The Editors of the Proceedings of the Nutrition Society *accept no responsibility for the abstracts of papers read at the Society's meetings for original communications.*

PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Fifty-third Meeting of the Nutrition Society for the presentation of oral communications was held as parallel sessions in the Ernest Walton and Jonathan Swift Lecture Theatres, Trinity College, Dublin, on Monday, Tuesday and Wednesday, 25–27 July 1988.

https://doi.org/10.1079/PNS19890018 Published online by Cambridge University Press

Why is there increased lipid droplet size in brown adipose tissue of genetically obese (*ob/ob*) mice? By M. HOULDEN and J. F. ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Irish Republic*

Qualitative studies have reported a tendency towards multilocularity in active brown fat cells and this has been demonstrated quantitatively in the hamster (Ahlabo & Barnard, 1974). By examining the droplet surface area per unit volume of tissue (interfacial area) we have attempted to identify a function of droplet structure which may, in extreme circumstances such as those which occur in the obese mouse, be of significance in determining the thermogenic capacity of brown adipose tissue (BAT).

Animals, reared in the cold (17°) or warm (28°), were killed by cervical dislocation and BAT samples removed from the interscapular site at the time points indicated in the Table. After removal, samples were fixed in osmium tetroxide and embedded in resin. Semi-thin sections were cut and stained with toluidine blue for 1 min. Sections were then examined under oil immersion. A MOP videoplan unit with a 'Bildanalyse' software package was used to measure areas of individual droplets; 100 from each animal were measured. Areas of individual droplets were converted to surface areas and volumes using standard stereological formulae.

			Surface area of droplets ($\mu m^2/\mu m^3$)					
		Age . . .	26		35		56	
Ambient temperature (°)	Genotype	*n*	Mean	SD	Mean	SD	Mean	SD
28	Lean	5	1·649	0·114	2·415	0·590	1·982	0·371
28	Obese	5	1·104***	0·197	1·190***	0·255	0·782***	0·113
17	Lean	5	2·898†††	0·264	2·426	0·605	2·866††	0·369
17	Obese	5	2·054**†††	0·282	2·098†††	0·328	2·609†††	0·553

Significantly different from animals raised at same temperature: $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.
Significantly different from same genotype at 28°: $\dagger\dagger P<0{\cdot}01$, $\dagger\dagger\dagger P<0{\cdot}001$.

The results demonstrate a significant increase in interfacial area brought about by cold rearing in both genotypes. It is also noted that at 28°, obese mice had a consistently lower interfacial area than lean animals, while such a difference was only significant at 26 d for cold-reared mice. The tendency towards what is an energetically less costly unilocular state also reduces the interfacial area between the lipid droplets and cytoplasm. The energy saved by maintaining unilocularity is due to the reduction of surface tension per unit volume of lipid. If multilocularity *per se* requires more energy to maintain, it must have a functional significance, perhaps related to substrate availability for lipolysis. Thus the presence of unilocularity in *ob/ob* mice, as well as being due to reduced activity in BAT, may itself prevent complete activation in response to an acute stimulus.

Ahlabo, I. & Barnard, T. (1974). *Journal of Ultrastructure Research* **48**, 361–376.

Results refuting volatile fatty acids *per se* as signals of satiety in ruminants. By W. L. GROVUM and W. W. BIGNELL, *Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1*

Acetate (Ac) in the rumen and propionate (Pr) in the ruminal veins and the liver have come to be regarded as signals of satiety in ruminants (Baile & Forbes, 1974; Anil & Forbes, 1980) even though Ternouth & Beattie (1971) demonstrated conclusively that intraruminal injections of sodium Ac and NaPr were no more effective in depressing food intake by hungry sheep than equimolar loads of Na chloride. Doubts about volatile fatty acids as satiety signals were raised again by Grovum (1987) because the rumen was found to be the site mediating the anorexic effects of ruminal NaCl loads (Carter & Grovum, 1988) and water was the control for intraruminal loads of NaAc that depressed intake in the majority of studies. Also, osmotic controls were not used for vascular infusions (ruminal vein, portal vein) of NaPr when it was infused at high rates to depress intake. Furthermore, the Pr effect, if any, may be mediated by insulin as Pr stimulates the release of insulin which depresses food intake in sheep (Deetz & Wangsness, 1980).

Seven lambs were fitted with rumen cannulas and fed *ad lib.* on lucerne pellets. On an experimental day, they were fed from 08.00 to 09.00 hours and then deprived of food but not water until 14.00 hours. At 13.50 hours in Expt 1, drinking water was withdrawn and the rumen of each sheep was loaded by gravity flow with 283 ml saline (control at 275 Osmol, 0·4 Osmol NaCl, NaAc or PEG-400, or 0·8 Osmol of these substances according to a 7×7 Latin-square design. In Expt 2, NaPr replaced NaAc.

		Mean food intake (g) from 14.00 to 14.30 hours	
	Osmol	Expt 1	Expt 2
Saline control	275	314[a]	367[a]
NaCl	0·4	242[b]	286[b]
NaAc	0·4	224[bc]	—
NaPr	0·4	—	271[b]
PEG-400	0·4	247[b]	283[b]
NaCl	0·8	186[cd]	182[cd]
NaAc	0·8	146[d]	—
NaPr	0·8	—	173[d]
PEG-400	0·8	167[d]	218[c]

[a–d] Within experiments the overall treatment effects were significant ($P<0·001$), and values with different superscript letters were significantly different ($P<0·05$).

The results showed no response to Ac *per se* in the rumen over and above its osmotic effect and no effect of NaPr above that due to NaCl at both osmotic loads and that due to PEG-400 at the lower load. There would have been ample time for absorption of Pr into the ruminal veins and portal system during the 30 min feeding period so the results do not support the commonly held view that Pr specifically is sensed in the ruminal veins or the liver to depress food intake.

Ternouth & Beattie (1971) were largely right in relegating the intake-depressing effects of intraruminal Ac and Pr loads to their effects on the osmotic pressure of rumen fluid. Insulin could, however, mediate part of the effect of Pr once it reaches the systemic circulation. Silage intakes could be particularly affected by rumen tonicity.

Funding from NSERC (A2377) and OMAF is acknowledged.

Anil, M. H. & Forbes, J. M. (1980). *Journal of Physiology* **298**, 407–414.
Baile, C. A. & Forbes, J. M. (1974). *Physiological Reviews* **54**, 160–214.
Carter, R. R. & Grovum, W. L. (1988). *Proceedings of the Nutrition Society* **47**, 155A.
Deetz, L. E. & Wangsness, P. J. (1980). *Journal of Nutrition* **110**, 1976–1982.
Grovum, W. L. (1987). In *Feed Intake by Beef Cattle*, pp. 1–40 [F. N. Owens, editor]. Stillwater: Oklahoma State University.
Ternouth, J. H. & Beattie, A. W. (1971). *British Journal of Nutrition* **25**, 153–164.

Brown adipose tissue: structural changes related to nutritional status in the mouse. By CHRISTINE MURPHY, ELINOR A. ARBUTHNOTT and J. F. ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Irish Republic*

There have been many observations relating biochemical indices of brown fat function to nutritional status, including studies specific to the Aston strain of mouse (Trayhurn *et al.* 1982). As related structural changes have not been studied we have examined the histological appearance of brown adipose tissue in underfed, *ad lib.*-fed and overfed male lean Aston mice.

Food intake was measured in *ad lib.*-fed controls (pelleted stock diet). The underfed mice were individually housed and were given 50% by weight of their average daily intake in the pre-experimental period. Overfed mice were offered chocolate *ad lib.* in addition to access to stock diet. Chocolate has been shown to stimulate energy intake in Aston strain mice (Younger & Trayhurn, 1984) and can be readily quantified. Animals were maintained at thermoneutrality (28°) on a 12 h light–12 h dark cycle. Body-weight and energy intake were recorded. After 21 d on the dietary regimen animals were killed by cervical dislocation. Interscapular brown fat pads were dissected out and immediately fixed in glutaraldehyde (30 g/l). Tissues were dehydrated and resin-embedded by standard techniques. Semi-thin sections (1 μM) were cut and analysed for average lipid droplet size by quantitative image analysis. Mean results are given in the Table.

Nutritional status	*n*	Body-wt (% initial wt) Mean	SD	Energy intake (kJ/d) Mean	SD	Lipid droplet size (μm²) Mean	SD	Mitochondrial activity
Underfed	6	85·3*	3·7	29·9*	6·0	71	65	Inactive
Ad lib.-fed	6	97·8	2·9	52·0	8·9	309	195	Inactive
Overfed	6	115·0*	3·5	89·4*	3·2	27*	13	Active

Significantly different from *ad lib.*-fed group: $*P<0·05$.

In the overfed animals lipid droplet size decreased. Such an appearance is characteristic of active brown adipose tissue in cold-exposed animals, as demonstrated by Ahlabo & Barnard (1974). Lipid droplet size fell in underfed animals too, but with much less total lipid per field, presumably a consequence of the depletion of fuel stores as reflected in the weight loss. This evidence parallels the biochemical changes (Trayhurn *et al.* 1982) and supports the hypothesis that brown adipose tissue has an energy regulatory function.

Ahlabo, I. & Barnard, T. (1974). *Journal of Ultrastructure Research* **48,** 361–376.
Trayhurn, P., Jones, P. M., McCTURE Research **48,** 361–376.
Trayhurn, P., Jones, P. M., McGuckin, M. M. & Goodbody, A. E. (1982). *Nature* **295,** 323–325.
Younger, K. M. & Trayhurn, P. (1984). *Irish Journal of Medical Science* **153,** 409.

Polyunsaturated fatty acid (PUFA) content of rabbit meat: a potential source of PUFA for human nutrition. By T. W. GRIFFITHS*, G. GANDEMER, MICHELE VIAU and P. VEDRENNE, *Laboratoire d'Etude des Interactions des Molécules Alimentaires, Institut National de la Recherche Agronomique, rue de la Geraudiere, 44072 Nantes, France*

Recent papers show that lean meat contains an appreciable amount of long-chain polyunsaturated fatty acids (PUFA), especially pork and chicken muscle (Sharma *et al.* 1987; Ackman *et al.* 1988). Little is known of the lipid composition of the rabbit. The aim of the present study was to determine the lipid content and fatty acid composition of the main edible portions of the rabbit carcass.

Twelve female rabbits (hybrid N2 Californian 67) fed on a commercial diet were killed at the age of 11 weeks. The liver tissue and three muscles, longissimus dorsi (LD), biceps femoris (BF) and psoas major (PM) were dissected and their lipid contents and fatty acid compositions determined. Results are given in the Table.

	Liver		LD		BF		PM	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Lipid content (g/kg meat)	66	6	11	1	17	4	10	2
Fatty acids (%)								
Saturated (S)	42·1	0·9	36·9	1·0	37·2	3·7	39·8	1.3
Monounsaturated	17·5	1·6	25·6	1·4	25·7	2·6	22·8	0·9
Polyunsaturated (P)	40·5	1.0	37·6	2·2	37·6	2·3	37·4	0·8
18:2ω6	27·9	0·7	24·4	1·8	25·7	2·6	25·9	1·2
Long chain*	7·2	0·5	6·8	1·2	5·5	1·1	6·3	1·2
18:3ω3	3·7	0·4	3·7	0·6	4·5	0·5	3·2	0·4
Long chain†	1·6	0·2	2·7	1·2	2·0	0·9	2·0	0·9
P:S	0·96	0·02	1·02	0·09	1·01	0·06	0·94	0·05

*20:0ω6 + 20:3ω6 + 20:4ω6 + 22:4ω6 + 22:5ω6.
†20:5ω3 + 22:5ω3 + 22:6ω3.

Liver tissue had a higher lipid content than muscle. The lipid content of rabbit muscle was close to that reported for pork and chicken meat. Both liver and muscle tissues contained a high proportion of PUFA. The P:S ratio was higher than that of pork and chicken meat. ω6 PUFA accounted for 80% of total PUFA. Linoleic acid and arachidonic acid accounted for 80 and 16% respectively of the ω6 fatty acids. Both liver and muscles contained a small proportion of long-chain PUFA as 22:4ω6 and 22:5ω6. ω3 PUFA were mainly linolenic acid but very-long-chain ω3 PUFA were present in appreciable amounts in liver as well as in muscles.

On the basis of its lipid composition, rabbit meat would seem to be a suitable component of the human diet.

Ackman, R. G., Lamothe, F., Hulan, H. W. & Proudfoot, F. G. (1988). *N-3 News* **3** (1), 1–4.
Sharma, N., Gandemer, G. & Goutefongea, R. (1987). *Meat Science* **19**, 121–128.

*Present address: The National Food Centre, Dunsinea, Castleknock, Dublin 15, Irish Republic.

Morphological changes in brown adipose tissue lipids during cold acclimation in the golden hamster (*Mesocricetus auratus*). By GABRIELLE MCKEE and J. F. ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Irish Republic*

In the cold-acclimated rat, brown adipose tissue (BAT) is the primary organ of heat production (Foster & Frydman, 1979). Chaffee *et al.* (1964) pointed to BAT as the chief source of lipids as fuel for thermogenesis in acute cold exposure in the golden hamster. We have examined the time course of increased thermogenesis and parallel changes in BAT lipids and body-weight in the hamster.

Oxygen consumption (24 h), BAT lipid content (measured by microscopy) and body-weights were measured. Interscapular BAT samples were fixed in osmium tetroxide, dehydrated and embedded in resin. A standard area (200 μm^2) of an electron micrograph was used to assess percentage lipid content.

Oxygen consumption, body-weight and BAT lipid content in cold-acclimated hamsters

	Summer conditions†	Period of cold acclimation‡ (weeks)							
		24 h	1	2	3	5	6	7	10
Total O_2 consumption (ml/g per 24 h) (*n* 8):									
Mean	72·3		109·8*	134·4*	150·9*	147·5*	160·7*	136·5*	116·9
SD	6·6		42·6	30·8	30·2	40·4	31·1	37·9	34·8
Body-wt (g) (*n* 20):									
Mean	112·24		103·51	91·47*	90·75*	91·73*	98·06*		
SD	15·48		11·52	10·21	9·81	10·61	13·31		
BAT lipid content (mg/g):									
Mean	677	403*	563		672	518		602	534
SD	120	224	119		67	92		123	104
n	4	8	8		5	9		10	9

*Significantly different from 'summer' values ($P<0·05$).
†Continuous light, 30°. ‡Complete darkness, 10°.

On day 1 of cold exposure, decreasing lipid content along with increasing lipid droplet number indicated BAT activity (Ahlabo & Barnard, 1974). It is likely that during this period BAT was a main site and source of substrate for thermogenesis. Later during cold acclimation (1–3 weeks) BAT maintained its active form but with no further decrease in lipid content. Body-weight decreased during this period. Thus dietary intake appeared to be inadequate for thermogenic requirements in this period and body stores were used. During the next period of cold acclimation (3–7 weeks) there was no significant change in BAT lipid levels or body-weight, while BAT maintained its active form. Thermogenic needs during this period were probably met by intake alone. Thermogenesis eventually began to decline at week 7 of cold acclimation.

The results show that BAT may play an important role in cold acclimation in the golden hamster.

Ahlabo, I. & Barnard, T. (1974). *Journal of Ultrastructural Research* **48**, 361–376.
Chaffee, R. R. J., Hoch, F. L. & Lyman, C. P. (1964). *American Journal of Physiology* **207**, 1211–1214.
Foster, D. O. & Frydman, M. I. (1979). *Canadian Journal of Physiology and Pharmacology* **57**, 257–270.

Dietary availability and rumen solubility of calcined magnesite sources. By E. R. PARKER, N. S. RITCHIE and R. G. HEMINGWAY, *Department of Veterinary Animal Husbandry, Glasgow University Veterinary School, Bearsden, Glasgow G61 1QH*

Both temperature of calcination and particle size have important effects on the dietary availability of calcined magnesites (Wilson & Ritchie, 1981). Wilson (1981) demonstrated a wide range of availabilities for calcined magnesites of different origin.

As part of a comprehensive investigation of commercially available materials, four products (two Spanish, one Greek, one Chinese) were obtained during the same week. These were compared with magnesium hydroxide made from sea water.

Twenty-four wether sheep (40 kg, 9 months), housed in metabolism cages, were given 0·8 kg dried grass cubes plus 0·2 kg rolled barley providing 1·73 g Mg/d. Four sheep were given each of the supplements in amounts to provide 2·0 g Mg/d. There was a 7 d run-in period and 7 d collection. In a balanced replicated block design this procedure was repeated twice so that twelve sheep in total received each supplement or no addition.

Dietary availability was assessed as that part of the supplementary Mg not recovered in the faeces. The Agricultural Research Council (1980) indicated that supplementary Mg should be readily soluble in rumen fluid. This was determined by placing 10-g quantities in duplicate of all the calcined magnesites in nylon bags (24 μm mesh) in the rumen of each of three hay-fed cows fitted with a rumen fistula for 48 h.

Supplement . . .	A Spanish Agma 85	B Spanish Navarros	C Greek Granular	D Chinese Granular	E $Mg(OH)_2$	F Nil	SEM
Particle diameter (μm):							
<150	24	22	3	10	100		
150–1000	61	78	69	42	—		
>1000	15	0	28	48	—		
Mg:							
Intake (g/d)	3·73	3·73	3·73	3·73	3·73	1·73	—
Faeces (g/d)	2·46	2·48	2·69	2·78	2·41	1·00	0·078
Urine (g/d)	0·66	0·66	0·60	0·68	0·73	0·44	0·033
Retention (g/d)	0·61	0·59	0·44	0·27	0·59	0·29	0·049
Availability (%)	27·0	26·0	15·5	11·0	29·5		
Solubility in rumen (%)	17·2	7·7	9·5	1·6	ND		1·44
Significance:							
Faeces	$P<0·001$, A,B,C,D,E>F; $P<0·01$, D>A,B,E; $P<0·05$, C>A,B,E						
Urine	$P<0·001$, A,B,C,D,E>F; $P<0·01$, E>C						
Retention	$P<0·001$, A,B,E>D,F; $P<0·05$, A,B,E>C						
Availability	$P<0·01$, A,B,E>C,D						
Solubility in rumen	$P<0·001$, A>B,C,D; $P<0·001$, C>D; $P<0·01$, B>D						

ND, not determined.

Significantly less Mg was found in the faeces and significantly more Mg was apparently retained when the sheep were given the two Spanish calcined magnesites or magnesium hydroxide. The calculated dietary availabilities were significantly less for both the Greek and Chinese products. Spanish Agma 85 had the highest and Chinese calcined magnesite had the lowest solubility in the rumen.

Agricultural Research Council (1980). *The Nutrient Requirements of Ruminant Livestock*. Slough: Commonwealth Agricultural Bureaux.

Wilson, C. L. (1981). PhD Thesis, University of Glasgow.

Wilson, C. L. & Ritchie, N. S. (1981). *Journal of the Science of Food and Agriculture* **32**, 993–994.

https://doi.org/10.1079/PNS19890018 Published online by Cambridge University Press

Continuous cold *v.* choice of cold: effect of different foraging regimens on brown fat acclimation in mice. By M. E. JAKOBSON, *Department of Biology and Biochemistry, North East London Polytechnic, London E15 4LZ* and S. MCBENNETT and J. F. ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Irish Republic*

Traditional studies on cold-induced changes in brown adipose tissue (BAT) impose sudden continuous cold (Himms-Hagen, 1986). In the present study, acclimation after 1, 7 and 14 d cold was compared with warm (WA) using: (1) the traditional 'cold room' model (CA) (mice transferred from 30° to 10°); and (2) a new 'cold-choice' model (CC) (mice free to choose between a warm and cold room (cages connected by plastic tubes), food and water available *ad lib.* in the cold room only). Forty-eight female LACA mice (26·5 (SD 2·5) g) were caged in groups of four. In each CC group (n 4), period of time in the food cage was estimated in one mouse by radiotelemetry and averaged 25% before cold exposure, and 17% in the 1st week and 19% in the 2nd week of CC.

CA (n 4) and CC (n 12) mice had greater body-weight gain over 14 d than WA mice (n 4) (+2·9, +2·6 and +1·4 g respectively, F 3·83, $P<0·05$). CA and CC groups removed more food than WA animals (1·9 × and 1·7 × WA rates in week 2). Unexpectedly, mean interscapular BAT (IBAT) weights (85–99 mg) were broadly similar across WA, CA and CC groups at 7 d (F 0·15, not significant) and 14 d (F 1·97, not significant). Mean weights of endometrial white fat pads were similar across most groups (181–285 mg) except 7 d CC mice (460 mg; F 9·83, $P<0·01$).

Histologically, cold-adapted BAT has high multilocularity of fat within each cell (Heldmaier, 1975). Haematoxylin/eosin-stained sections of IBAT confirmed that WA mice had very low multilocularity. CA mice (7 and 14 d) all had consistently high multilocularity, as did ten of twelve 14 d CC mice. The other two CC mice had a mixture of high and low multilocularity cells.

The acute 1 d response varied within and between groups. 1 d CA mice had (i) 48% drop in IBAT weight (t 5·55 *v.* WA, $P<0·001$), (ii) cells with a mixture of large and small fat globules, (iii) less than 50% BAT area with fat (estimated visually from cryostat sections, fat stained with Sudan Red), compared with well over 70% in all other mice. 1 d CC IBAT weights and locularity were indistinguishable from WA, as was that of one 7 d CC mouse. The remaining three 7 d CC mice had a mixture (high multilocularity cells more common than low).

Thus, frequent short voluntary foraging visits into moderate cold (total <5 h nightly) can initiate BAT acclimation as effectively as CA, although a little slower but with less acute trauma.

Heldmaier, G. (1975). *Journal of Comparative Physiology* **98**, 161–168.

Himms-Hagen, J. (1986). In *Brown Adipose Tissue*, pp. 214–268 [P. Trayhurn and D. G. Nicholls, editors]. London: Edward Arnold.

Sodium bicarbonate and calcined magnesite supplementation for lactating dairy cows. By R. G. HEMINGWAY, E. R. PARKER and N. S. RITCHIE, *Veterinary Animal Husbandry Department, Glasgow University Veterinary School, Bearsden, Glasgow G61 1QH*

Sodium bicarbonate (BIC, about 10 g/kg) and calcined magnesite (MgO, about 8 g/kg), both separately and combined in complete diets (*ca.* 400 g maize silage and 600 g concentrates/kg), have improved feed intakes and milk fat production in dairy cows. The mean results of five experiments conducted by Erdman *et al.* (1978, 1980*a*,*b*, 1982) were:

Supplement . . .	Nil	BIC	MgO	BIC+MgO
Feed intake (kg/d)	17·9	19·5	18·8	19·0
Milk fat (kg/d)	1·09	1·22	1·24	1·31

Thomas *et al.* (1984) considered fine particle MgO to be preferred. Edwards & Poole (1983) found BIC improved hay intake and fat yields (+0·13 kg). BIC improved grass silage intake, reduced live weight losses and improved conception.

Sixteen cows at peak lactation (mean 30 kg milk/d, 55 d post-calving) had 24 h access to grass silage and were group-fed on 2 kg molassed sugar-beet pulp, 2 kg wheat distillers' dark grains and 10 kg fresh brewers' grains. These were estimated to provide for 14 kg milk/d. Four groups each of four cows were formed. Four diets were given in balanced 4×4 Latin squares with 28 d feeding periods. Milk yields and compositions were recorded in the last 14 d of each period. The diets were: A, 50 g MgO in 1·67 kg feed; B, 225 g BIC in 1·67 kg feed; C, diet A plus diet B; and D, no supplement. Diets A, B and C were given via out-of-parlour feeders. Diet D was given in the milking parlour in additional amounts to make the total concentrate allocation 0·4 kg/kg milk produced after allowing for that provided by diets A, B and C. At the mean overall milk yield of 26·5 kg/d the various total diets all provided (g/kg) about 600–680 cereals, 200–220 vegetable proteins, 20–30 animal protein and 8–16 fat. All diets were readily consumed.

Supplement	Yield (kg)	Fat (g/kg)	Protein (g/kg)	Fat (g/d)	Protein (g/d)
Nil	26·7	37·6	29·7	1·01	0·79
BIC	26·6	37·4	30·5	0·99	0·81
MgO	26·2	37·6	29·7	0·98	0·78
BIC+MgO	26·7	37·2	30·2	0·99	0·80
SEM	0·38	0·50	0·20	0·012	0·010

There were no significant differences in the yield or composition of the milk. The results tend to indicate that positive effects from BIC and MgO supplementation are most likely to be seen where cows consume acidic silage and large amounts of cereal-based concentrates.

Edwards, S. A. & Poole, D. (1983). *Animal Production* **37**, 183–186.
Erdman, R. A., Botts, R. L., Hempken, R. W. & Bull, L. S. (1978). *Journal of Dairy Science* **61**, Suppl. 1, 172–173.
Erdman, R. A., Botts, R. L., Hempken, R. W. & Bull, L. S. (1980*a*). *Journal of Dairy Science* **63**, 923–930.
Erdman, R. A., Hempken, R. W. & Bull, L. S. (1980*b*). *Journal of Dairy Science* **63**, Suppl. 1, 146–147.
Erdman, R. A., Hempken, R. W. & Bull, L. S. (1982). *Journal of Dairy Science* **65**, 712–731.
Thomas, J. W., Emery, R. S., Breaux, J. K. & Liesman, J. S. (1984). *Journal of Dairy Science* **67**, 2532–2545.

Thermogenic activity and capacity of brown adipose tissue during the seasonal hibernation cycle in Richardson's ground squirrel. By R. E. MILNER, P. TRAYHURN and L. C. H. WANG, *Nutrition and Metabolism Research Group, Departments of Medicine, Foods & Nutrition and Zoology, University of Alberta, Edmonton, Alberta, Canada*

Hibernating species generally exhibit substantial seasonal changes in body-weight. In parallel with the increase in body fat occurring between spring and summer, the thermogenic capacity of brown adipose tissue (BAT) increases in preparation for hibernation. In the present study we have examined the thermogenic activity and capacity of BAT (axillary) during the seasonally-linked hibernation cycle in Richardson's ground squirrel (*Spermophilus richardsonii*). Squirrels were examined post-hibernation in April or May, and during hibernation and arousal. Indices of thermogenesis in BAT were measured as previously (Trayhurn *et al.* 1987), except that uncoupling protein (UCP) was determined by immunoblotting.

	Post-hibernation			Hibernation			Arousal		
	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*
Body-wt (g)	327	13	6	543*	21	6	506*	30	7
Axillary BAT wt (mg)	990	110	6	5080*	360	6	4330*	330	7
Cytochrome *c* oxidase† activity (μmol cytochrome *c* oxidized/min)	119	29	6	3021*	376	6	2506*	113	7
GDP bound (pmol/mg protein)	435	33	6	503	55	6	998*	91	7
Uncoupling protein (μg/mg protein)	93	9	5	96	7	10	108	15	7

Statistically significant from post-hibernation group: *$P<0{\cdot}001$.
†*EC* 1.9.3.1.

The Table shows that body-weight and the amount of axillary BAT were both increased in the hibernating squirrels. Cytochrome *c* oxidase activity in BAT was also greatly increased. Mitochondrial GDP binding was similar in the post-hibernation and hibernating animals. GDP binding was, however, markedly increased during arousal ($P<0{\cdot}001$). There was no significant difference between the groups in the specific mitochondrial concentration of UCP, the concentration being high in each group.

It is concluded that the mitochondrial content of BAT increases substantially during pre-hibernation fattening in Richardson's ground squirrel, but that there is no change in the specific mitochondrial concentration of UCP. The UCP content of BAT in this species is therefore modulated exclusively by alterations in mitochondrial content. Arousal is associated with an acute increase in GDP binding, resulting from an unmasking of binding sites on pre-existing UCP.

Trayhurn, P., Ashwell, M., Jennings, G., Richard, D. & Stirling, D. M. (1987). *American Journal of Physiology* **252**, E237–E243.

Effects of dietary methionine supplementation on copper status in the rat. By S. M. LYNCH and J. J. STRAIN, *Biomedical Sciences Research Centre, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB*

Dietary methionine supplementation (MET) can lower the activity of the copper-dependent enzyme cytochrome *c* oxidase (*EC* 1.9.3.1, CCO) and this effect has been attributed to inhibition of sulphydryl-sensitive (SH) enzymes by methionine metabolites (Finkelstein & Benevenga, 1986). The aim of the current study was to investigate possible effects of MET on measurements of Cu status and on the activities of other SH enzymes which are not Cu-dependent.

Groups (*n* 5) of male, weanling Wistar rats were housed individually, provided with deionized water and pair-fed on low-Cu (0·5 mg Cu/kg) diets containing either 2·0 or 20·0 g methionine/kg for 49 d.

Hepatic, cardiac and erythrocyte Cu–zinc superoxide dismutase (*EC* 1.15.1.1, CuZnSOD) activities were significantly reduced in MET compared with control animals. Hepatic CCO activity, liver Cu and plasma caeruloplasmin (*EC* 1.16.3.1) were also significantly lowered in the MET group. Although fumarase (*EC* 4.2.1.2) activity was significantly lowered, the MET diet significantly increased glutathione peroxidase (*EC* 1.11.1.9, GSH-Px) activity and did not affect the activities of the other SH enzymes; lipoamide dehydrogenase (*EC* 1.6.4.3, LDH), creatine kinase (*EC* 2.7.3.2, CK) and glutathione-S-transferase (*EC* 2.5.1.12, GSH-Tase).

	Control		MET	
	Mean	SE	Mean	SE
Liver:				
Cu (μg/g dry weight)	24·10	3·58	5·20**	2·19
CCO (U/mg protein)	0·41	0·06	0·20*	0·01
CuZnSOD (U/mg protein)	97·50	13·60	47·50*	10·38
Fumarase (U/mg protein)	12·41	1·43	7·88*	0·93
LDH (U/mg protein)	17·97	1·22	16·25	1·22
CK (U/mg protein)	168·19	39·67	107·85	18·05
GSH-Tase (U/mg protein)	302·14	20·62	350·72	20·33
GSH-Px (U/mg protein)	0·61	0·04	0·78*	0·04
Heart:				
CuZnSOD (U/mg protein)	308·00	7·50	149·00***	12·16
Blood:				
Caeruloplasmin (U/l)	90·32	16·32	36·50*	14·34
Erythrocyte CuZnSOD (U/mg protein)	99·65	17·81	35·73**	7·28

Significantly different from control animals (analysis of variance): *$P<0·05$, **$P<0·01$, ***$P<0·001$.

These results indicate that MET resulted in lower Cu status and the toxic effects of methionine metabolites do not appear to be due to their postulated general inhibitory effects on SH enzymes.

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

Finkelstein, A. & Benevenga, N. J. (1986). *Journal of Nutrition* **116**, 204–215.

The effect of overfeeding following cold-acclimation on energy balance and brown adipose tissue thermogenesis in mice. By K. M. YOUNGER* and P. TRAYHURN†, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

It has been suggested (Rothwell & Stock, 1980) that thermoregulatory non-shivering thermogenesis (NST), and diet-induced thermogenesis (DIT), are mediated by the same mechanism, present in brown adipose tissue (BAT). The present study addresses the question as to whether the stimulation of DIT might be enhanced by a cold-induced 'priming' of the tissue. Young male 'Aston' mice were acclimated to 5° (CA) or 23° (WA) for 3 weeks. The animals at each temperature were then divided into two groups, a stock-fed control group and a chocolate-supplemented group. Full energy balance measurements were carried out and, after 3 weeks, activity of BAT was investigated in these animals.

The animals acclimated to 5° incurred a significant body energy deficit; however, these mice then achieved higher gross efficiencies (energy gain/digestible energy (DE) intake) than the WA mice and had 'caught up' with their WA littermates after a subsequent 3 weeks at 23°. Both of the chocolate-supplemented groups exhibited a similar hyperphagia (27% of DE in WA and 20% in CA), and deposited the same proportion (41%) of their extra DE intake in the carcasses. The thermogenic activity of the BAT from the overfed WA and CA animals was increased to a similar extent, as measured by the binding of GDP to the mitochondrial 'uncoupling' protein considered to be responsible for heat production (122 (SE 9·4) pmol GDP/mg mitochondrial protein in the WA control group and 190 (SE 15·8) in the WA chocolate-supplemented group, $P<0·01$; 127 (SE 14·8) in the CA controls and 176·4 (SE 14·1) in the supplemented group, $P<0·05$). The total cytochrome *c* oxidase (*EC* 1.9.3.1) activity and protein content of the BAT from the four groups were similar. These mice therefore showed little evidence of a persisting effect of cold-acclimation on energy balance or BAT activity. This is perhaps because such an effect would be detrimental to the complete recovery (at 23°) of the substantial body energy deficit incurred by these young growing mice during their severe cold stress. Control of thermogenesis in BAT thus appears to be a highly flexible adaptive mechanism in mice.

Rothwell, N. J. & Stock, M. J. (1980). *Canadian Journal of Physiology and Pharmacology* **58**, 842–848.

Present addresses: *Division of Nutritional Sciences, Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, James's Street, Dublin 8, Irish Republic. †Nutrition and Metabolism Research Group, 318 Robert Newton Research Building, University of Alberta, 11315–87 Avenue, Edmonton, Alberta, Canada.

Effects of dietary changes in zinc and copper levels on rabbit liver metallothionein-like protein. By S. R. R. RAO and W. H. PARRY, *Nutrition Group, Bristol Polytechnic, Coldharbour Lane, Bristol BS16 1QY*

Three groups of male rabbits were fed on semi-synthetic diets differing in their copper and zinc concentrations. The control group was given 100 mg Zn/kg and 25 mg Cu/kg; whilst one experimental group was given 1000 mg Zn/kg with 25 mg Cu/kg (high-Zn group); the other group was given 200 mg Cu/kg with 100 mg Zn/kg (high-Cu group). After 4 weeks on the respective diets, the rabbits in each group were killed and liver samples homogenized. Liver cytosol from each group was chromatographed on a 900 × 26 mm column packed with Sephadex G-75. Protein fractions were eluted with 0·02 M-ammonium carbonate at a flow rate of 50 ml/h (Kimura *et al.* 1979). A metallothionein-like protein with a molecular weight of approximately 10 000 daltons was detected prominently in the high-Zn group but not so prominently in the high-Cu group.

Concentrations (μg/ml) of Zn and Cu in the liver cytosol fractions obtained from control, high-Zn and high-Cu groups

Fraction no.	Control		High Zn		High Cu	
	Zn	Cu	Zn	Cu	Zn	Cu
4	0·12	0·02	–	–	0·05	0·04
6	0·10	0·02	–	–	0·07	0·05
8	0·11	0·11	–	–	0·12	0·06
10	0·66	0·35	–	–	0·44	0·11
12	1·31	0·24	1·20	0·07	0·30	0·21
14	0·80	0·26	1·27	0·24	0·47	0·38
16	0·92	0·41	0·96	0·18	0·25	0·26
18	1·05	0·10	0·87	0·08	0·15	0·18
20	0·93	0·32	0·92	0·11	0·06	0·37
22	0·99	0·35	0·76	0·20	0·02	0·26
24	0·43	0·06	0·61	0·03	0·07	0·11
26	0·22	0·04	1·05	–	0·21	0·27
28	0·06	0·04	1·42	–	0·08	0·30
30	0·03	0·05	0·44	–	–	0·20
32	0·03	0·05	0·17	–	–	0·07
34	0·06	0·06	0·29	–	–	0·08
36	0·08	0·06	0·45	–	–	0·08
38	0·07	0·05	0·47	–	–	0·07
40	0·06	0·04	0·50	–	–	0·06
42	0·06	0·03	0·36	–	–	0·05
44	0·06	0·03	0·40	–	–	0·05

The dietary ratios of Zn:Cu in the control, high-Zn and high-Cu groups were 4:1, 40:1 and 1:2 respectively. An interesting observation was the difference between the two experimental groups with respect to the apparent binding of the metallothionein-like protein for Zn and Cu. The protein in the high-Zn group bound more Zn and no Cu, compared with the protein in the high-Cu group which bound less Zn but an appreciable amount of Cu. There is evidence to show that the binding sites on this protein for Zn and Cu were influenced antagonistically by the dietary levels of these metals.

Kimura, M., Otaki, N. & Imano, M. (1979). In *Metallothionein*, pp. 163–168 [J. H. R. Kagi and M. Nordberg, editors]. Basel/Boston/Stuttgart: Birkhauser Verlag.

Growth and weights of abdominal and carcass fat in sheep immunized against adipose cell membranes. By A. P. MOLONEY and P. ALLEN (introduced by J. O'GRADY), *An Foras Taluntais, Grange, Dunsany, Co. Meath, Irish Republic*

The recent prohibition on the use in the European Community of steroid hormones in livestock production, makes the development of alternative approaches to improving growth and carcass composition imperative. Passive immunization of rats with antiserum to rat adipose cell plasma membranes significantly reduces both internal and subcutaneous fat (Flint *et al.* 1986). Accordingly, the feasibility of this procedure as a means of altering fat deposition in sheep was examined.

Subcutaneous and abdominal adipose tissue was obtained from sheep at slaughter and a plasma membrane fraction prepared by homogenization and density-gradient centrifugation in Percoll (Belsham *et al.* 1980). The presence and enrichment of plasma membranes was confirmed by reference to the marker enzyme 5′-nucleotidase (*EC* 3.1.3.5). Female donkeys were actively immunized with 0·75 mg membrane protein in Freund's complete adjuvant and routinely boosted at 10-week intervals with plasma membranes in Freund's incomplete adjuvant.

Sixteen Finnish Landrace crossbred sheep (eight ewes and eight wether lambs) were subcutaneously immunized with 50 ml normal donkey serum or donkey antiovine plasma membrane serum on three consecutive days. The animals were offered a pelleted concentrate ration for 12 weeks and then slaughtered. Following slaughter, the internal fat was removed and weighed and carcass composition determined by dissecting the right side into separable fat, lean and bone.

	Control	Immunized	SEM	Statistical significance
Average daily wt gain (g)	126	75	17	$P<0·06$
Feed conversion efficiency (gain/intake)	0·097	0·071	0·013	NS
Omentum fat (g/kg live wt)	55·8	41·3	3·7	$P<0·05$
Kidney and pelvic fat (g/kg live wt)	40·0	34·4	2·3	$P<0·05$
Killing-out rate (g carcass/kg live wt)	492	512	5·7	$P<0·05$
Fat depth (mm):				
Loin	7·09	4·03	1·08	NS
Leg	14·57	12·81	1·61	NS
Intermuscular fat (g/kg carcass)	117·1	112·7	8·9	NS
Subcutaneous fat (g/kg carcass)	157·8	147·5	11·3	NS
Total dissectable fat (g/kg carcass)	274·9	260·2	15·7	NS

NS, not significant.

Passive immunization against adipose cell plasma membranes significantly reduced the weights of omentum and kidney and pelvic fat. These reductions contributed to a significant increase in killing-out rate. The fat depth of the loin was reduced from 7·09 to 4·03 mm and the separable carcass fat was reduced from 274·9 to 260·2 g/kg carcass. Both these effects were not significant. Live-weight gain was reduced ($P<0·1$) in line with the decrease in abdominal fat, while the efficiency of feed utilization also tended to be decreased by treatment. The efficiency of carcass production, however, was not affected. It is concluded that immunization against fat cells may have potential as a means of altering fat deposition in sheep.

Belsham, G. J., Denton, P. M. & Tanner, M. J. A. (1980). *Biochemical Journal* **192,** 457–467.
Flint, D. J., Coggrave, H., Futter, C. E., Gardner, M. J. & Clarke, T. J. (1986). *International Journal of Obesity* **10,** 69–77.

The effects of acute and chronic ingestion of *n*-3 and *n*-6 polyunsaturated fats on ethanol-induced gastric haemorrhage in the rat. By B. HUNTER and M. J. GIBNEY, *Division of Nutritional Sciences, Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, Dublin 8, Irish Republic*

Several studies have shown that the administration of exogenous arachidonic acid (20:4*n*-6) by gastric gavage significantly reduces the extent of ethanol-induced gastric haemorrhage in rats by a process dependent on prostaglandin synthesis (Hollander *et al.* 1982). The purpose of the present study was to compare the relative effects of the *n*-3 polyunsaturated fatty acids (PUFA) and the *n*-6 PUFA, administered acutely or chronically to rats, on ethanol-induced gastric haemorrhage.

Female Wistar weanling rats were given either a stock diet (low-fat control) or a stock diet enriched with (100 g/kg) maize oil (*n*-6 PUFA), fish oil (*n*-3 PUFA) or butter fat for 5 weeks. Rats (*n* 12) were fasted overnight and killed 1 h after an intragastric dose of 2 ml ethanol. In a second experiment, rats (*n* 12) maintained on a low-fat stock diet for 5 weeks received a stomach gavage of arachidonic acid, eicosapentaenoic acid (20:5*n*-3) or linoleic acid (18:2*n*-6) in micellar solution (120 mM; taurocholic acid) 1 h before a stomach gavage with ethanol. The rats were killed 1 h later. The stomachs of all animals were scored macroscopically for the area (% of total) covered by gross haemorrhagic lesions (see Table).

Percentage of stomach lesioned (n *12)*

4 week feeding	Mean	SD	Acute dose	Mean	SD
Maize oil diet	15·3	13·2	Linoleic acid	21·4	10·4
Fish oil diet	12·2	8·2	Eicosapentaenoic acid	26·5	12·0
Butter fat diet	23·8	8·1	Arachidonic acid	8·3	7·3
Low-fat diet	29·3	14·0			
Difference ($P<0·05$)	8·3			9·4	

Dietary maize oil (*n*-6 PUFA) and dietary fish oil (*n*-3 PUFA) both led to a reduction in the extent of stomach lesions. In contrast only arachidonic acid (*n*-6 PUFA) reduced haemorrhagic lesions when the free acids were acutely administered. The phospholipid fatty acid compositions of stomach scrapings from rats acutely administered with free fatty acids showed no remarkable differences while the changes in the chronically fed animals were as expected, i.e. reduced ratio of 20:4*n*-6/20:5*n*-3 in fish-oil-fed animals and elevated 18:2*n*-6 in maize-oil-fed animals.

Hollander, D., Tarnawski, A., Ivey, K. J., de Feery, A., Zipser, R. D., McKenzie, W. N. & McFarland, W. D. (1982). *Journal of Laboratory and Clinical Medicine* **100**, 296–308.

Chronic effects of endotoxin infusion on energy balance and brown adipose tissue in the rat. By J. ARNOLD[1,3], R. A. LITTLE[1] and NANCY J. ROTHWELL[2], [1]*North Western Injury Research Centre*, [2]*Department of Physiological Sciences* and [3]*Department of Surgery, University of Manchester, Manchester M13 9PT*

Injection of bacterial endotoxin induces a pronounced fever in laboratory rodents and has therefore been used as a means of studying the central control and effector mechanisms of fever. In addition to causing a rise in metabolic rate associated with increased thermogenic activity of brown adipose tissue (BAT) (Jepson *et al.* 1987), acute injection of endotoxin also alters metabolic substrate turnover rates. However, tolerance to the febrile effects of endotoxin rapidly develops during repetitive administration. The objective of the present study was to assess the chronic effects of endotoxin treatment on energy balance and BAT thermogenesis in the rat.

Bacterial endotoxin (*Escherichia coli* 0127:B8, Sigma Chemical Co., Poole, Dorset) was continuously infused (12 μg/h) via osmotic minipumps implanted subcutaneously (halothane anaesthesia) in male, Sprague-Dawley rats (starting weight approximately 230 g). Complete energy balance measurements were made in a 7 d study while in separate experiments, the effects of 2 and 4 d infusions of endotoxin on BAT properties were also investigated. Control rats were infused with saline (9 g sodium chloride/l).

Food intake was significantly reduced in endotoxin-infused rats (by almost 25%) compared with controls over the first 4–5 d, but thereafter returned to control values. In the 7 d study, an extra group of animals was pair-fed to the same intake as endotoxin-infused rats. Body-weight gain was also suppressed in animals infused with endotoxin for the first 4–5 d, but increased in the latter part of the study.

Metabolizable energy intake, from the 7 d study, was reduced almost 20% in the endotoxin-infused rats. While protein and fat gains were lowest in the infected rats, there appeared to be a selective loss of protein when considered as a percentage of final body-weight. Compared with pair-fed controls, endotoxin-infused rats had an elevation in energy expenditure of nearly 15%. Gross efficiency was unchanged between *ad lib.* and pair-fed controls (20%), while it was 40% reduced in endotoxaemic rats.

Significant increases in colonic temperature of endotoxin-infused rats were observed on days 1–5 of the study with a peak fever occurring on day 1 (control 37·8 (SEM 0·2)°, endotoxin 38·5 (SEM 0·3)°, pair-fed 37·7 (SEM 0·2)°; $P<0·01$).

The activity of the thermogenic proton conductance pathway in BAT mitochondria was assessed from in vitro binding of GDP. Specific GDP binding was increased by 30% after 2 or 4 d of endotoxin infusion compared with free-feeding controls. After 7 d, GDP binding (pmol/mg protein) remained elevated by 22% in endotoxin-infused (112 (SEM 5)) compared with control rats (92 (SEM 3), $P<0·01$) and was almost 50% above the values for pair-fed animals (76 (SEM 3)).

These results demonstrate that chronic infusions of endotoxin cause significant alteration in energy balance of the rat. Likewise endotoxin induces modest increases in BAT activity which are sustained even after the fever has declined.

Jepson, M. M., Cox, M., Bates, P. C., Rothwell, N. J., Stock, M. J., Cady, E. B. & Millward, D. J. (1987). *American Journal of Physiology* **252,** E581–E587.

Electrogenic ion secretion in the small intestine of the pseudo-germ-free rat. By A. YOUNG, ALISON J. EDWARDS, R. J. LEVIN, HELEN C. NZEGWU and M. M. C. PEREIRA, *Department of Biomedical Science, The University, Sheffield S10 2TN*

A useful and economic preparation to complement the true germ-free rat is the pseudo-germ-free rat produced by allowing healthy male rats to drink an antibiotic cocktail containing bacitracin, streptomycin and neomycin (1 mg of each/ml) for 7 d as previously described (Rowland & Wise, 1985). Little is known concerning fluid and electrolyte secretion in the intestine of the pseudo-germ-free rat. Electrogenic ion transport was measured as the short-circuit current (Isc) in the proximal duodenum, jejunum and ileum using standard techniques.

Both basal Isc and maximal increases in Isc above basal level ($\triangle$Isc) in response to secretagogue challenge were measured. The secretagogues chosen were Bethanecol (1 mM, a muscarinic cholinergic agonist which acts via Ca^{2+}); prostaglandin E_2 (PgE_2, 1 μg/ml, acts via cAMP) and *Escherichia coli* enterotoxin STa (STa, 50 units/ml, acts via cGMP). All were applied serosally, except STa which was added mucosally. Caecal length and weight, as well as the wet and dry weights of the small intestinal segments used were also monitored.

The caeca of the pseudo-germ-free rats were grossly enlarged compared with control rats, in terms of length (+77%, $P<0{\cdot}001$), full wet weight (+166%, $P<0{\cdot}001$), empty wet weight (+77%, $P<0{\cdot}001$) and dry weight (+73%, $P<0{\cdot}001$). No statistically significant changes in the wet or dry weights of the duodenal, jejunal and ileal segments were observed.

The basal Isc in the duodenum and jejunum of the pseudo-germ-free rat were not significantly reduced compared with controls (duodenum −18%, $P>0{\cdot}05$; jejunum −16%, $P>0{\cdot}05$). In the ileum, however, the reduction in basal Isc was significant (−34%, $P<0{\cdot}01$). The responses to Bethanecol and STa did not differ between the controls and pseudo-germ-free rats in all areas of the small intestine. Interestingly the pseudo-germ-free intestine always responded to PgE_2 challenge to a lesser extent than the controls (duodenum −43%, $P<0{\cdot}01$; jejunum −16%, $P<0{\cdot}05$; ileum −29%, $P<0{\cdot}05$).

Thus the pseudo-germ-free rat small intestine displays alterations in electrogenic ion movement under both basal and PgE_2-stimulated conditions.

Supported by the University of Sheffield Research Fund. The authors thank Boots plc, May & Baker and Wellcome Laboratories for the generous donation of antibiotics.

Rowland, I. R. & Wise, A. (1985). *Critical Reviews in Toxicology* **16** (1), 31–103.

Hypothermic and pyrogenic effects of recombinant tumour necrosis factor α in rats. By D. C. BIBBY and R. F. GRIMBLE, *Human Nutrition Department, Southampton University Medical School, Southampton SO9 3TU*

Bacterial endotoxins are potent stimulators of cytokine production by macrophages (Old, 1987). Tumour necrosis factor α (TNFα) is one of the cytokines produced. In rats, endotoxin has been shown to be pyrogenic at low doses (intravenous; i.v.) and to produce a hypothermia at high doses (Lang *et al.* 1985). The present study examines whether the type of temperature response obtained could be related to the quantity of cytokines produced.

Male Wistar rats (154 ±1 g) from the Southampton Medical School Colony, caged separately and allowed free access to food and water, received i.v. injections of sterile non-pyrogenic saline (9 g sodium chloride/l) of various doses (10, 20, 50, 100, 200, 300, 400 μg/kg body-weight) of human recombinant TNFα (endotoxin content <0·137 ng/mg protein) in similar saline. Injections commenced at 08.00 hours and rectal temperatures were measured before and at intervals (t_n hours) after injection, for 8 h. Rats were stunned, decapitated and blood collected for serum zinc assay at this time.

	Dose of TNFα (μg/kg body-wt)							
Rectal temperature (°) (Δ $t_0 \rightarrow t_n$)	0	10	20	50	100	200	300	400
t_1	−0·2	0·1	0·1	0·1	0·1	−0·6	−1·3**††	−1·2*††
t_2	−0·3	0·3	0·5**††	0·9**††	0·7**††	−0·1	−0·2	−0·3
t_3	−0·4	0·1*	0·1**	0·9**††	0·9**††	0	0·2	0·1
t_4	−0·6††	−0·1	−0·2	0·7**††	0·7**††	0·1*	0·4**	0·2
t_5	−0·6††	−0·3	−0·5††	0·5**†	0·3**†	−0·1	0·1	0·1
t_6	−0·6††	−0·5†	−0·5††	0·2**†	0**	−0·1	−0·2	−0·1
t_8	−0·7††	−0·4	−0·6††	−0·4	−0·1**	−0·5	−0·4	−0·2
Serum Zn (μg/ml)	1·91	2·02	1·64	1·25**	0·81**	0·67**	0·86**	0·79**

Values significantly different from saline (0) group (analysis of variance): *P<0·05, **P<0·01.
Values significantly different from t_0 value (analysis of variance): †P<0·05, ††P<0·01.

Serum Zn was significantly depressed by doses of 50 μg/kg or more of TNFα. The rectal temperature response to TNFα changed in intensity and character with increasing dosage. Between 20 and 100 μg/kg a fever with a latency of 2 h and of increasing intensity and duration occurred. At doses of 300 μg/kg or more a transient hypothermia occurred 1 h after injection. The temperature response to bacterial endotoxins could therefore depend on quantitative aspects of cytokine release.

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

Lang, C. H., Bagby G. J. & Spitzer, J. J. (1985). *American Journal of Physiology* **248,** R471–R487.
Old, L. J. (1987). *Nature* **330,** 602–603.

The effect of *Escherichia coli* enterotoxin STa on electrogenic secretion in the starved and undernourished rat colon. By HELEN C. NZEGWU, M. M. C. PEREIRA, A. YOUNG and R. J. LEVIN, *Department of Biomedical Science, The University, Sheffield S10 2TN*

Famine or hunger diarrhoea is usually, but controversially, attributed to infectious agents. Little is known concerning the actions of bacterial enterotoxins on the secretory response of the intestine from undernourished or starved victims. We have previously reported that nutritional reductions (starvation, acute and chronic undernutrition) induce a hypersecretory state in the rat large intestine following secretagogue challenge (Levin *et al.* 1987; Nzegwu *et al.* 1988). The present study investigates the effects of enterotoxin *E. coli* STa on electrogenic ion secretion across in vitro sheets of rat proximal colon (MPC), mid-colon (MC) and distal colon (DC) using standard techniques. *E. coli* STa is a heat-stable enterotoxin which activates brush-border guanylate cyclase (*EC* 4.6.1.2) and elevates intracellular cGMP levels (Cohen *et al.* 1986). Electrogenic secretion was monitored as the short-circuit current (Isc) under basal and stimulated conditions (ΔIsc = max Isc−basal Isc) following mucosal application of STa (50 units/ml).

Three groups of male rats were used: fed control, 72 h starved and acutely undernourished (fed 33% (8g) of the control's daily food intake per day for 9 d). The results are shown in the Table. Statistical comparison of the data was performed using the Kruskal-Wallis Anova and specific differences located using Conover's multiple *t* test.

ΔIsc ($\mu A/cm^2$ serosal area)

		MPC		MC		DC	
	n	Mean	SE	Mean	SE	Mean	SE
Fed	12	26·6	1·9	13·8	1·4	1·4	0·2
72 h starved	11	10·6	1·8	14·5	2·3	4·6	0·6
Acute undernourished	13	13·6	1·9	22·4	2·4	1·8	0·6

STa caused a small but distinct secretory response in all areas of the colon, but to a greater extent in the MPC and MC. In the MPC, both starvation and undernutrition caused decreases in the secretory response (72 h undernourished, −60%, $P<0·001$; acute undernourished, −49%, $P<0·001$). In the MC only acute undernutrition altered the secretory response causing an increase in secretion (+62%, $P<0·001$). Responses from the DC were very much lower than those observed in either the MPC or MC, although 72 h starvation increased the secretion by some 229% ($P<0·001$).

Undernutrition significantly altered electrogenic secretion in the rat colon in response to STa, with pronounced segmental variations. The 72 h starved and acutely undernourished MPC responded with a decrease in secretion but the acutely undernourished MC and 72 h starved DC responded with an increase in secretion.

Cohen, M. B., Moyer, M. S., Luttrell, M., Gianella, R. A. (1986). *Pediatric Research* **20** (6), 555–560.
Levin, R. J., Nzegwu, H. C. & Young, A. (1987). *Journal of Physiology* **396**, 33P.
Nzegwu, H. C., Pereira, M. M. C., Warren, M. A., Levin, R. J. & Young, A. (1988). *Proceedings of the Nutrition Society* **47**, 164A.

An enzyme-linked immunosorbent assay for the measurement of uncoupling protein in brown adipose tissue. By ELLEN CHAN and R. SWAMINATHAN, *Department of Chemical Pathology, Prince of Wales Hospital, Shatin, Hong Kong*

Brown adipose tissue (BAT), which is involved in thermogenesis, has a unique 32 kDa protein, the uncoupling protein (UCP), in the inner mitochondrial membrane. UCP was purified by the method of Lin & Klingenberg (1980) and rabbits were immunized with purified UCP with Freund adjuvant, and an enzyme-linked immunosorbent assay was developed using protein A linked to β-galactosidase (*EC* 3.2.1.23). The lower detection limit of the assay is 10 ng and the precision of the assay is 9·6% at 4 μg/ml. Using this assay the time course of change in UCP content of BAT in response to cold adaptation was followed. Rats were housed at 4° and BAT was removed from groups of rats at 0, 1, 4 and 6 d after exposure to 4°. BAT was weighed and a homogenate was prepared. After centrifugation at 8800 ***g*** for 5 min (Eppendorf centrifuge 5413) the pellet was dissolved in 10% Triton X-100 and the UCP content was measured by the enzyme-linked immunosorbent assay, and cytochrome *c* oxidase (*EC* 1.9.3.1) activity was measured by the method of Wharton & Tzagoloff (1967).

Period at 4° (d) . . .	0		1		4		6	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body-wt (g)	179·5	2·9	167·6	6·14	165·6	4·6	153·2***	3·3
Wt of interscapular and cervical BAT (mg)	322	21	307	10	451	46	466*	27
Total protein content of BAT (mg)	21·0	1·5	26·8	2·0	38·8**	2·3	39·5***	3·2
Total UCP content of BAT (μg)	513	20	546	29	979***	54	892***	71
Total cytochrome *c* oxidase activity of BAT (μmol cytochrome *c* oxidized/min)	799	73	439**	46	1047	69	1087*	79
UCP: cytochrome *c* oxidase	0·659	0·047	1·245***	0·092	0·958*	0·107	0·826*	0·051

n 5 in each group.
Significantly different from day 0 value: **P*<0·05, ***P*<0·01, ****P*<0·001.

Taking cytochrome *c* oxidase activity as an index of mitochondria the results clearly show an increase in mitochondria as well as an increase in UCP content per mitochondria. We suggest that reliable results can be obtained using a simple homogenate and the enzyme-linked immunosorbent assay.

Lin, C. S. & Klingenberg, M. (1980). *FEBS Letters* **113**, 299–303.
Wharton, D. C. & Tzagoloff, A. (1967). *Methods in Enzymology* **10**, 245–246.

The use of weighed dietary records and the doubly-labelled ($^2H_2{}^{18}O$) water method to compare energy intake and expenditure. By M. B. E. LIVINGSTONE, J. J. STRAIN, G. B. NEVIN, M. E. BARKER, R. J. HICKEY and P. G. MCKENNA, *Biomedical Sciences Research Centre, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB* and A. M. PRENTICE, W. A. COWARD and R. G. WHITEHEAD, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

The 7 d weighed dietary record (WDR) is regarded as the most precise method of assessing dietary intake (DI) but its absolute validity is uncertain (Bingham, 1987). The WDR was compared with doubly-labelled ($^2H_2{}^{18}O$) water estimates of total energy expenditure (TEE) in sixteen male and sixteen female, healthy, free-living subjects who were selected to represent a range of energy intakes from previous participation in a larger randomized dietary survey. The following protocol was observed: TEE over 15 d; seven consecutive day WDR during TEE measurement; basal metabolic rate (BMR) by indirect calorimetry. Metabolizable energy was calculated from food tables. DI results from one subject were excluded due to incomplete recording.

		DI (MJ/d)		TEE (MJ/d)		DI:TEE		DI:BMR		TEE:BMR	
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Males	16	11·21	2·48	14·23*	2·95	0·81	0·22	1·49	0.30	1·88	0·28
Females	15	8·00	1·88	9·87**	1·49	0·82	0·21	1·43	0·33	1·76	0·17
Total	31	9·66	2·72	12·15***	3·20	0·82	0·21	1·46	0·31	1·82	0·23

Significantly different from DI value (paired *t* test): $^*P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.

Mean DI was 81 (SD 22)% (males) and 82 (SD 21)% (females) of TEE values, with individual discrepancies ranging from −55% (−5·3 MJ) to +40% (+3·8 MJ). Allowing for normal day-to-day variation in DI, giving a mean intra-individual coefficient of variation of 23% (males) and 26% (females) and in TEE values of approximately 5%, individual discrepancies of up to ±11% would be anticipated even if both methods were perfectly accurate. However, only seven subjects fell within this range. In two subjects DI exceeded TEE by 17% and 40% but in nineteen subjects DI fell short of TEE by >20%. DI values of four subjects were less than BMR and were clearly invalid estimates of habitual intake. Even when these subjects were excluded, mean DI was 86 (SD 18)% of TEE values (*n* 27). The DI of the three obese (>135% ideal body-weight, IBW) subjects fell short of TEE by 16, 32 and 55%, but there was no consistent discrepancy between DI and TEE in moderately overweight subjects (>115% IBW, *n* 9). It is concluded that DI:TEE discrepancies may be due to under-reporting or conscious or subconscious downward shift in habitual energy intake, or both.

These results confirm the suspected 'observer effect' in dietary surveys. Discrimination between these effects would require detailed concomitant analyses of quantitative and qualitative changes in body composition.

This work was supported by the Department of Health and Social Services (Northern Ireland).

Bingham, S. A. (1987). *Nutrition Abstracts and Reviews* **57**, 705–742.

Studies on the purine nucleotide binding site of the uncoupling protein of brown adipose tissue mitochondria. By T. PEACHEY, M. GORE and D. A. YORK, *Departments of Human Nutrition and Biochemistry, School of Biochemical and Physiological Sciences, Southampton University, Southampton SO9 3TU*

Purine nucleotides bind to the uncoupling protein (UCP) of brown adipose tissue (BAT) and inhibit the thermogenic proton translocation through the inner mitochondrial membrane (Nichols & Locke, 1984). While there is considerable knowledge of the physiological control of BAT function, less is known of the mechanism and control of the proton channel at the membrane level.

The effect of pyridoxal phosphate (6 mM) on GDP binding to both purified UCP and isolated mitochondria has been studied. Pyridoxal phosphate, which reacts with lysine residues, reduces GDP binding to BAT mitochondria isolated from control (24°) or cold-exposed (4°) rats. Scatchard analysis showed that this reduction resulted from an increase in the K_d without any change in the maximal number of binding sites (B_{max}) (Table). Similar results were observed with the purified UCP.

		Mitochondria			
		24°		4°	
Pyridoxal phosphate		Mean	SEM	Mean	SEM
−	K_d (μM)	0·61	0·4	0·66	0·05
−	B_{max} (pmol/mg)	265	12	444	21
+	K_d (μM)	1·92	0·35	2·26	0·36
+	B_{max} (pmol/mg)	323	43	467	56

These results suggest that a lysine residue is near or at the nucleotide binding site of UCP.

Tryptic digestion cleaved the UCP of mitochondria into two major fragments of molecular weight 21 000 and 11 000 kDa approximately. The digestion pattern was influenced by the presence of GDP in the incubation medium and by pH, additional fragments appearing at higher pH. UCP has also been photolabelled with 8-azidoadenosine γ-^{32}[P]triphosphate and subsequently subjected to proteolytic digestion. The ^{32}P-labelled cleavage fragments have been separated. The photolabel was located in the 11 000 kDa fragment. It is hoped that sequence analysis will localize the nucleotide binding fragment to a specific region of the peptide.

T.P. was supported by a SERC studentship.

Nichols, D. G. & Locke, R. M. (1984). *Physiology Review* **64**, 1–65.

Body fat in lean and obese women measured by six methods. By G. McNeill[1], P. A. Fowler[2], R. J. Maughan[3], B. A. McGaw[1], S. Gvozdanovic[2], D. Gvozdanovic[2] and M. F. Fuller[1], [1]*Rowett Research Institute, Bucksburn, Aberdeen* and *Departments of* [2]*Bio-Medical Physics and Bio-Engineering* and [3]*Environmental and Occupational Medicine, University of Aberdeen, Aberdeen*

To investigate the differences between estimates of body fat content in individual women by different methods we made estimates of body fat content in seven lean (body mass index (BMI, weight/height^2) 20·6 (SD 1·8)) and seven obese women (BMI 31·1 (SD 3·3)). Age was very similar in the lean (36·9 (SD 9·5)) and obese groups (37·1 (SD 13·0) years), and height was not significantly different (lean 1·66 (SD 0·058) m; obese 1·62 (SD 0·041) m) (*t* test: $P>0·05$).

Estimates of body fat content were made by four established methods: four-site skinfold thickness (SFT), underwater weighing (UWW), whole body counting (^{40}K) and deuterium dilution of body water (D_2O); and by two more recent methods: tetra-polar bio-electrical impedance (BEI) (BMR 2000 analyser; Berkeley Medical Research Co., USA), and magnetic resonance imaging (MRI) based on estimates of adipose tissue volume from seventeen to twenty-eight transverse sections of the body using a whole body NMR imager. Measurements of SFT, BEI and D_2O were carried out after a standard breakfast on the same morning in all women, with other measurements made within the following 0–8 d.

The mean values for body fat (g/kg body-weight) measured by the six methods are shown in the Table:

Method . . .		SFT	UWW	^{40}K	D_2O	BEI	MRI	SED
Lean (*n* 7)	Mean	286	254	277	274	247	282	20·6
	SD	49	61	75	65	49	42	
Obese (*n* 7)	Mean	398	424	435	437	396	441	18·3
	SD	29	51	64	61	49	40	

Data for each group were analysed by ANOVA, which showed that differences between the estimates by all six methods were not significant ($P=0·348$) in the lean women. In the obese women the differences between the methods approached significance at the 5% level ($P=0·056$), and the results by SFT and BEI were significantly lower than those by ^{40}K, D_2O or MRI ($P<0·05$). The highest correlation between two methods was between UWW and MRI, with correlation coefficients of +0·93 ($P<0·01$) in the lean subjects and +0·78 ($P<0·05$) in the obese subjects. When the two groups were combined, all correlations between methods were significant, the highest being between UWW and MRI (r+0·96; $P<0·001$) and the lowest being between SFT and ^{40}K (r+0·71; $P<0·01$).

The results illustrate that despite high correlations between estimates of body fat by two different methods in a group of subjects with a wide range of body fat content, there may be substantial disagreement between estimates obtained by different methods in individual subjects.

Effect of fasting and refeeding on uncoupling protein-mRNA levels in rat brown adipose tissue. By ODETTE CHAMPIGNY and D. RICQUIER (introduced by J. F. ANDREWS), *Centre de Recherches sur la Nutrition, CNRS, F-92190 Meudon, France*

It has been established that brown adipose tissue (BAT) is under nutritional control. In young rats an excess of energy intake can stimulate heat production in BAT through an increase in its uncoupling protein (UCP) content (Rothwell & Stock, 1979). Inversely, starvation is known to reduce BAT mass, mitochondrial protein content and UCP level (Desautels, 1985; Trayhurn & Jennings, 1986). Refeeding for 24 h is sufficient to restore UCP to the prefasting level. This nutritional control of BAT UCP would be mediated by the hypothalamus and the sympathetic nervous system and modulate UCP gene expression. We have chosen to follow the variations of UCP-mRNA in rat BAT during a period of fasting and refeeding.

Male Wistar rats, acclimated to 23° and previously fed on a stock diet, were fasted for 48 h. After that time, some animals were refed on the stock diet for 5 or 24 h, while others continued fasting for 5 or 24 h. After killing the animals, the interscapular BAT was rapidly removed and frozen. Total RNA was extracted and analysed on Northern blots for UCP-mRNA through hybridization with a rat UCP cDNA (Bouillaud *et al.* 1985).

UCP-mRNA decreased in BAT of rats fasted for 48 h and was highly stimulated by 5 or 24 h refeeding, reaching a higher level than that in fed controls. Unexpectedly a marked peak of UCP-mRNA was observed after 53 h of fasting; it was followed by a decrease, so that after 72 h fasting the level of UCP-mRNA was similar to that of fed controls. This striking increase of UCP-mRNA between 48 and 53 h of fast suggests that 2 d of fasting induced a need for non-shivering thermogenesis (NST). This suggestion is supported by recent preliminary results of an experiment in which the environmental temperature was increased to 28° after 48 h of fasting: UCP-mRNA showed no peak, but a gradual decrease when rats continued fasting, while it increased as usual when rats were refed.

Therefore under the present experimental conditions, diet-induced thermogenesis and NST responses have been clearly dissociated.

Bouillaud, F., Ricquier, D., Thibault, J. & Weissenbach, J. (1985). *Proceedings of the National Academy of Sciences, USA* **82,** 445–448.
Desautels, M. (1985). *American Journal of Physiology* **249,** E99–E106.
Rothwell, N. J. & Stock, M. J. (1979). *Nature* **281,** 31–35.
Trayhurn, P. & Jennings, G. (1986). *Bioscience Reports* **6,** 805–810.

Energy intake during the menstrual cycle: little change at ovulation. By CATHERINE MCCOY, B. DONNE and J. F. ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Irish Republic*

Dalvit (1981) determined energy intake (by daily recall interview) related to phase of the menstrual cycle and observed a step-function decrease in mean intake of *ca.* 25% in the first ten post-menstruation days compared with the last ten pre-menstruation days ($P<0{\cdot}004$). Dalvit (1981) supposed this effect to be related to the hormonal changes associated with ovulation. We have repeated the study but by determining the day of ovulation have related food intake specifically to that event. For comparison we have also measured food intake during a 'cycle' regulated by a contraceptive pill, where ovulation is suppressed.

Twelve healthy young women with normal menstrual cycles and six women receiving an oestrogen/progesterone-based contraceptive pill were studied. Food intake was reported by daily diet sheet during the course of a full cycle or a 21-d-on, 7-d-off pill 'cycle'. Energy intake was calculated from food tables (Paul & Southgate, 1985). Day of ovulation was established by daily determination of oral temperature.

The results in the Table show that although mean energy intake was slightly higher in the post-ovulation period (8%) this increase was not significant and fell far short of the 25% increase observed by Dalvit (1981). Subjects receiving the contraceptive pill showed no change at all in food intake when the first and second phases of the 'cycle' were compared.

	Normal menstrual cycle (n 12)			
	8 d pre-ovulation		8 d post-ovulation	
	Mean	SE	Mean	SE
Energy intake (kJ/d)	8274	273	8950	340
	Contraceptive-pill-regulated 'cycle' (n 6)			
	days 1–21		days 22–28	
	Mean	SE	Mean	SE
Energy intake (kJ/d)	7384*	277	7279*	227

*Significantly different from normal group ($P<0{\cdot}05$).

In conclusion we are unable to confirm a large increase in food intake associated with ovulation. However, a small effect cannot be ruled out, the method used being inadequate to enable this to be confirmed statistically. The complete lack of change in dietary intake in the pill group would suggest that if there is an effect it is unlikely to be driven by progesterone:oestrogen changes.

The only significant observation was a marked decrease in energy intake in the pill group (11% comparing normal post-ovulation with the off pill phase).

Dalvit, S. P. (1981). *American Journal of Clinical Nutrition* **34**, 1811–1815.

Paul, A. A. & Southgate, D. A. (1985). *McCance and Widdowson's The Composition of Foods*. Fourth edn. London: H.M. Stationery Office.

https://doi.org/10.1079/PNS19890018 Published online by Cambridge University Press

Effects of cold exposure on mitochondrial GDP binding and uncoupling protein concentration in brown adipose tissue of lean and obese (*ob/ob*) mice. By R. E. MILNER and P. TRAYHURN, *Nutrition and Metabolism Research Group, Departments of Medicine and Foods & Nutrition, 536 Newton Research Building, University of Alberta, Edmonton, Alberta, Canada*

Mature *ob/ob* mice do not exhibit a significant increase in GDP binding in brown adipose tissue (BAT) mitochondria following acute exposure to cold, in contrast to lean animals (Himms-Hagen & Desautels, 1978). However, cold acclimation does lead to increased GDP binding (Hogan & Himms-Hagen, 1980). In the present study the time-course of the effects of cold exposure on GDP binding and the specific mitochondrial concentration of uncoupling protein in *ob/ob* mice have been examined.

Female lean and obese mice (Aston strain), aged 5–6 weeks, were adapted to 28° for 3 weeks. The mice were then exposed to 13° for periods from 1 h to 20 d. GDP binding to BAT mitochondria was measured as described previously (Mercer & Trayhurn, 1986).

	GDP bound (pmol/mg mitochondrial protein)						
	Lean			Obese			
Period of time at 13°	Mean	SEM	*n*	Mean	SEM	*n*	*P*
0	66	5	10	75	9	6	NS
24 h	283	22	10	102	13	7	<0·001
3 d	336	11	5	304	38	5	NS

NS, not significant.

The Table shows that after 24 h at 13° there was a large increase in GDP binding in the lean mice, but a non-significant change in the obese. However, after 3 d at 13° GDP binding had risen sharply in the obese, to a similar level to that in the lean. The uncoupling protein concentration of the mitochondria was significantly increased in the lean mice after 24 h in the cold (from 10·1 (SE 1·9) to 18·7 (SE 1·0) μg/mg), and following 3 d of cold-exposure in the obese (from 8·1 (SE 1·7) to 15·0 (SE 2·6) μg/mg protein). Molar binding ratios indicated an unmasking of GDP binding sites in the lean after 1 h at 13°, but not until 3 d in the obese.

While mature *ob/ob* mice fail to exhibit a rapid unmasking of GDP binding sites on acute exposure to cold, prolonged cold exposure leads to unmasking. Since insulin resistance is an important factor in the defective acute cold-induced activation of the proton conductance pathway in *ob/ob* mice (Mercer & Trayhurn, 1986). Given the effects of cold exposure on insulin sensitivity (Vallerand *et al.* 1987), the unmasking of GDP binding sites in the obese following 3 d of exposure to 13° could result from a cold-induced restoration of insulin sensitivity in BAT.

Himms-Hagen, J. & Desautels, M. (1978). *Biochemical and Biophysical Research Communications* **83**, 628–634.

Hogan, S. & Himms-Hagen, J. (1980). *American Journal of Physiology* **239**, E301–E309.

Mercer, S. W. & Trayhurn, P. (1986). *FEBS Letters* **195**, 12–16.

Vallerand, A. L., Perusse, F. & Bukowiecki, L. J. (1987). *American Journal of Physiology* **253**, E179–E186.

Folic acid and vitamin B_{12} status of a sample of the Northern Ireland population. By J. J. STRAIN, MARGO E. BARKER, KATE A. THOMPSON, MARION E. WRIGHT, SALLY I. MCCLEAN, P. G. MCKENNA, NORMA G. REID and A. P. WILLIAMSON, *Centre for Applied Health Studies, University of Ulster at Coleraine, Cromore Road, Coleraine BT52 1SA*

Folic acid and vitamin B_{12} status of a representative sample of the Northern Ireland population was assessed from blood samples taken from subjects (18–64 years), who participated in the Northern Ireland Diet and Health Study. Blood samples were obtained from 230 males and 292 females, representing 87% of the eligible participating sample.

A single non-fasted venous blood sample was taken from each subject in the evening. Blood samples were analysed within 48 h in the Royal Victoria Hospital laboratories (which subscribe to the UK National External Quality Assessment Scheme). Serum and erythrocyte (RBC) folic acid and serum vitamin B_{12} were determined using a dual radioassay kit (utilizing ^{125}I and ^{57}Co as tracers). A total of 508 valid results were obtained for both serum folic acid and vitamin B_{12} and 490 valid results were obtained for RBC folic acid.

Vitamin values were normally distributed and there were no statistical differences (ANOVA) with respect to age or sex in levels of RBC folic acid (males (*n* 215) 368 (SD 188·7) μg/l; females (*n* 275) 374 (SD 190·1) μg/l), serum folic acid (males (*n* 224) 3·31 (SD 1·589) μg/l; females (*n* 284) 3·39 (SD 2·284) μg/l) or vitamin B_{12} (males (*n* 224) 271 (SD 100·1) ng/l; females (*n* 284) 274 (SD 106·1) ng/l). Classification of subjects according to folic acid and vitamin B_{12} status is given in the Table. Cut-off points have been taken from Herbert (1985, 1987). The two lower cut-off points for RBC folic acid (<100 and <150 μg/l) denote folic-acid-deficiency anaemia and folic-acid-depletion phases respectively, while RBC folic acid levels (>200 μg/l) are considered to be normal. The cut-off points for serum folic acid classify negative folic acid balance (<3 μg/l), and normal (≥5 μg/l) and high (>16 μg/l) serum levels. Subjects with low serum vitamin B_{12} levels (<100 ng/l) are considered to be vitamin-B_{12}-deficient while vitamin B_{12} levels (>200 ng/l) are classified as normal.

	RBC folic acid (μg/l)			Serum folic acid (μg/l)			Serum vitamin B_{12} (ng/l)	
	<100	<150	>200	<3	≥5	>16	<100	>200
Male (%)	7·0	10·2	85·1	37·5	13·4	0	1·8	76·3
Female (%)	5·8	10·2	82·2	43·0	16·2	0·3	1·4	73·6

Using these criteria the prevalence of low-folic-acid status may be a cause for concern.

This work was supported by the Health Promotion Research Trust.

Herbert, V. (1985). *Laboratory Investigation* **52**, 3–19.
Herbert, V. (1987). *American Journal of Clinical Nutrition* **46**, 387–402.

Effects of moderate and rapid weight loss on body composition in obese (*fa/fa*) Zucker rats. By MICHAEL J. STOCK, *Department of Physiology, St George's Hospital Medical School, Tooting, London SW17 0RE*

The present study was designed to test the effects of reducing body-weight by restricting energy intake to levels equivalent to human low- (LED) and very-low- (VLED) energy diets. Two groups (*n* 8) of weight-matched (558 g) obese (*fa/fa*) male rats (age 3·5 months) were fed on either 3 g/d (47 kJ/kg body-weight$^{0·75}$ per d; VLED group) of a commercial VLED preparation (Cambridge Nutrition Ltd, Norwich), or 11 g/d (208 kJ/kg body-weight$^{0·75}$ per d; LED group) of a rodent stock diet (PRD; C. Hill Group Ltd, Dorset) until their average weight had fallen to that (421 g) of an age-matched group of lean (*Fa/?*) Zucker rats fed on the stock diet *ad lib*. The energy intakes were equivalent to 1380 kJ (330 kcal) (VLED) and 5860 kJ (1400 kcal) (LED) for a 90 kg human being. Weight- and age-matched groups of lean and obese rats were killed at the start of the experiment to determine initial body composition.

The rate of weight loss (mean VLED 8·6 (SE 0·1); LED 4·5 (SE 0·2) g/d) was significantly ($P<0·001$) greater in the VLED group, which took 15 d compared with 30 d (LED group) to reach the same body-weight. Average daily energy expenditure (carcass balance method) and resting oxygen consumption (measured on days 8 and 14) were depressed equally in both groups to 55–60% of levels in the lean rats. The body composition (g/kg body-weight) of the obese rats before and after weight loss is shown in the Table.

	Initial (*n* 8)		VLED (*n* 8)		LED (*n* 8)	
	Mean	SE	Mean	SE	Mean	SE
Water	404[a]	5	396[a]	4	380[b]	5
Fat	408[a]	8	418[a]	5	441[b]	8
Protein	121[a]	2	145[b]	3	140[b]	2

[a,b]Values in rows with the same superscript letter are not significantly different (Scheffe): $P<0·05$.

There was an increase in protein concentration in both treatment groups, but the proportion of fat and water in the VLED group remained unaltered, whereas the fat increased and the water decreased in the LED group. There were no significant differences in organ weights (heart, liver, kidney) between the VLED and LED groups.

It is concluded that the depression in metabolic rate induced by severe energy restriction in genetically obese rats is no greater than that seen during moderate energy restriction, and that very rapid weight loss does not produce a greater loss of lean tissue than that produced by a more gradual reduction in body-weight.

The relative effects of recent dietary intakes and adipose tissue levels of linoleic acid on the linoleic acid content of plasma free fatty acids of healthy volunteers. By J. KEARNEY and M. J. GIBNEY, *Division of Nutritional Sciences, Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, Dublin 8, Irish Republic*

Adipose tissue levels of linoleic acid (18:2*n*-6) are an accurate long-term indicator of dietary levels of linoleic acid. Consequently, biopsies of adipose tissue are being increasingly used in epidemiological and case-control studies of the role of linoleic acid intake in disease. However, a high level of adipose tissue linoleic acid does not necessarily indicate a high level of metabolically available linoleic acid. This is possible because of the likely existence within adipose tissue of at least two pools: a small pool with a short half-life in slow equilibrium with a large pool with a long half-life. The purpose of the present study was to determine the relative importance of a high recent dietary intake of linoleate and a high long-term intake of linoleate (i.e. high adipose 18:2) on fasting free fatty acid linoleate levels.

Fat biopsies were taken from fourteen healthy volunteers who, on the basis of previous biopsies, were known to have low (group 1) or high (group 2) levels of adipose tissue linoleate. Fasting blood was taken 10 h after a 48 h period of restricted intake of linoleic acid and after a 48 h period of high intake of supplementary linoleic acid (23·72 (SE 1·32) g/d). The fatty acid composition of the free fatty acid fraction of lipoprotein-deficient plasma (density $<1{\cdot}21 \times 40\,000$ rpm $\times$ 48 h) was determined. The results are summarized in the Table for linoleic acid.

	Group 1 (*n* 7)		Group 2 (*n* 7)	
	Mean	SE	Mean	SE
Adipose tissue linoleate (% wt/wt)	11·1	1·9	17·6	3·9
Plasma free fatty acid linoleate (% wt/wt):				
Restricted linoleate intake	12·7	2·5	12·6	2·5
High-linoleate intake	18·0	2·8	15·3	3·2

Analysis of variance found a highly significant effect ($P<0{\cdot}001$) of dietary linoleate on plasma free fatty acid linoleate with no significant effect of adipose tissue linoleate. There was no significant correlation between adipose tissue and free fatty acid levels of any of the major fatty acids. These results show that high levels of adipose tissue linoleate do not immediately lead to high levels of linoleate in the plasma free fatty acid fraction, the normal route for the transport of fatty acids out of adipose tissue.

Effect of voluntary exercise training and detraining on energy intake and body-weight gain: implications for existence of regulatory thermogenesis. By C. FORD, P. C. FOSTER and J. J. WARING, *School of Applied Biology, Lancashire Polytechnic, Corporation Street, Preston PR1 2TQ*

The debate on whether exercise training results in an adaptation which causes an alteration in resting metabolic rate or regulatory thermogenesis, or both, is at present unresolved.

Twenty-four male Wistar rats (198 (SEM 3) g) were divided into three groups (A, B, C). Group A was *ad lib.*-fed and given unrestricted access to exercise wheels for 6 weeks. Sedentary groups B and C were restricted fed so that they achieved the same body-weight gain as group A. At the end of the 6 week period the wheels were removed and a detraining period, lasting 4 weeks, was commenced, during which sedentary group C was transferred to a pair-feeding regimen in which metabolizable energy (ME) intake was adjusted to equal that of group A. Food intake, body-weight and amount of voluntary exercise were recorded daily throughout the experiment.

All members of the exercise group used the wheels on a regular basis (amount of exercise 2735 (SEM 597) m/d). The ME intake of all groups was significantly higher in the detraining period compared with the training period ($P<0{\cdot}05$ group A, $P<0{\cdot}01$ group B, $P<0{\cdot}001$ group C).

	Group A (*n* 8)		Group B (*n* 8)		Group C (*n* 8)	
	Mean	SEM	Mean	SEM	Mean	SEM
ME intake, training period (kJ/d)†	330·90	11·02	282·03***	8·82	310·74*	7·00
ME intake, detraining period (kJ/d)‡	352·33	7·23	313·58**	5·59	353·57	6·65
Wt gain, training period (g/d)†	2·05	0·22	1·90	0·35	2·30	0·33
Wt gain, detraining period (g/d)‡	2·98	0·24	2·97	0·26	2·92	0·41

Significantly different from group A: *$P<0{\cdot}05$, **$P<0{\cdot}01$, ***$P<0{\cdot}001$.
†Values for period 2 weeks immediately before start of detraining.
‡Values for period 2 weeks immediately after start of detraining.

The Table shows that there was no significant difference in the ME intake nor body-weight gain of group A compared with group C during the first 2 weeks after the start of detraining. This suggests that no adaptation which leads to an alteration in resting metabolic rate or regulatory thermogenesis occurred as a result of exercise training. In the same period, however, group B achieved a body-weight gain which was not significantly different from that of group A but at the same time had a significantly lower ME intake. This indicates that the energy expenditure of the exercise-trained rats was greater than that of sedentary group B rats. However, this is not inconsistent with the above findings since it might be expected that group B would have a lower energy expenditure as a result of the lower ME intake.

These results support the view that exercise training does not result in an adaptation of energy expenditure.

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

The energy and nutrient intakes of a representative sample of adults (aged 16–64 years) were assessed as part of the Northern Ireland Diet and Health Study. This study also obtained information on the social, psychological, anthropometric and biomedical characteristics of the population. A total of 616 subjects kept 7 d weighed intake records, representing a response rate of 77·3% (calculated from the effective sample of 797 addresses). Of these, twenty-four subjects produced incomplete or doubtful records and were excluded from the analysis.

Energy and nutrient intakes were examined in relation to the variables of sex, age, and household socio-economic group (HSEG). Subjects were categorized into four age groupings (16–29, 30–39, 40–49 and 50–64 years) and four HSEGs (non-manual, manual, unemployed and others). The ANOVA test was used to examine differences in intakes of energy and all nutrients with the exception of alcohol.

As expected, male subjects (n 258) had significantly ($P<0·001$) greater mean daily intakes of energy, protein, fat and carbohydrate than female subjects (n 334). Alcohol intake was also greater in the male group. Age had a statistically significant effect on energy and nutrient intakes in both the male and female groups. In general, intakes of all nutrients were lowest in the 50–64 age group and greatest in the 30–39 age group (males) and 16–29 age group (females). HSEG did not significantly affect overall intakes of energy and nutrients, although protein intake showed a significant interaction with sex and HSEG. There were striking differences with regard to alcohol consumption between the HSEGs: in the male group the unemployed reported intakes considerably in excess of any other group.

	Males		Females	
Intake	Mean	SD	Mean	SD
Energy (MJ/d)	10·6	2·44	7·1	1·86
(kcal/d)	2526	582·6	1670	445·0
Protein (g/d)	85·0	19·44	59·7	15·02
Fat (g/d)	108·8	29·63	75·5	23·57
Carbohydrate (g/d)	292·2	81·50	198·7	56·87
Alcohol (g/d)	15·3	25·31	4·8	9·52

Energy and nutrient intakes have been contrasted with comparable studies of other regions of the UK and are, in general, similar. It is concluded that in Northern Ireland there is considerable scope for dietary change in order to comply with recommended nutritional goals.

This work was supported by the Health Promotion Research Trust.

Perinatal changes in iodothyronine 5′-deiodinase activity in rat brown adipose tissue. By M. GIRALT, I. MARTIN, T. MAMPEL, F. VILLARROYA, R. IGLESIAS and O. VIÑAS (introduced by J. F. ANDREWS), *Departament de Bioquímica, Universitat de Barcelona, 08071-Barcelona, Spain*

We have investigated the changes in brown adipose tissue (BAT) iodothyronine 5′-deiodinase activity (15′D) in the fetal rat and in the period immediately after birth, as well as the role of stage of birth and environmental temperature in the observed changes.

In Expt 1, fetuses were obtained by Caesarian section and neonates were studied at different times after spontaneous delivery. In Expt 2, at-term or premature fetuses were removed by Caesarian section in a thermostatic chamber (37°); half the litter was placed at 21° and the other half remained at 37°. Interscapular BAT was removed and 15′D was determined as already reported (Iglesias *et al.* 1987).

Table 1. *Changes in rat BAT I5′D (fmol I^-/h per mg protein) during the fetal and immediately postnatal periods*

(Mean values, with their standard errors for seven to eight litters per group)

	Fetus (gestation period, d)					Neonate (postnatal period, h)				
	17	18	19	20	21	0	2	6	12	24
I5′D: Mean	26	42	168	368	205	56	88	62	42	32
SEM	5	13	6	20	17	7	14	11	6	3

Table 2. *Effect of environmental temperature on BAT 15′D (fmol I^-/h per mg protein) in neonatal rats at different times after Caesarian section*

(Mean values with their standard errors for seven to eight litters per group)

	At-term (21·5 d gestation period)							Premature (20·5 d gestation period)						
Temperature . . .	37°				21°			37°				21°		
Period of housing (h) . . .	0	2	12	24	2	12	24	0	2	12	24	2	12	24
I5′D: Mean	122	72	49	28	59	78	49*	226	71	35	23	70	74	45
SEM	17	6	10	8	7	11	5	35	14	5	7	6	16	11

*$P<0·05$, when neonates of the same age maintained at different environmental temperatures were compared.

As shown in Table 1, there was a progressive increase in I5′D during the fetal period which reached its peak on the 20th day of gestation, but there was a progressive decrease in the last days of fetal life. Birth was associated with a decline in I5′D, both in spontaneous delivery and in Caesarian sections of at-term fetuses. Caesarian section of premature fetuses caused a sudden drop in I5′D. During the 1st day of life there was no important changes in I5′D caused by the environmental temperature (Table 2).

It is concluded that in the fetus and period immediately after birth, the known concurrence between BAT I5′D and thermogenic activities occurring in the adult rat is not present. It is suggested that the main physiological regulation of BAT I5′D in the perinatal period is different from the mainly noradrenergic modulation in the adult rat. It is speculated that high fetal I5′D has the function of generating 3,5,3′-triiodothyronine (T_3) *in situ* in BAT, so aiding the processes of maturation and differentiation.

Iglesias, R., Fernandez, J. A., Mampel, T., Obregon, M. J. & Villarroya, F. (1987). *Biochimica et Biophysica Acta* **923**, 233–240.

https://doi.org/10.1079/PNS19890018 Published online by Cambridge University Press

Selenium content of commercial dietetic products used in the Irish Republic. By K. R. O'SULLIVAN and P. M. MATHIAS, *Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Irish Republic*

Selenium is largely associated with the protein component of foods (Young *et al.* 1982). The processing of elemental feeds and artificial formulations used in clinical dietetic practice can, therefore, result in significant losses of Se from these products. Hence, the Se status of patients consuming these feeds may be compromised. In the present study a selection of commercial formulae typically used for enteral feeding, total parenteral nutrition (TPN), and for patients with cystic fibrosis (CF) and phenylketonuria (PKU) were analysed for Se using a modified single test-tube microfluorimetric assay (Jordan *et al.* 1987). The Table gives results for those feeds found to contain insufficient dietary Se for daily needs, based on suggested 'safe intakes' of the US Food and Nutrition Board, National Research Council (1980) of 0·05–0·2 mg Se/d for adults and 0·02–0·06 mg Se/d for infants, 6–12 months of age.

Product	Powder (P) or Liquid (L)	Manufacturer	Typical use of feed	Se (ng/g P or ng/ml L)	Se intake (mg/kcal)	Se intake (mg/kJ)
Triosorban	P	Merck	Enteral	42	0·018/2000	0·018/8368
Nutricomp	P	Braun	Enteral	66	0·030/2000	0·030/8368
Clinifeed ISO	L	Roussel	Enteral	2·1	0·005/2250	0·005/9414
Clinifeed Favour	L	Roussel	Enteral	3·3	0·008/2250	0·008/9414
Isocal	L	Mead Johnson	Enteral	8·0	0·02/2400	0·02/10041
Travenol Bag	L	Travenol	TPN	None	0	
Portagen	P	Mead Johnson	CF	32	0·004/650	0·004/2720
Nutramigen	P	Mead Johnson	CF	26	0·005/650	0·005/2720
Pregestimil	P	Mead Johnson	CF	33·2	0·005/650	0·005/2720
Lofenalac	P	Mead Johnson	PKU	46·5	0·008/640	0·008/2677
PKU Milk Matilde	L	Danish Long Life Milks	PKU	None	0	

The results show that a number of enteral feeds, and especially those used for patients with CF and PKU and those on TPN, are lacking in Se. Low serum Se concentrations and Se deficiency have been reported in patients with CF and PKU, and particularly in those on TPN (Lombeck *et al.* 1975; Stead *et al.* 1985; Brown *et al.* 1986). Thus an investigation of the Se status of patients on these formulations may indicate the need for Se supplementation.

Brown, M. R., Cohen, H. J., Lyons, J. M., Curtis, T. W., Thunberg, B., Cochran, W. J. & Klish, W. J. (1986). *American Journal of Clinical Nutrition* **43**, 549–554.

Jordan, K., Shortt, H. S. & Mathias, P. M. (1987). *Proceedings of the Nutrition Society* **46**, 116A.

Lombeck, I., Kasperek, K., Feinendegen, L. G. & Bremen, H. J. (1975). *Clinica Chimica Acta* **64**, 57–61.

Stead, R. J., Redington, A. N., Hinks, L. J., Clayton, B. E., Hodson, B. E. & Batten, J. C. (1985). *Lancet* **ii**, 862–863.

US Food and Nutrition Board, National Research Council (1980). *Recommended Dietary Allowances*, 9th edn. Washington DC: National Academy of Sciences.

Young, V. R., Nahepetian, A. & Janhorbani, M. (1982). *American Journal of Clinical Nutrition* **35**, 1076–1088.

Bovine brown adipose tissue iodothyronine 5′-deiodinase activity during development. By O. VIÑAS[1], L. CASTEILLA[2], M. GIRALT[1], T. MAMPEL[1], R. IGLESIAS[1] and F. VILLARROYA[1] (introduced by J. F. ANDREWS), [1]*Departament de Bioquímica, Universitat de Barcelona, 08071-Barcelona, Spain*, [2]*Laboratoire de Production de Viande, INRA, Theix, France* and *Centre de Recherches sur la Nutrition, CNRS, Meudon-Bellevue, France*

We have studied the developmental changes in iodothyronine 5′-deiodinase activity (I5′D) in bovine perirenal brown adipose tissue (BAT) in the fetus and after birth, as well as the occurrence of I5′D in different bovine adipose tissue sites. I5′D was assessed from the ^{125}I produced from ^{125}I-labelled rT_3, as already reported (Leonard & Rosenberg, 1980). From previous characterization studies we have established optimal standard conditions for the bovine BAT I5′D assay as 2 μM-rT_3, 100 mM-DTT, 5 min incubation time.

I5′D (pmol I^-/h per mg protein) in the perirenal adipose tissue of bovine fetuses and neonates

	Fetuses				
Gestation period (d) . . .	<150	151–180	181–210	211–240	241–birth
Number of animals . . .	3	6	3	2	7
I5′D: Mean	173	678	1955	2388	517
SEM	86	231	140	181	129

	Neonates					
Postnatal period (d) . . .	0–2	3–9	15	38	100	Adult
Number of animals . . .	2	3	1	1	1	1
I5′D: Mean	126	276	87	71	35	Undetectable
SEM	53	54				

As the Table shows, there were peak values of I5′D in the perirenal adipose tissue of the fetus between the 7th and the 8th month of gestation. I5′D declined before birth and decreased progressively after parturition, becoming very low after day 15 of birth and finally undetectable in the adult cow. I5′D was also present in other adipose tissue sites of the neonatal calf, such as the pericardiac, intramuscular and peritoneal sites, whereas it is always undetectable in the subcutaneous site. The parallel between the reported results on I5′D and the cytological and biochemical features of the adipose depots of bovine fetuses and neonates (Casteilla *et al.* 1987) indicates that the occurrence of I5′D is a specific feature of BAT in the bovine species and is not shared by white adipose tissue. When the changes in I5′D during development is compared with the corresponding changes of other specific markers of BAT, the high fetal enzyme activity appears to be an early event in the prenatal differentiation of cells in BAT.

Casteilla, L., Forest, C., Ricquier, D., Robelin, J., Lombet, A. & Ailhaud, G. (1987). *American Journal of Physiology* **252,** E627–E636.

Leonard, J. L. & Rosenberg, N. (1980). *Endocrinology* **107,** 1376–1383.

Sex differences in blood antioxidant enzyme activities in human beings. By D. G. M. CARVILLE, J. J. STRAIN, R. W. WELCH and M. E. BARKER, *Biomedical Sciences Research Centre, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB*, and D. A. RICE and P. YOUNG, *Veterinary Research Laboratories, Stoney Road, Stormont, Belfast BT4 3SI*

There is increasing speculation about the involvement of oxygen free radicals (OFR) in human diseases, particularly in ischaemic heart disease and in cancer. This, in turn, has led to speculation about the aetiological role of dietary nutrients which help to maintain the integrity of antioxidant defences (Machlin & Bendich, 1987). It is probable, however, that physiological determinants apart from diet are involved in the body's defence potential against OFR. For example, sex differences in the status of provitamin A carotenoids and retinol have been reported (Thurnham & Flora, 1988).

Blood samples were obtained from 209 males and 252 females who participated in the Northern Ireland Diet and Health Study. Plasma, washed erythrocytes and whole blood were stored at −20° before analysis for caeruloplasmin (*EC* 1.16.3.1, CP), erythrocyte superoxide dismutase (*EC* 1.15.1.1, CuSOD), catalase (*EC* 1.11.1.6) and whole blood glutathione peroxidase (*EC* 1.11.1.9, GSH-Px). Activities of CP and CuSOD followed a skewed distribution and were normalized. Two-way analysis of variance (ANOVA) was performed on the data to investigate sex and age differences. Arithmetic means with standard deviations (SD) are given in the Table.

	Men		Women		
Enzyme	Mean	SD	Mean	SD	ANOVA
CP (U/l)	692	202	821	273	$P<0{\cdot}0001$
CuSOD (U/mg Hb)	17·2	3·08	18·3	4·73	$P<0{\cdot}05$
Catalase (U/g Hb)	84·1	48·4	94·5	53·7	$P<0{\cdot}05$
GSH-Px (U/g Hb)	51·9	18·9	58·4	25·5	$P<0{\cdot}01$

Hb, Haemoglobin.

Females had significantly higher levels than males of all four blood antioxidant enzymes, while age appeared to have no significant effect. These preliminary findings suggest that there are sex-related differences in blood enzymic antioxidants that may be relevant to the different susceptibilities of men and women to various chronic diseases where OFR are involved. Furthermore, since CuSOD and GSH-Px have been used as indicators of copper and selenium body levels respectively, the present results may reflect increased body status for these two trace elements in women.

This work was supported by the British Heart Foundation.

Machlin, L. J. & Bendich, A. (1987). *FASEB Journal* **1**, 441–445.
Thurnham, D. I. & Flora, P. S. (1988). *Proceedings of the Nutrition Society* **47**, 181A.

Some effects of β receptor agonists and antagonists on oxygen consumption and lipolysis in brown adipocytes of the neonatal rabbit. By T. W. G. JONES, SUSAN BROWN, LISA CARVER and NICOLA SPENCER, *Department of Biological Sciences, Coventry Polytechnic, Coventry CV1 5FB*

β Receptors of brown adipocytes (BA) have been variously proposed to be β_1, a mixed population of β_1 and β_2 or a novel type (Arch *et al.* 1984), tentatively designated β_3 with different binding characteristics from those of β_1 and β_2 receptors for agonists and antagonists. Evidence supporting this view has come from comparing the effective concentration causing 50% of maximal response (EC_{50}) for β receptor-mediated effects in different tissues of a range of β-agonists and pA_2 (natural logarithm of concentration of antagonist causing 50% reduction in response to a sub-maximal dose of agonist) values for agonist–antagonist interactions.

Working with BA isolated by collagenase (*EC* 3.4.24.3) digestion from the cervical fat pads of New Zealand White neonate rabbits, we determined the EC_{50} for isoprenaline and BRL28410-stimulated oxygen consumption (V_{O_2}) and lipolysis, and pA_2 values for isoprenaline–propranolol and BRL28410–propranolol. BRL28410 is a novel β-agonist developed by Beecham Research Laboratories. These values are compared with those of Wilson & Lincoln (1984), obtained from isolated rabbit atria, and with those of J. R. S. Arch (personal communication) using rat atria.

	EC_{50} (M)					
	Isoprenaline			BRL28410		
	Mean	SEM	*n*	Mean	SEM	*n*
Rabbit BA V_{O_2}	(a) $4{\cdot}5\times10^{-6}$	$0{\cdot}64\times10^{-6}$	4	$2{\cdot}5\times10^{-5}$	$0{\cdot}16\times10^{-5}$	3
	(b) $1{\cdot}7\times10^{-6}$	$0{\cdot}57\times10^{-6}$	6	$2{\cdot}95\times10^{-5}$		1
Rabbit BA glycerol release	(a) $2{\cdot}0\times10^{-6}$	$1{\cdot}14\times10^{-6}$	4	$3{\cdot}0\times10^{-5}$	$0{\cdot}72\times10^{-5}$	2
Rabbit right atrial rate	(c) $6{\cdot}1\times10^{-9}$			$6{\cdot}2\times10^{-5}$		
EC_{50} BA:EC_{50} atria	4481			0·5		

	pA_2					
	Isoprenaline–propranolol			BRL28410–propranolol		
	Mean	SEM	*n*	Mean	SEM	*n*
Rabbit BA V_{O_2}	(a) 6·80	0·17	4	4·05	0·24	2
	(b) 6·95	0·4	14	4·96	0·16	11
Rabbit BA glycerol release	(a) 6·75	0·57	4	4·95	0·8	2
Rat right atrial tension	(d) 8·7			8·7		
K_d BA:K_d atria	73·6			6025·6		

K_d, dissociation constant.

(a) Carver, L. (1984). BSc (Hons) Thesis, Coventry Polytechnic; (b) Spencer, N. (1985). BSc (Hons) Thesis, Coventry Polytechnic; (c) Wilson & Lincoln (1984); (d) J. R. S. Arch, personal communication.

The ratios of EC_{50} and pA_2 obtained for each tissue suggest that BRL28410 is more selective for BA than isoprenaline. Together with the marked differences in propranolol pA_2 between isoprenaline and BRL28410 on BA the view is also supported that in the rabbit, as in the rat, the β_1 receptors mediating changes in right atrial rate or tension are different from β receptors mediating increases in BA lipolysis and respiration.

Arch, J. R. S., Ainsworth, A. T., Cawthorne, M. A., Piercy, V., Sennitt, M. V., Thody, V. E., Wilson, C. & Wilson, S. (1984). *Nature* **389,** 163–165.

Wilson, C. & Lincoln, C. (1984). *Journal of Cardiovascular Physiology* **6,** 1216–1221.

Effect of physiological ligands on zinc uptake by syncytiotrophoblast microvillous plasma membrane vesicles from human placenta. By G. QUINN and A. FLYNN, *Department of Nutrition, University College, Cork, Irish Republic*

Zinc in human plasma is bound mainly to albumin (~80%) and α_2-macroglobulin (~20%), with a small fraction (<2%) bound to amino acids (histidine, cysteine). As Zn is very tightly bound to α_2-macroglobulin it has been suggested that only that Zn bound to albumin and amino acids is readily available for tissue uptake.

Syncytiotrophoblast microvillous plasma membrane vesicles (SMPMV) were prepared from frozen human placenta by cold saline extraction and differential centrifugation (Smith *et al.* 1974). Zn uptake was studied by suspending vesicles with ^{65}Zn in 10 μM-$Zn(NO_3)_2$ in isotonic HEPES buffer, pH 7·4, at 37°. Uptake was stopped with ice-cold 0·5 mM-$Zn(NO_3)_2$ in buffer, pH 7·2, and vesicles were collected and washed on Whatman GF-C filters which were then counted in a well-gamma counter.

	Zn uptake (ng/min per mg protein)			
	Histidine (*n* 6)		Cysteine (*n* 6)	
Amino acid concentration (μM)	Mean	SE	Mean	SE
0	140	20	192	17
20	158	12	168	7
40	150	15	172	14
100	90	10	156	15
500	38	7	70	5

Zn uptake was temperature- and osmolarity-dependent, indicating uptake into SMPMV. At 37°, human serum albumin at 0·4 and 1·6 mg/ml (1–4% of its plasma concentration) reduced Zn uptake by 78 and 91% respectively. Zn was readily taken up by SMPMV at physiological concentrations (20–100 μM) of histidine and cysteine, but higher concentrations (500 μM) inhibited Zn uptake. These results suggest that tissue Zn uptake may occur from the small pool of amino acid-bound Zn in plasma which can be replenished from the albumin-Zn pool. This may serve as a large reservoir of available Zn in plasma.

Smith, N. C., Brush, M. G. & Luckett, S. (1974). *Nature* **252**, 302.

Enhanced thermogenic effects of D-fenfluramine following neurotoxic lesions of central 5-hydroxytryptamine in the rat. By ANN LATHAM, ROS A. LE FEUVRE and NANCY J. ROTHWELL, *Department of Physiological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT*

Central serotonergic pathways are involved in the regulation of food intake and body temperature. Recently, stimulatory effects of the serotonergic system on brown adipose tissue (BAT) thermogenesis have also been reported (Rothwell & Stock, 1987). Peripheral injection of D,L-fenfluramine stimulates metabolic rate and BAT activity in the rat, whilst central serotenergic lesions result in hyperphagic obesity, associated with reduced BAT thermogenesis (Fuller *et al.* 1987).

The serotonergic control of BAT activity was investigated by studying the effects of selective neurotoxic lesions, and of D-fenfluramine administration on thermogenesis in the rat. Depletion of brain content of 5-hydroxytryptamine (5HT) (30%), and its metabolite 5-hydroxy-indole acetic acid (5HIAA) (50%), were achieved by bilateral intracerebroventricular (i.c.v.) injections of 5,7-dihydroxytryptamine (150 μg/20 μl; 5,7-DHT) under sodium pentobarbitone anaesthesia in rats pre-treated with desipramine (25 mg/kg intraperitoneally (i.p.)), to protect noradrenergic neurons. Indwelling lateral ventricular cannulas were implanted into the brain, and the interscapular BAT depot was unilaterally denervated.

Body-weight gain, food intake and resting oxygen consumption (V_{O_2}) were not significantly affected by 5,7-DHT lesions. A reduced thermogenic capacity of lesioned rats was suggested by a decreased V_{O_2} response to peripheral injection of noradrenaline (250 μg/kg i.p., control 41 (SEM 9)% increase, lesioned 25 (SEM 2)%). However, the thermogenic effect of D-fenfluramine was significantly ($P<0{\cdot}05$) enhanced in lesioned rats in response to peripheral (5 mg/kg i.p., control 15 (SEM 2), lesioned 30 (SEM 4)% increase in $\dot{V}_{O_2}$) and central (50 μg i.c.v., control 14 (SEM 2)%, lesioned 26 (SEM 3)%) administration. Similarly, enhanced responses were observed in lesioned rats at other doses of D-fenfluramine (1 and 10 mg/kg i.p. or 20 μg i.c.v.).

BAT activity (in vitro mitochondrial GDP binding) was slightly suppressed in lesioned animals (control 68 (SEM 5) pmol/mg protein, lesioned 47 (SEM 3) pmol/mg protein, $P<0{\cdot}05$). Denervation also reduced BAT activity, but to a greater extent in control than in lesioned rats. Peripheral injection of D-fenfluramine (10 mg/kg i.p.) significantly ($P<0{\cdot}001$) increased GDP binding by 78 (SEM 13)% in lesioned animals (n 13), compared with 15 (SEM 7)% in control animals (n 13). No changes in activity in response to D-fenfluramine were seen in the denervated lobes of either group.

In summary, the serotonergic lesions induced by 5,7-DHT did not induce obesity, and therefore differ from the effects of *p*-chlorophenyl alanine lesions reported earlier. BAT activity was, however, slightly reduced in lesioned rats. Thermogenic responses to fenfluramine apparently resulted from sympathetic activation of BAT, and were markedly enhanced in lesioned animals. This effect indicates that D-fenfluramine can act as a direct 5HT agonist, and the enhanced responses in lesioned animals could result from an elevated 5HT receptor density or increased post-receptor sensitivity, or both.

Fuller, N. J., Stirling, D. M., Dunnet, S., Reynolds, G. P. & Ashwell, M. (1987). *Bioscience Reports* **7**, 121–127.

Rothwell, N. J. & Stock, M. J. (1987). *International Journal of Obesity* **11**, 319–324.

Comparison of iron and zinc absorption in rat pups from human milk extrinsically labelled with ^{59}Fe and ^{65}Zn. By M. BRENNAN, A. FLYNN and P. A. MORRISSEY, *Department of Nutrition, University College, Cork, Irish Republic*

The sucking rat pup has been proposed as a model for investigating the bioavailability of Zn in human milk and infant formulae (Sandstrom *et al.* 1983). The present report outlines a method for simultaneous studies on iron and Zn absorption from human milk in rat pups.

Pooled mature human milk was extrinsically labelled with both ^{59}Fe (1 μCi/ml) and ^{65}Zn (0·5 μCi/ml). After allowing 24 h for isotopic equilibrium to be attained, 0·6 ml milk was given by gavage to 16-d-old Wistar rats, fasted for 16 h. Animals were killed 6 h later and tissues collected (stomach, small intestine, caecum–colon and liver). Small intestines (SI) were perfused with 6 ml saline (9 g sodium chloride) and divided into three segments of equal length. ^{59}Fe and ^{65}Zn were determined in tissues by counting in a well-gamma counter using a channels ratio method.

	Percentage of dose (*n* 8)			
	^{59}Fe		^{65}Zn	
Organ/tissue	Mean	SE	Mean	SE
Stomach	0·5	0·1	1·9***	0·2
Duodenum	1·3	0·2	8·3***	0·8
Jejunum	8·3	2·5	10·4	1·2
Ileum	30·9	3·8	8·1***	1·5
SI total	40·6	4·4	26·7**	1·4
SI perfusate	2·9	1·5	2·3	0·5
Caecum–colon	3·1	0·4	2·1	0·6
Liver	5·3	1·0	23·4***	0·2

Significantly different from ^{59}Fe values (paired *t* test): $^{**}P<0·01$, $^{***}P<0·001$.

Practically all ^{59}Fe and ^{65}Zn were cleared from stomach and SI lumen in 6 h, little had reached the caecum–colon and about 94% of each isotope was absorbed. There were significant differences in the disposition of absorbed ^{59}Fe and ^{65}Zn. More ^{59}Fe than ^{65}Zn was retained in SI tissue, more ^{65}Zn (67%) than ^{59}Fe (53%) was transferred to the carcass and a greater proportion of ^{65}Zn was taken up by the liver. While ^{65}Zn was evenly distributed throughout the length of the SI, ^{59}Fe was mainly concentrated in the distal segment. These results indicate that Zn in human milk is more readily absorbed than Fe. This may be due to the association of a significant amount of Fe with lactoferrin, which is difficult to digest (Lonnerdal, 1985).

Lonnerdal, B. (1985). In *Composition and Physiological Properties of Human Milk*, pp. 3–14 [J. Schaub, editor]. London: Elsevier.

Sandstrom, B. M., Keen, C. L. & Lonnerdal, B. (1983). *American Journal of Clinical Nutrition* **38**, 420–428.

Neuropeptide Y and noradrenaline together cause significant weight loss in obese rats. By A. AL-ARABI and J. F. ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Irish Republic*

Neuropeptide Y (NPY) has been reported to co-exist with noradrenaline (NA) within catecholaminergic neurons of the central and peripheral nervous systems (Allen *et al.* 1984; Everitt *et al.* 1984). The functional significance of NPY in these neurons has been related to its vasomotor effects that complement and interact with NA which is known to have central and peripheral effects on resting metabolic rate (RMR), food intake and body-weight of rats.

We have studied the influence of chronic peripheral administration of NPY on the effect of NA on metabolism, food intake and body-weight in two groups of adult male rats (twenty lean, Wistar strain; twenty obese (*fa/fa*), Zucker strain). Animals were acclimated to 28° (thermoneutrality). Each group was divided into five subgroups: (1) untreated controls, (2) carrier-treated controls, (3) NPY-treated, (4) NA-treated and (5) NPY+NA-treated. In subgroups 1–5 of each group, Alzet® (2002) osmotic minipumps were implanted under the skin in the interscapular region. Pumps were filled with carrier alone, 0·1 M-L-ascorbic acid and 0·02 M-L-Tiron® (Sigma Chemical Co, St Louis, Mo) (subgroup 2); carrier plus 0·3 μM-NPY (subgroup 3); carrier plus 0·3 M-NA (subgroup 4); or carrier plus both (subgroup 5). Delivery rate of NPY was calculated to be 0·5 μg/h, and of NA 20 μg/h extending over a period of 14 d. The RMR of the animals was measured on days 2, 8 and 14 (indirectly as minimal oxygen consumption using a multi-channel closed-circuit system). Cumulative food intake and cumulative change in total body-weight were determined every 2 d starting from day 2. The values at day 14 are presented in the Table.

Subgroup	1 Untreated		2 Carrier		3 NA		4 NPY		5 NPY+NA	
Genotype†	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Minimal O_2 consumption (ml/kg lean body mass per min)									
Obese	20·1	2·3	23·9	3·2	25·2	4·3	21·9	1·2	32·6*	1·8
Lean	14·8	1·6	16·3	0·9	17·5*	0·8	14·9	0·5	19·9*	2·2
	Cumulative food intake (g/rat)									
Obese	355·1	8·1	342·2	35·5	425·7*	10·0	431·5*	13·3	249·8*	13·3
Lean	209·8	3·5	209·3	3·6	229·5*	6·7	312·1*	11.5	351·9*	5·3
	Cumulative change in body-wt (g total body-wt)									
Obese	33·7	1·2	48·6	27·8	−1·7*	6·1	25·9	4·2	−63·2*	23·0
Lean	32·8	4·2	32·3	4·7	31·5	2·9	42·0	7·6	33·3	3·1

Significantly different from subgroup (2) carrier: *$P<0·05$.
†Values are for four rats per subgroup except for the untreated obese animals (n 3).

NA alone caused increased RMR of both groups, while NPY alone had the opposite effect. Combined treatment with NPY and NA caused significant increase in RMR of both groups, particularly evident in the obese group. Separate treatments enhanced food intake of both groups, while the combined treatment caused hyperphagia in the lean animals and satiety in the obese. With combined NPY and NA treatment, lean animals maintained a normal rate of weight gain, however, the obese rapidly lost weight to a significant degree.

NPY deficiency may be a factor related to the obesity of the Zucker (*fa/fa*) rats.

Allen, Y. S., Roberts, G. W., Bloom, S. R., Crow, T. J. & Polak, J. M. (1984). *Brain Research* **321**, 357–362.

Everitt, B. J., Hokfelt, T., Terenius, L., Tatemoto, K., Mutt, V. & Goldstein, M. (1984). *Neuroscience* **11**, 443–462.

Salivary and serum antibodies to gliadin in treated and untreated coeliac disease. By CAITRIONA BRADY, M. J. GIBNEY, C. KELLY and D. G. WEIR, *Division of Nutritional Sciences, Department of Clinical Medicine, Trinity College Medical School, St James's Hospital, Dublin 8, Irish Republic.*

Elevated levels of serum antibodies to the wheat protein, gliadin, are frequently reported for patients with untreated coeliac disease. To date, little information exists on the impact of coeliac disease on secretory antibodies to specific antigens. Access to secretory fluids is difficult and is generally confined to intestinal aspirates. Saliva is a good source of secretory IgA, originating from duct-associated lymphoid tissue and might have use in the evaluation of patient progress on gluten-free diets. The present study set out to determine the pattern of salivary IgA antibodies to gliadin in coeliac disease.

Thirty patients with coeliac disease were recruited (fifteen treated, fifteen untreated). A group (n 15) of patients with Crohn's disease acted as disease controls and a group (n 15) of healthy volunteers provided a further control. Fasting blood samples and stimulated parotid saliva samples were analysed by an enzyme immunoassay for antibodies to total gliadin, α-gliadin and casein.

As expected, anti-α-gliadin serum IgG and IgA antibodies were significantly elevated ($P<0{\cdot}05$) in patients with untreated coeliac disease compared with healthy controls. With treated coeliac disease, only the IgG serum antibody levels were elevated ($P<0{\cdot}05$). Even then, the degree of elevation over the controls was slight. In general, total gliadin gave lower specificity than α-gliadin. No elevations in serum antibodies to casein were observed with either group of coeliac patients. Crohn's patients showed a significant ($P<0{\cdot}05$) lowering of anti-casein serum IgG.

Salivary secretory IgA antibodies to α-gliadin, total gliadins and casein in coeliac patients (treated and untreated), Crohn's disease patients and normal healthy controls

(Results are expressed as percentage of reference/mg total IgA)

	Coeliac patients				Crohn's patients		Normal controls	
	Treated		Untreated					
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total gliadins	17·5	11·9	16·0	11·1	14·0	4·6	15·8	8·0
α-Gliadin	16·3	18·9	11·5	6·2	11·0	4·8	11·9	7·8
Casein	13·4	11·3	12·9	8·4	8·1	3·9	13·0	8·0

In contrast, no significant differences were observed between the four groups in any variable studied in saliva, which included flow-rate, total IgA and secretory IgA antibodies to α-gliadin, total gliadins and casein. Clearly saliva is of little use in the detection of an immune response to wheat proteins in coeliac disease.

https://doi.org/10.1079/PNS19890018 Published online by Cambridge University Press

Polymyxin B suppresses the stimulation of lipogenesis in brown adipose tissue of rats after an oral load of glucose. By ALISON E. TEDSTONE, STEWART W. MERCER and DERMOT H. WILLIAMSON, *Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE*

Polymyxin B (PMN), a cyclic decapeptide antibiotic, counteracts the hypoglycaemic effects of exogenous insulin in mice and rats (Amir *et al.* 1987). In vitro studies suggest that PMN acts by inhibition of the insulin-dependent activation of glucose transport in muscle and adipocytes (Amir *et al.* 1987; Griméaux *et al.* 1987). In the present work the effects of PMN on the stimulation of lipogenesis in brown adipose tissue (BAT) after an oral load of glucose (Agius & Williamson, 1980) were studied.

Female rats (starved 24 h) were injected subcutaneously with either PMN or colistin (1 mg/kg body-weight); the latter differs from PMN by a single amino acid substitution in the ring structure and is inactive. The rats were then given 2·0 ml glucose (2 mM) by gavage and ^3H_2O incorporation into lipid (lipogenesis) was measured in vivo in liver, interscapular BAT and parametrial white adipose tissue (WAT), between 30 and 90 min. Relative blood flow was measured with intravenous [^{14}C]DDT (1,1,1-trichloro-2, 2-bis(*p*-chlorophenyl) ethane) (Herd *et al.* 1968) over the same period.

		Lipogenesis†			Relative blood flow‡				ATP content in BAT§
		Liver	WAT	BAT	Liver	Heart	WAT	BAT	
Colistin (*n* 4–6):	Mean	6·1	10·0	128	1·16	0·30	0·13	2·13	2·19
	SE	0·3	3·7	16	0·20	0·08	0·02	0·14	0·31
Polymyxin (*n* 4–6):	Mean	5·7	5·4	23**	1·03	0·38	0·16	0·48*	1·12*
	SE	0·3	1·5	5	0·22	0·08	0·08	0·08	0·17

Significantly different from colistin: *$P<0·05$, **$P<0·01$.
†μmol ^3H_2O incorporated into saponified lipid/h per g tissue.
‡Accumulation of [^{14}C]DDT as a percentage of injected dose per g.
§μmol per g.

PMN administration caused hyperglycaemia (blood glucose (μmol/ml): colistin 7·2 (SEM 0·22) *n* 6 *v.* PMN 13·9 (SEM 2·0) *n* 6; $P<0·01$) and this was accompanied by virtually complete inhibition of the stimulation of lipogenesis in BAT (see Table); no significant change occurred in liver or WAT. Blood flow to BAT was decreased (80%) by PMN and there was 51% decrease in the ATP content of the tissue.

The effect of PMN on BAT lipogenesis may be due to inhibition of insulin-stimulated glucose transport. However, the impaired blood flow may also play a role by decreasing the delivery of substrates and hormones to the tissue. The possibility that PMN has a central action to bring about both effects cannot be excluded.

Agius, L. & Williamson, D. H. (1980). *Biochemical Journal* **190,** 477–480.
Amir, S., Sasson, S., Kaiser, N., Meyerovitch, J. & Schecter, Y. (1987). *Journal of Biological Chemistry* **262,** 6663–6667.
Griméaux, T., Tanti, J.-F., Van Obberghen, E. & Le Marchand Brustel, Y. (1987). *American Journal of Physiology* **252,** E248–E254.
Herd, J. A., Goodman, H. M. & Grose, S. A. (1968). *American Journal of Physiology* **214,** 263–268.

The effect of a low-dose intravenous dopamine infusion on gastric pressure. By CERI J. GREEN, M. N. HARTLEY, R. SARGINSON, R. DUCKWORTH, N. PARR and I. T. CAMPBELL, *University Departments of Anaesthesia and Surgery, Royal Liverpool Hospital, Prescot Street, PO Box 147, Liverpool L69 3BX*

Dopamine is an endogenous catecholamine used pharmacologically in the critically ill to support renal function (at 2 μg/kg body-weight per min) and myocardial function (at 5 μg/kg per min and above). It stimulates noradrenaline release at 5 μg/kg per min and increases metabolic rate at 10 μg/kg per min (Campbell *et al.* 1988). Dopamine produces gastric relaxation and delays gastric emptying in dogs at 3 μg/kg per min and in man at 9 μg/kg per min when given for 25 min (Valenzuela & Liu, 1982). The effect of dopamine on gastric relaxation in normal volunteers has been studied when infused at 2 μg/kg per min for 150 min.

Gastric corpus fundus pressure was measured using a micro-pressure transducer in the distal end of a nasogastric tube contained within an 800 ml plastic bag passed into the stomach. The bag was inflated with air at a rate of 800 ml/min for 30 s. Gastric pressure was measured during the filling period and over a further 150 s. Average pressure during filling (dynamic P) and during the following 150 s (static P) were derived from the area under the curve.

The subjects were five healthy males, aged 29–41 (median 32) years. The bag was inserted in the stomach and the subjects rested for 30 min. Dynamic and static P were then measured three times at 15-min intervals, then again after 15, 30 and 45 min of intravenous dopamine (in dextrose 50 g/l) infusion at 2 μg/kg per min, and after 120, 135 and 150 min of infusion. The results were compared with the values obtained during infusion of the corresponding volume of dextrose (50 g/l) alone. The order of infusion (dopamine or dextrose (50 g/l) alone) was randomized.

During the 1st hour of dopamine infusion both mean static and dynamic P were significantly lower during dopamine infusion than during infusion of dextrose alone (dynamic P: 96 (SEM 10) *v.* 146 (SEM 8) mm H_2O; static P: 91 (SEM 11) *v.* 136 (SEM 5) mm H_2O). During the 3rd hour of infusion, gastric pressure in three subjects had decreased further but in two it had returned to pre-infusion levels so that there was no significant difference between infusion of dopamine and infusion of dextrose alone.

It is concluded that dopamine at 2 μg/kg per min produces gastric relaxation during the 1st hour of infusion. This may delay gastric emptying. In some individuals this relaxation persists over 3 h, in others gastric pressure returns to normal. Prolonged dopamine infusion at 2 μg/kg per min may thus delay gastric emptying in some patients and so cause difficulties in nasogastric feeding.

Campbell, I. T., Regan, C., Duckworth, R., Fairhurst, J., Maycock, P. & Frayn, K. N. (1988). *Proceedings of the Nutrition Society* **47**, 60A.

Valenzuela, J. E. & Liu, D. P. (1982). *Scandinavian Journal of Gastroenterology* **17**, 293–296.

Activation of non-shivering thermogenesis, brown adipose tissue respiration and uncoupling protein by short daily cold exposure. By H. WIESINGER[1], S. KLAUS[1], O. CHAMPIGNY[2], D. RICQUIER[2] and G. HELDMAIER[1], [1]*Philipps University, Department of Biology, D–3550 Marburg, Federal Republic of Germany,* [2]*Centre de Recherches sur la Nutrition (CNRS, LP 1511), 9 rue Jules Hetzel, F–92190 Meudon, France*

It is well documented that chronic cold exposure does improve non-shivering thermogenesis (NST) in small mammals. We studied the effect of short daily cold exposures (two times 2 h, one times 4 h) on the acclimation of in vivo NST and heat generating properties of brown adipose tissue (BAT).

The experiments were performed with Djungarian hamsters, raised and kept at 23° (thermoneutral) with 16 h light – 8 h dark. Three groups of animals were exposed to 5° daily either once for 4 h, twice for 2 h (with a 12 h interval) or permanently for 24 h. After 13 d of acclimation, noradrenaline-induced NST-capacity was measured. Total BAT was removed 2 d later in order to estimate the uncoupling protein (UCP)-mRNA. For each group total mRNA of four animals was used for Northern blots. The hamsters' UCP-mRNA was detected with ^{32}P-labelled rat cDNA. Radioautographs were scanned and analysed with a densitometer. In a parallel experiment the respiratory capacity of BAT (cytochrome *c* oxidase (*EC* 1.9.3.1) activity) and the UCP content (GDP binding) were measured.

Temperature (°) . . .	23	5		
Period of exposure (h) . . .	24	4	Two × 2	24
NST-capacity (ml O_2/h): Mean	161·3	192·9	220·5	233·1
SE	13·5	14·1	14·0	13·2
Total cytochrome *c* oxidase (U/BAT): Mean	107·2	162·7	174·4	235·3
SE	12·8	9·8	17·1	20·0
GDP binding (pmol/mg mitochondrial protein): Mean	236·1	440·1	460·5	607·4
SE	29·4	29·2	21·0	70·1
UCP-mRNA (arbitrary units): Mean	123	289	367	527

As shown in the Table, short daily periods of cold exposure were sufficient to enhance significantly the in vivo NST-capacity as well as BAT respiration, UCP content and UCP-mRNA. Two times 2 h and one times 4 h cold exposure caused similar improvements of NST and BAT. The NST-capacity achieved by short daily cold exposure did not differ from the effect of chronic cold exposure. Cytochrome *c* oxidase, GDP binding and UCP-mRNA were found to be slightly less than those in chronically cold exposed hamsters.

In their natural environment burrowing rodents will experience fluctuations in ambient temperature, rather than a chronic cold load. We conclude that short daily cold exposure of a few hours is sufficient to activate transcription of UCP-mRNA, synthesis of mitochondrial protein and improvements of NST.