. BURNS, K. C. H. FEARON, K. CARTER and A. SHENKIN, *University Departments of Surgery and Pathological Biochemistry, Royal Infirmary, Glasgow G31 2ER*

It has been proposed that the synergistic action of the catabolic hormones adrenaline, cortisol and glucagon (ACG), acting after injury and sepsis in man, produces the observed increase in energy expenditure (EE) (Bessey *et al.* 1984). To carry out studies of this response a model system of inflammation is important. Previous studies have shown that intraperitoneal and subcutaneous injection of lipopolysaccharide (endotoxin) in rats produces a febrile response (Grimble & Holden, 1987). However, using a similar model with injection or intra-arterial infusion of either 055:B5 (phenol extract of *Escherichia coli*) or 0127:B8 (butanol extract of *E. coli*) endotoxin (10–30 μg/kg) in Sprague-Dawley (SD) rats, a consistent febrile response was not obtained at room temperature nor at thermoneutrality (28°). Infusion of 0127:B8 endotoxin produced a hypothermic response in two animals (*n* 6) with no alteration in core temperature of the remaining four animals. However, a severe inflammatory reaction with fever has been consistently obtained using intramuscular injection of 0·6 ml turpentine.

Fifteen male SD rats (mean weight 262 g) were studied using indirect calorimetry following turpentine injection. Animal core temperature was continually recorded. Within 12 h of injection there was a significant rise in EE from 8·11 (SD 1·16) to 10·05 (SD 2·14) watts/kg ($P<0·05$) (Wilcoxon Signed Ranks test) followed by a non-significant decrease in EE to 24 h (8·07 (SD 2·61) watts/kg) compared with the equivalent control period (8·77 (SD 2·37) watts/kg). A biphasic rise in core temperature was recorded. An initial increase of approximately 1·5° occurred within 30 min of injection. Temperature plateaued for approximately 5 h before rising once more to reach a new level approximately 2·5° above pre-injection values at 24 h.

Ten male SD rats (282 g) were also studied during an initial 24 h period of saline infusion followed by a simultaneous infusion of the catabolic hormones adrenaline (45 ng/kg per min), corticosterone (3·5 μg/kg per min) and glucagon (4·5 ng/kg per min) for up to 24 h. Within the first 12 h of ACG infusion there was a significant decrease in EE from 8·43 (SD 1·73) to 7·13 (SD 1·08) watts/kg ($P<0·05$) (Mann-Whitney test). Core temperature remained stable during ACG infusion. Infusion of saline alone produced no significant alterations in either EE or core temperature.

The reason for reduced EE following ACG is unclear. It may be due in part to reduced activity. Our results show that in rats, the metabolic and temperature responses observed during the inflammatory response differ from those recorded during catabolic hormone infusion. These differences in response may be due to involvement of other factors such as cytokines.

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Dietary molybdenum may enhance the inflammatory reaction to and hence rejection of gut nematodes in lambs. By N. F. SUTTLE, D. P. KNOX, K.W. ANGUS, F. JACKSON and R. L. COOP, *Moredun Research Institute, Edinburgh EH17 7JH*

A small dietary supplement of molybdenum (5 mg/kg dry matter (DM)) reduced the establishment of the intestinal parasite *Trichostrongylus vitrinus*, given as a trickle larval infection to lambs, but increased the pathogenicity, as measured by the severity of hypoalbuminaemia and growth retardation, without inducing hypocupraemia (Suttle *et al.* 1988). Since the form in which Mo is present in the digesta changes along the tract, effects on an abomasal parasite were studied.

Groups of six worm-free lambs were given challenge infections of *Haemonchus contortus*, with or without (controls) the dietary Mo supplement. Histological signs of inflammation in the abomasal mucosa were assessed and activity of Cu:Zn superoxide dismutase (SOD, *EC* 1.15.1.1) in homogenates of duodenal and jejunal mucosa were measured. In Expt 1, lambs were given a trickle infection of 500 larvae/d, 5 d/week, for 6 weeks and were slaughtered after 8 weeks. In Expt 2, lambs were given a single large dose of 10 000 larvae and slaughtered after 10 d. The diets were based on whole barley coated with a sugar solution containing urea; vitamins A, D and E; calcium sulphate and other minerals, with or without sodium molybdate: they contained 3 mg Cu/kg DM and were given from 2 weeks before challenge. Results of Expt 1 are shown in the Table.

Dietary Mo supplement	Total worm burden	Log_{10} IEMC in abomasum*	SOD (U/g protein)		Bathocuproin-reactive Cu (μmol/g protein)		Final ÷ initial liver Cu concentration
			Duodenum	Jejunum	Duodenum	Jejunum	
Without	4167	0·461	6537	14 511	0·78	1·44	0·54
With	907	1·585	5373	4863	0·86	0·73	0·44
SED	567	0·139	1707	2101	0·154	0·234	0·083

*Mean cell count in forty fields from an abomasal fold viewed at × 40.

Fewer worms were retrieved from the abomasum of Mo-supplemented lambs than from controls at the end of Expt 1 and intraepithelial mast cell (IEMC, or globule leucocyte) counts, indicative of acute-type hypersensitivity reactions (Miller, 1984), were correspondingly increased compared with controls ($P<0·01$). Mo reduced Cu concentrations and SOD activities in jejunal homogenates, but did not accelerate the depletion of liver Cu from the initial mean concentration of 300 (SE 29·6) mg/kg DM (nor induce hypocupraemia) in the lambs. Mo did not inhibit protease activity in or secreted by cultured *H. contortus* adults although it had done so with *T. vitrinus* (Suttle *et al.* 1988). In Expt 2, Mo did not impair the early invasive activity of the parasite nor alter histological changes in the abomasal mucosa. Results are consistent with the hypothesis that Mo is either necessary for the inflammatory reaction of lambs to prolonged challenge with nematode larvae or enhances that reaction by causing a localized depletion of Cu and, in particular, Cu:Zn SOD in the gastrointestinal mucosa.

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Thermogenic response to parenteral nutrition in septic man. By J. ARNOLD[1,2], K. SHIPLEY[1], N. SCOTT[1], R. A. LITTLE[2] and M. H. IRVING[2], [1]*Department of Surgery* and [2]*North Western Injury Research Centre, University of Manchester, Manchester M13 9PT*

Activation of the sympathetic nervous system (SNS) has been linked to nutrient (diet)-induced thermogenesis in man (Welle *et al.* 1980). Similarly, there is evidence to suggest that sepsis and trauma also stimulate the SNS (Wolfe & Shaw, 1985). The purpose of the present study was to investigate the combined effects of sepsis and parenteral nutrition on metabolic rate in man. To that end, the thermogenic response to intravenously infused parenteral nutrition (dextrose (400 g/l), amino acids (Synthamin 9) and micronutrients) was investigated in five septic (mean sepsis score of 17; Elebute & Stoner, 1983) and six non-septic (home parenteral nutrition) patients using indirect calorimetry.

Subjects had been receiving parenteral nutrition via a central venous catheter for at least 4 weeks before the study. Body-weight was measured and percentage body fat estimated from skinfold measurements at four sites immediately before each test. Resting metabolic rate (RMR) was measured with the ventilated canopy system for 1 h pre-infusion (after a minimum of 7 h fasting) and for 2 h during which approximately 323 kJ of the nutrient solution (2150 kJ in total) were infused. During the test, blood samples were periodically obtained from an indwelling catheter.

While septic patients had a resting oxygen uptake 19% higher than that of controls (4·70 (SE 0·03) *v.* 3·94 (SE 0·02) ml/kg lean body mass per min, $P<0·01$), both groups responded in a similar fashion to the nutrient solution. By the end of the 3 h study, RMR had risen in both groups by approximately 30% and, after accounting for the pre-infusion differences in RMR, no difference in nutrient-induced thermogenesis was revealed between the groups. Respiratory quotient, initially 8% higher in the septic patients (0·77 *v.* 0·89, $P<0·05$), was not significantly different between the two groups following 2 h of nutrient infusion. Respiratory quotient rose 9% in the non-septic patients during that period ($P<0·01$). Plasma noradrenaline (NA) levels were elevated pre-infusion in the septic patients (4·47 (SE 0·20) *v.* 2·46 (SE 0·12) nmol/l, $P<0·01$). While NA rose almost 25% during the first hour of nutrient infusion in the non-septic subjects, no change in plasma NA was noted in septic patients during the study. The results highlight the influence that nutrient-induced thermogenesis may have on the energy balance of parenterally fed patients.

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Energy balance in acute illness. By CERI J. GREEN, P. MCCLELLAND, A. A. GILBERTSON, R. G. WILKES, J. M. BONE and I. T. CAMPBELL, *Intensive Therapy Unit, University Department of Anaesthesia and Department of Renal Medicine, Royal Liverpool Hospital, Liverpool L69 3BX*

The sickest group of patients in hospital are those on intensive care. Previous measurements of energy expenditure (EE) in this group of patients have either been done over short periods and the results extrapolated to 24 h or have been made over one period of 24 h only. No studies have attempted to standardize energy intake (Damask *et al.* 1987).

We have measured the energy balance of ventilated patients on intensive care to (a) characterize energy balance, and (b) determine whether acute renal failure or (c) whether feeding all non-protein energy as carbohydrate or as a 50:50 mixture of fat and carbohydrate made any clinically significant difference to EE.

The criterion for admission was the need for respiratory support using intermittent positive pressure ventilation. EE was measured continuously using an Engstrom Metabolic Computer (Gambro Engstrom AB, Bromma, Sweden). All energy was intravenous and individual target energy intakes were standardized in proportion to the patient's fat free mass (FFM) calculated from body-weight and skinfold thickness. The standard was a patient of 70 kg body-weight and 250 g fat/kg. Patients were randomized to be given either 4·2 MJ carbohydrate as glucose, 0·5 litres 20% Intralipid (Kabivitrum Ltd, Uxbridge) and 1 litre Vamin 14 (Kabivitrum) over 24 h, or 8·4 MJ glucose and 1 litre Vamin 14.

Twenty-one patients (fourteen male, seven female) aged 18–76 (median 58) years, were studied for between 3 and 25 (median 11) d. In seven patients the respiratory failure was secondary to cardiovascular or respiratory disease and in fourteen to sepsis or trauma. Eleven patients were given glucose/fat/amino acids and ten were given glucose/amino acids only. Six of the glucose/fat group and seven of the glucose only group were in acute renal failure.

Individual patients were given the full regimen for between 0 and 100 (median 54)% of the time they were studied. One patient was given 50% more than the standard regimen for 8 d (32% of her study period). Energy intake when 'fully fed' was 180 (SE 4·2) kJ/kg FFM. Median 24 h EE was 142 (range 107–204) kJ/kg FFM per 24 h. EE of eleven patients lay within 8·4 kJ of this value. It represents 120·9 (range 89·2–148)% of basal EE predicted from the Harris Benedict equation. Variation in 24 h EE within individuals as represented by the coefficient of variation, was 5·4 (1·5–12·8)%.

There was no correlation between age and EE and no difference in EE between days when patients were fully fed and days when they received less than 84 kJ/kg FFM. There was no difference in 24 h EE between the patients in renal failure (146 (107–204) kJ/kg FFM) and those whose kidneys continued to function (140 (132–188) kJ/kg FFM). Protein metabolism contributed to (mean (SE)) 22·3 (1·8)% of the 24 h EE of the renal-failure group compared with 16·4 (1·8)% in the non-renal-failure group ($P<0·02$). There was no difference in EE whether non-protein energy was given entirely as carbohydrate or as a mixture of fat and carbohydrate.

Fourteen of the patients were in positive energy balance on the days when they received the full feeding regimen but only eight were in positive balance over the period of study and only two in positive balance by more than 4·2 MJ. No relation was apparent between energy balance and mortality.

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Damask, M. C., Schwarz, Y. & Weissman, C. (1987). *Critical Care Clinics* **3**, 71–76.

The nutritional intake of schoolchildren in Northern Ireland—a comparison of two school-meal systems. By MARGO E. BARKER, KATE A. THOMPSON, EILEEN M. T. EVASON and ROBERTA WOODS, *Centre for Applied Health Studies, University of Ulster, Coleraine BT52 1SA*

It has been demonstrated that the lunchtime meal eaten at school is an important factor in the diets of children in Great Britain (Wenlock *et al.* 1986). The 1980 Education Act gave Local Authorities the freedom to determine the form, content and price of school meals. As a result, some schools in Northern Ireland adopted a cash-cafeteria meal system providing extensive food choice, while others have retained the traditional meal system providing limited food choice. The present study assessed the nutrient intake of schoolchildren (all of whom were receiving free school meals) in the two systems mentioned.

Daily energy and nutrient intakes were calculated by 24 h recall in conjunction with the direct weighing of the school meal. The study was based on data from four single-sex schools, two girls-only and two boys-only, with each meal system being represented equally. Initially twenty-five pupils were randomly selected from each school. The sample was stratified by age, in the case of the girls the mean age (SD) was 12 (0·15) years, the boys' mean age being 13·3 (0·93) years. Nine children were absent from school on the recall-day, giving a final sample size of ninety-one. The mean daily intake (SD) of energy and nutrients by school-meal system are given in the Table.

	n		Energy (MJ)	Protein (g)	Fat (g)	Carbo-hydrate (g)	Dietary fibre (g)	Sugars (g)
Boys:								
Traditional	25	Mean	11·0	72·4	121·0	334·9	23·2	116·3
		SD	3·47	14·09	42·62	107·66	5·84	45·31
Cafeteria	22	Mean	12·3	64·4	140·6	381·0	26·2	108·0
		SD	2·68	18·54	31·52	92·84	6·49	38·23
Girls:								
Traditional	22	Mean	9·2	55·3	108·4	266·1	15·1	108·0
		SD	2·57	12·53	30·16	92·70	5·73	53·18
Cafeteria	22	Mean	13·8	84·8	162·8	398·3	26·1	133·4
		SD	3·59	19·43	40·86	124·70	7·66	83·66

The energy intakes of those children receiving the cafeteria meal tended to be greater than those receiving the traditional meal. The difference was particularly marked for the girls. A similar trend was apparent for fat, carbohydrate and dietary fibre intakes. Protein and sugar intakes were slightly greater in boys receiving the traditional meal. When the nutrient content of the school meals was calculated it was evident that their high fat content was exacerbated by the home diet. This effect was especially apparent for children taking the cafeteria meals, where food intake patterns at home were reflected in food choice at school.

Although the dietary information is particular to only one day, the implications with regard to fat intake are far-reaching in a community which experiences extraordinarily high mortalities from ischaemic heart disease.

Wenlock, R. W., Disselduff, M. M., Skinner, R. W. & Knight, I. (1986). *The Diets of British Schoolchildren*. London: H.M. Stationery Office.

Urinary excretion of 5-oxoproline in severe inflammatory illness. By B. MORAN[1], C. PERSAUD[2] and A. A. JACKSON[2], *Departments of* [1]*Surgery* and [2]*Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

Total parenteral nutrition (TPN) represents one of the most significant developments of the last decade in the perioperative management of patients with intestinal failure. However, nutritional support for severely ill patients, especially those with marked sepsis, is still less than optimal. The evidence is accumulating to show that glycine is a conditionally essential amino acid in a range of physiological and pathological states where the metabolic demand exceeds endogenous synthesis of the amino acid. There is circumstantial evidence which suggests that the demands for glycine may be particularly high following sepsis or trauma. The urinary excretion of 5-oxoproline can be used as an index of glycine status (Jackson *et al.* 1987). We have looked at the urinary excretion of 5-oxoproline in a series of patients referred for TPN.

Urine (24 h) was collected from twelve adults, who acted as controls, and fourteen patients who were referred for TPN for major perioperative complications. Twelve of the fourteen patients had obvious severe sepsis, with eight having a perforated bowel. 5-Oxoproline was isolated by short column chromatography, and measured enzymically as glutamic acid following acid hydrolysis. Creatinine was measured by Jaffe's method.

		5-Oxoproline (μmol/d)		Creatinine (mmol/d)		5-Oxoproline/ creatinine (μmol/mmol)	
	n	Mean	SE	Mean	SE	Mean	SE
Controls	12	237	58	12·9	1·1	15	3·7
Patients	14	567	102	8·8	1·9	73	10·0
Statistical significance: $P<$		0·01		0·09		0·001	

The daily excretion of 5-oxoproline in urine was significantly increased in the patients, expressed absolutely or as a ratio to creatinine excretion. The highest rate of excretion was found in the sickest group. The two patients without overt sepsis had an excretion of only 200 μmol/d, whereas those who died excreted 596 μmol/d on average.

These results might suggest that in severely ill patients requiring nutritional support by TPN, the ability to synthesize glycine in the body is insufficient to satisfy the demand.

This work was supported in part by Nestlé Nutrition Research Foundation and B. Braun.

Jackson, A. A., Badaloo, A. V., Forrester, T., Hibbert, J. M. & Persaud, C. (1987). *British Journal of Nutrition* **58**, 207–214.

Nitrogen and glucose metabolism by the liver of forage- and forage–concentrate-fed cattle.

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The experiment was undertaken to examine the hypothesis that either the pattern of supply of nutrients to, or their metabolism within, the liver could contribute to the reduced efficiency of utilization of amino-nitrogen, and hence less realization of growth potential previously observed (Beever & Siddons, 1985) in forage-fed animals.

Four Friesian steers (live weight 160–170 kg) were allocated to a 2×2 cross-over design and fed hourly on a forage (grass pellets) or forage–concentrate (50:50, grass:flaked maize pellets) diet at levels of 24 g dry matter (DM)/kg live weight (grass) and 19 g DM/kg live weight (grass/flaked maize) to provide equal metabolizable energy intakes. The forage diet and the forage–concentrate diet contained estimated in vivo organic matter digestibilities of 0·62 and 0·78 (DM basis) and 510 and 290 g neutral-detergent fibre/kg respectively.

Indwelling catheters were inserted into portal, hepatic and mesenteric veins and a carotid artery. Blood flow was measured using a dye-dilution technique with *p*-amino hipperic acid (PAH) which was infused from 90 min before, and during the sampling period.

		Forage			Forage–concentrate		
		PA	HP	SA	PA	HP	SA
Ammonia (mmol/min)	Mean	2·381	–2·049	0·332	1·113*	–1·483*	–0·370*
	SD	0·282	0·265	0·125	0·098	0·140	0·145
Urea (mmol/min)	Mean	–1·051	2·884	1·833	–1·093	0·932*	–0·161*
	SD	0·300	0·219	0·302	0·310	0·156	0·325
Glucose (mmol/min)	Mean	–1·404	3·858	2·454	–0·628	1·384*	0·756*
	SD	0·198	0·104	0·123	0·240	0·103	0·100

	Portal		Hepatic		Portal		Hepatic	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood flow (l/min)	7·51	1·10	8·73	1·20	6·83	1·00	8·04	1·20

PA, portal absorption of nutrients from the gut; HP, hepatic production of nutrients by the liver; SA, absorption by the splanchnic bed (liver and gut).

Negative values indicate uptake/absorption, positive values indicate output/production.

*Significantly different between diets at $P<0·05$ or better (Student's *t* test). *n* 3 or 4.

More ammonia (PA) was presented to the liver on the forage diet, with a correspondingly higher hepatic production (HP) of urea. Output of glucose by the liver of animals on the forage diet was also higher (all dietary differences $P<0·05$). However, the urea production by the liver (on both diets) could not be fully accounted for by the ammonia arriving at the liver in portal blood. It has previously been observed that glucose production by the liver was not fully accounted for by the amount of its major precursor, propionate, arriving at the liver.

One possible source of both N for urea synthesis and carbon for glucose synthesis would be amino acids; if the requirement for N and C from amino acids is greater on the forage diet, there could be less amino acids available for growth.

Beever, D. E. & Siddons, R. C. (1985). *Proceedings VIth International Symposium on Ruminant Physiology, Banff, Canada*, pp. 479–497.

Energy metabolism and fuel selection during high-energy feeding: a case study. By E. PULLICINO and M. ELIA, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

The purpose of the present study was to assess (1) the changes in thermogenesis and fuel selection in a malnourished patient who was fed intravenously with progressively increasing amounts of energy and (2) to relate these changes to the changes in body composition.

The patient was a 28-year-old cachectic male (53 kg) with a small intestinal fistula and inactive Crohn's disease. He was fed intravenously by nocturnal cyclic total parenteral nutrition (TPN) for a period of 62 d. The initial energy intake of 10 MJ/d was increased to 21 MJ/d in three stepwise increments of about 4 MJ/d. The increments were made in the glucose component of the feed; the fat (approximately 4 MJ/d) and amino acid intakes (12·2 g nitrogen/d) were kept constant. Forty-four measurements of resting energy expenditure (REE) were made using a computerized ventilated hood system; twenty-seven of these measurements were made towards the end of the daily TPN infusion which lasted 15 h, and seventeen measurements were carried out about 9 h after the infusion was stopped. Body composition was assessed by anthropometry.

Throughout the period of study, REE during the on and off feed periods differed by only about 0·5 kJ/min. However, REE increased from about 3·9 kJ/min at the beginning of the study to 7·4 kJ/min towards the end of the 9 week feeding period. This was accompanied by an increase in body-weight (from 53 to 72 kg) and estimated fat free mass (from 50·5 to 61·2 kg). REE rose by a progressively greater amount (ΔREE) with each increment of energy intake (ΔEI) (ΔREE/ΔEI; 0·12–0·26 for measurements made 4–7 d after each increment). The respiratory quotient (RQ) increased progressively to a value of up to 1·2, and persisted above 1·0 even 9 h after the feed was stopped. Both REE and RQ decreased sharply during the 3 d starvation period which followed high-energy feeding (RQ 0·7 and REE 5·7 kJ/min).

The results suggest: (1) the increase in REE observed at the end of the 9 week feeding period was due to a combination of factors including an increase in lean body mass and diet-induced thermogenesis, (2) the increase in REE due to diet-induced thermogenesis accounts for dissipation of only a small proportion of total energy infused, (3) net lipid synthesis from carbohydrate occurred during both the on and off feeding periods.

The effect of acute undernutrition on rat jejunal and ileal secretion in vitro. By M. M. C. PEREIRA, R. J. LEVIN and A. YOUNG, *Department of Biomedical Science, The University, Sheffield S10 2TN*

An acute restriction in food intake to 33% of normal for 9 d (AU; equivalent to 8 g/d) makes the rat small intestine hypersecrete in response to cholinomimetic challenge (Young *et al.* 1988). We have investigated this phenomenon further in the rat jejunum and ileum using both Ca^{2+}-mobilizing agonists and agents which act via adenylate cyclase/cAMP.

Tissues were obtained from male Sheffield-strain Wistar rats (230–250 g) fed on diet CRM (Labsure) and killed by intraperitoneal injections of sodium pentabarbitone (60 mg/kg body-weight). Electrogenic secretion in vitro was assessed as the short-circuit current (Isc) across sheets of jejunum and ileum stripped of their external muscle layers, previously removed from fed (*ad lib.*) control and acutely undernourished (AU) rats. Both basal Isc and maximal increases in Isc above the basal level (ΔIsc) in response to secretagogue challenge were measured. The secretagogues used were acetylcholine (ACh, 1 mM), bethanecol (BCh, 1 mM) and 5-hydroxytryptamine (5-HT, 50 μM) which all act via Ca^{2+}, as well as prostaglandin E_2 (PGE_2, 28 μM), dibutyryl cAMP (cAMP, 1 mM) and forskolin (F, 5 μM) which act via adenylate cyclase/cAMP.

The basal Isc of the AU rats did not differ from fed controls in any area of the small intestine (all $P>0{\cdot}05$). The Ca^{2+}-mobilizing agonists caused increases in the ΔIsc in the AU rats above fed levels in both the jejunum and ileum (jejunum: ACh +45% $P<0{\cdot}001$; BCh +91% $P<0{\cdot}001$; 5-HT +153% $P<0{\cdot}001$; ileum: ACh +59% $P<0{\cdot}001$; BCh +49% $P<0{\cdot}01$; 5-HT +48% $P<0{\cdot}001$).

However, none of the cAMP-acting agonists caused a significant increase in secretion over fed levels in either the jejunum or ileum (jejunum: cAMP +17% $P>0{\cdot}05$; PGE_2 +33% $P>0{\cdot}05$; F +14% $P>0{\cdot}05$; ileum: cAMP +5% $P>0{\cdot}05$; PGE_2 +22% $P>0{\cdot}05$; F +30% $P>0{\cdot}05$).

The results clearly show that the secretory activity of Ca^{2+}-mobilizing agonists is potentiated in the jejunum and ileum of AU rats, whilst that of agents acting via adenylate cyclase/cAMP is unaffected by a period of acute undernutrition.

Young, A., Nzegwu, H. C., & Levin, R. J. (1988). *Proceedings of the Nutrition Society* **47**, 127A.

An animal model of injury: the value of turpentine injections for studying the acute phase changes in tissue protein metabolism. By M. WUSTEMAN, G. JENNINGS and M. ELIA, *Dunn Nutritional Laboratory, Downhams Lane, Milton Road, Cambridge CB4 1XJ*

Various forms of trauma have been used as models for studying the metabolic response to injury, e.g. fractures, burns, ischaemic leg injury, infections and repeated injections of endotoxin. Aseptic abscesses resulting simply from single injections of turpentine have also been used for this purpose but little is known about their effects on tissue protein metabolism. Therefore we have used this form of 'injury' to confirm a range of acute phase responses and to further investigate the changes in intramuscular concentration of amino acids and protein synthesis.

Young male rats (38–40 d) were injected subcutaneously in two dorso-lumbar sites with mineral turpentine (5 ml/kg body-weight). Preliminary work has shown that turpentine administration reduces nutrient intake by about 30–55% ($P<0.025$) and therefore pair-feeding experiments were undertaken to distinguish the metabolic effects of aseptic abscesses from those of decreased nutrient intake. The Table illustrates the changes in circulating albumin and α_2 macroglobin concentrations (measured by specific antibody techniques) as well as the changes in muscle glutamine concentration (measured enzymically) and muscle protein synthesis (measured by the phenylalanine flooding technique of Jepson *et al.* (1986)) 48 h after the turpentine injection.

Effect of turpentine injections on some acute phase responses

	Fed *ad lib.*	Turpentine injected	Pair-fed controls
Serum albumin (g/l)	28·0	22·4*	28·1
Serum α_2 macroglobulin (g/l)	<0·05	4·7*	<0·05
Muscle glutamine (mmol/kg wet wt)	7·4	3·8*	7·3
Muscle protein synthesis (%/d)	17·5	6·8*	12·6†

*$P<0.005$ compared with the other two groups.
†$P<0.005$ compared with the *ad lib.*-fed group.

The results confirm the development of hypoalbuminaemia and the dramatic responsiveness of serum α_2 macroglobulin (an acute phase protein in the rat) to inflammatory stimuli. These changes were associated with depressed intramuscular glutamine concentrations and decreased muscle protein synthesis which could be distinguished from the effects of reduced dietary intake.

It is concluded that turpentine-induced inflammation in the rat reproduces several of the important changes in protein metabolism which follow trauma and sepsis and that this model can be used to study how different dietary regimens can modify the metabolic response to injury.

Jepson, M. M., Pell, J. M., Bates, P. C. & Millward, D. J. (1986). *Biochemical Journal* **235**, 329–336.

Effect of systemic infection on intestinal permeability in man. By M. ELIA, C. A. NORTHROP and P. G. LUNN, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL* and A. GOREN, *Department of Gastroenterology, University of Ankara, Turkey*

The intestinal mucosa provides a selective 'barrier' to the uptake of different exogenous substances. It has been suggested that systemic illness may affect this intestinal mucosal function and allow potentially harmful substances, including bacteria, to enter the body. In the present study we have used a sensitive triple intestinal permeability test (Elia *et al.* 1987*a*,*b*) to assess the possible effects of acute systemic infections (septicaemia, pneumonia, urinary tract infections) on gastrointestinal integrity in man. The subjects (*n* 7) were studied within 48 h of emergency admission to hospital. For comparison of results a group of normal subjects (*n* 24) and a group of patients with an enteropathy (coeliac disease, *n* 15) were also studied. All the subjects were fasted overnight and given an oral dose of a solution (50 ml) containing a mixture of lactulose (10 g), mannitol (5 g and 0·5 μCi) and [^{51}Cr]EDTA (30 μCi). Urine was collected between 0–6, 6–12, and 12–24 h after dosing. None of the subjects received non-steroidal anti-inflammatory drugs or alcohol, but the patients with bacterial infections received antibiotics. The infected patients were anorectic but previous work has shown that a diet containing as little as 1255 kJ (300 kcal)/d does not affect the intestinal permeability test (Elia *et al.* 1987*b*).

The pattern of excretion of lactulose, mannitol and [^{51}Cr]EDTA, and their relative urinary ratios, are indicated in the Table. None of the results of the infected group were significantly different from normal. Furthermore, the excretion of [^{51}Cr]EDTA between 6–12 h and 12–24 h, which is influenced by colonic absorption (Elia *et al.* 1987*b*), was also not significantly different from normal. In contrast, patients with coeliac disease showed a marked increase in the absorption and excretion of lactulose and [^{51}Cr]EDTA, and a decrease in the absorption and excretion of mannitol.

Excretion (0–6 h) of lactulose, mannitol and [^{51}Cr]EDTA (% of administered dose) and the ratio of lactulose:mannitol and [^{51}Cr]EDTA:mannitol, after oral dosing

	Lactulose		Mannitol		[^{51}Cr]EDTA		Lactulose: mannitol		[^{51}Cr]EDTA: mannitol	
Subjects	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Normal	0·26	0·02	13·3	0·9	0·38	0·04	0·021	0·003	0·030	0·005
Coeliac	1·02***	0·16	6·7***	1·1	1·23***	0·14	0·152***	0·023	0·184***	0·028
Infected	0·26	0·04	14·3	1·55	0·47	0·13	0·018	0·004	0·033	0·009

***$P<0·001$.

The results confirm the sensitivity of the triple permeability test in detecting the presence of enteropathy (coeliac disease; Behrens *et al.* 1987) but provides no evidence for the concept that systemic infections, studied under the conditions specified, alter gastrointestinal permeability. However, the study does not exclude the possibility that more severe infections, or infections before treatment, may produce significant changes in intestinal permeability.

Behrens, R. H., Szaz, K. F., Northrop, C., Elia, M. & Neale, G. (1987). *European Journal of Clinical Investigations* **17**, 100–105.

Elia, M., Behrens, R., Northrop, C., Wraight, P., & Neale, G. (1987*a*). *Clinical Science* **73**, 197–204.

Elia, M., Goren, A., Behrens, R., Barber, R. W. & Neale, G. (1987*b*). *Clinical Science* **73**, 205–210.

Extracellular proteolytic activity of hyperoxic-exposed preterm guinea-pigs. By G. PHILLIPS and F. J. KELLY (Introduced by R. F. GRIMBLE), *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

Hyperoxic exposure (95% oxygen) of premature guinea-pigs resulted in extensive lung injury which was associated with elevated numbers of inflammatory cells in the airways (Kelly & Phillips, 1989). These cells, when activated, release a number of proteolytic enzymes which if not inhibited will produce extensive connective tissue damage. The objective of the present study was to determine whether elaboration of inflammatory-cell-derived proteolytic enzymes overwhelmed the antiproteolytic defences of the preterm guinea-pig exposed to hyperoxia.

Bronchoalveolar lavage (BAL) was performed on 3 d-premature animals (term 68 d), exposed to either 21% O_2 (control) or 95% O_2 (hyperoxic) for up to 96 h. Lung effluent elastase activity was measured against [^{3}H]elastin (free activity) and the synthetic substrate *N*-succinyl-L-trialanyl-*p*-nitroanilide (SLAPN) (free and α_2-macroglobulin bound activity). α-1-Protease inhibitor activity (α_1-PI) was measured as BAL inhibitory activity on porcine pancreatic elastase (PPE) degradation of SLAPN.

BAL proteolytic and antiproteolytic activities (n *4–6*)

	Control (21% O_2)		Hyperoxic (95% O_2)	
Proteolytic activity	Mean	SD	Mean	SD
[^{3}H]elastin (ng PPE/ml BAL)	nd		nd	
SLAPN (ng PPE/ml BAL)	21·6	12·2	49·0*	1·5
α_1-PI activity (μg PPE/ml BAL)	1·63	0·61	18·4**	2·70

nd, Not detected.
Significantly different from control value: *$P<0·05$, **$P<0·001$.

Free elastase activity could not be detected in BAL samples from either control or hyperoxic exposed animals. However, elastolytic activity against the synthetic substrate, SLAPN, was elevated twofold following 96 h hyperoxia. This result indicates that the concentration of elastase bound to α_2-macroglobulin was increased in these animals. α_1-PI activity was increased twelvefold in lung effluent obtained from these animals. In combination, these results demonstrate an increase in extracellular proteolytic activity in the lungs of hyperoxic exposed animals. However, this is more than compensated for by the large increase in anti-protease activity, due probably to the increased leakage of serum α_1-PI into the alveoli.

This work was supported by the Wellcome Trust.

Kelly, F. J. & Phillips, G. (1989). *Proceedings of the Nutrition Society* **48**, 60A.

Effect of endotoxin and turpentine injections on intestinal permeability in the rat. By P. G. LUNN, C. A. NORTHROP, G. JENNINGS and M. ELIA, *Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

Systemic stress, trauma and endotoxin administration may reduce host defences and allow large molecules from the gut to enter the body. In addition systemic stress may allow bacteria to translocate from the gut into the mesenteric nodes and other parts of the body. These changes might be due to a number of factors, including an impairment of the host immune defences and an increase in intestinal permeability.

The present study aimed to assess whether systemically administered endotoxins and asceptic abscesses produced by turpentine injection increase gastrointestinal permeability. Intestinal permeability was assessed by measuring urinary excretion (0–5 h) of orally administered lactulose and mannitol. These substances are not metabolized by the tissues of the body but are quantitatively excreted in urine. Rats, 100–150 g body-weight, were dosed with lactulose (16 mg), mannitol (4 mg) and 0·25 μCi D-[1−^{14}C] mannitol in 100 μl water. Three types of endotoxin, *Escherichia coli* 0111:B4 (TCA extract), *E. coli* 0111:B4 (phenol extract) and *Salmonella typhosa* (phenol extract) (3 mg/kg body-weight) were injected subcutaneously on the evening of day 1, morning and evening of day 2 and morning of day 3. Turpentine (5 ml/kg body-weight) was given as a single subcutaneous dose on the evening of day 1. Saline (9 g sodium chloride/l) control and pair-fed animals were also studied.

The urinary lactulose:mannitol ratios are shown in the Table.

	Saline		*E. coli* (TCA)		*E. coli* (phenol)		*S. typhosa* (phenol)		Turpentine	
Day	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	0·68	0·05	0·72	0·10	0·66	0·05	0·70	0·05	0·76	0·08
2	0·74	0·06	0·97*	0·11	1·00*	0·12	0·67	0·07	0·86	0·10
3	0·78	0·08	0·81	0·10	0·79	0·11	1·50	0·49	0·83	0·16
4	0·75	0·11	0·88	0·07	0·74	0·08	0·75	0·04	0·75	0·09

*$P<0·05$ (paired t test), seven rats per group.

Both phenol and TCA extracts of *E. coli* endotoxin caused a significant rise in the permeability ratio on day 2. This change was due to a decrease in mannitol excretion rather than an increase in lactulose values. Neither *S. typhosa* nor turpentine administration had any significant effect on urinary levels of lactulose or mannitol. Animals pair-fed to those given endotoxin or turpentine showed no change in permeability.

The results suggest that systemic endotoxins at the dose given may produce a temporary change in intestinal permeability, but this is not associated with an increased permeability of the small intestine to large molecules (lactulose). However, it does not exclude the possibility that such a change might be seen in different nutritional states.

Hormone and nutrient interaction in the control of growth: role of growth hormone and insulin-like growth factor-1. By J. M. PELL, M. GILL, D. E. BEEVER, A. R. JONES and S. B. CAMMELL, *AFRC Institute for Grassland and Animal Production, Hurley, Berks SL6 5LR*

The aim of the present investigation was to determine the relations between nutrient intake and hormonal status and their interactions in the control of growth. In addition, the magnitude of the anabolic response to exogenous growth hormone (GH) is variable in ruminants but the factors which contribute to this have not been elucidated.

Seventy-two lambs were randomly allocated to one of three protein diets containing 120 (L), 160 (M) or 200 (H) g crude protein/kg (soya:fishmeal, 3:1) offered either *ad lib.* (A, average intake approximately 50 g/kg live weight) or at 30 g/kg live weight (R). (Diet formulation, kg/tonne: barley 740 (L), 645 (M), 545 (H); ground straw 170; protein 17 (L), 112 (M), 212 (H); molassine meal 50; limestone 23; mineral mix 1.) Within each diet, lambs were randomly allocated to daily injections of either saline (−, 9 g sodium chloride/l) or GH (+, 0·1 mg/kg per d). All treatments commenced at 9 weeks of age and continued for 10 weeks; the animals were weighed and a jugular blood sample was taken weekly.

Treatment	Live-wt gain (g/d)	Empty body-wt (kg)	Liver wt/empty body-wt (g/kg)	Total plasma IGF-1 at 10 weeks (ng/ml)
LR−	117·4	23·7	19·6	227
LR+	139·7	25·5	18·4	571
LA−	397·8	39·0	26·3	509
LA+	391·0	41·7	26·4	776
MR−	105·7	22·5	17·6	260
MR+	159·7	26·3	18·6	562
MA−	369·5	40·8	26·7	666
MA+	383·5	40·5	30·8	806
HR−	114·1	23·7	26·5	435
HR+	136·4	24·1	25·0	528
HA−	374·6	38·4	28·9	711
HA+	401·2	44·5	31·7	877
SD	47·7	4·3	4·3	156

Growth hormone treatment induced significant increases in plasma insulin-like growth factor-1 (IGF-1) concentrations ($P<0·001$), live-weight gain ($P<0·05$), empty body-weight ($P<0·02$) and liver weight ($P<0·001$). Increased protein intake resulted in additional increases in IGF-1 concentrations ($P<0·05$) and liver weight ($P<0·001$) but not of live-weight gain or empty body-weight.

Measurements of heat production for the lambs on diets HR+ and HR− were obtained by open circuit calorimetry at treatment weeks 0, 5 and 10 and revealed a significant decline in relation to animal age (8·07, 7·29 and 6·88 MJ/kg dry matter intake (DMI)) but no significant effect due to GH (−GH 7·52, +GH 7·30 MJ/kg DMI).

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Cross-sectional study of intestinal permeability in infants from a Gambian village. By C. A. NORTHTROP, P. G. LUNN and R. M. DOWNES, *Dunn Nutrition Laboratories, Cambridge CB4 1XJ* and *Keneba, The Gambia*

Diarrhoeal disease remains the major cause of growth faltering, morbidity and death of infants in the rural areas of The Gambia. Specific intestinal pathogens can be detected in only a minority of disease episodes and the chronic/intermittent nature of the illness suggests that some long-standing post-enteritis enteropathy may be responsible. The measurement of intestinal permeability using lactulose and mannitol provides a non-invasive technique for assessing pathogenic alterations in the structure and integrity of the small intestine mucosa and the method is ideally suited for field conditions.

In the present study all male children in the village of Keneba aged between 2 and 24 months were investigated. Between 08.00 and 09.00 hours on the day of the test, each child was given a drink which contained the two sugars, lactulose and mannitol. For each kg of body-weight the infant received 400 mg lactulose (in the form of Duphalac) and 100 mg mannitol in 2 ml water. Urine was collected in the following 5 h period by means of a urine bag, which was emptied as required into a bottle containing chlorhexidine gluconate (0·2 g/l). Total urine volume was noted and a portion was taken for analysis.

The ratio of lactulose:mannitol (L:M) and the recovery of these two sugars in the urine are shown in the Table.

				% Recovery			
		L:M		Lactulose		Mannitol	
Age group (months)	*n*	Mean	SEM	Mean	SEM	Mean	SEM
2–9	12	0·56**	0·07	0·36	0·03	2·31*	0·34
9–15	19	1·14	0·14	0·41	0·06	1·54	0·19
15–24	15	0·72	0·09	0·49	0·06	2·81**	0·35

Values significantly different from age 9–15 months (*t* test): *$P<0·05$, **$P<0·01$.

The L:M of children in the 9–15 month age group was significantly greater than either the younger or older children. The cause of this change in ratio is apparent from the recovery figures and was clearly the result of a marked reduction in urinary mannitol values. No significant difference in lactulose recovery was found in any of the age groups. These results suggest that there is indeed damage to the small intestinal mucosa, particularly in the 9–15 month age group, and it is probably of importance that this coincides with the time of poorest growth (Lunn *et al.* 1979).

Lunn, P. G., Whitehead, R. G., Cole, T. J. & Austin, S. (1979). *British Journal of Nutrition* **41**, 73–84.