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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Forty-first Meeting of the Nutrition Society for the presentation of oral communications and discussion of posters was held as parallel sessions in the Babbage and Zoology Lecture Theatres on the New Museum site of Cambridge University, on Thursday and Friday, 16/17 July 1987.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Estimation of 24 h energy expenditure by a portable accelerometer. By Y. SCHUTZ, F. FROIDEVAUX and E. JÉQUIER, *Institut de Physiologie, Faculté de Médecine, Université de Lausanne, CH-1005 Lausanne, Switzerland*

Over the years various indirect non-calorimetric techniques have been developed (e.g. heart rate measurement, $^2H_2{}^{18}O$) to obtain an estimate of energy expenditure in free living conditions. There is a need to develop cheaper and less sophisticated methods when larger groups of individuals are to be studied such as in epidemiological research. Recently a new computerized accelerometer ('Caltrac activity computer') has been described (Montoye *et al.* 1983) but no direct assessment of the accuracy of the device has been made over 24 h.

In order to compare the rate of energy expenditure (EE) obtained by means of the accelerometer with that measured by a whole body indirect calorimeter (Jéquier & Schutz, 1983), twenty-nine women (body-weight 45·3–106·1 kg, body mass index 17·3–40·7 kg/m^2) were studied over 24 h and divided into two groups (1) a sedentary group (*n* 17) with spontaneous physical activity (63·4 (SD 11·9) kg) and (2) a more active group (*n* 12) in which two 30 min exercise periods on a treadmill at 3·22 km/h (2 miles/h), 10% elevation, were prescribed (72·6 (SD 18·7) kg).

		24 h EE (kJ/d)				24 h EE (kcal/d)				'Caltrac' / Measured × 100	
		Measured		'Caltrac'		Measured		'Caltrac'			
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
All subjects	29	8372	1548	7138	1138	2001	370	1706	272	86	6
Group 1, sedentary	17	7623	715	6548	494	1822	171	1565	118	86	7
Group 2, with exercise	12	9431	1812	7979	1284	2254	433	1907	307	85	5

There was a significant correlation (r 0·92, $P<0·001$, standard error of the estimate 619 kJ/d) between the 24 h EE estimated by the accelerometer and that measured with the chamber. As shown in the Table, the accelerometer tended to systematically underestimate the rate of 24 h EE by an average of 14%. It is concluded that differences in the rate of 24 h EE between individuals can be fairly well assessed with the present accelerometer but assessment of absolute 24 h EE values gave more uncertain results.

Jéquier, E. & Schutz, Y. (1983). *American Journal of Clinical Nutrition* **38**, 989–998.
Montoye, H. J., Washburn, R., Servais, S., Ertl, A., Webster, J. G. & Nagle, F. J. (1983). *Medicine and Science in Sports and Exercise* **15**(5), 403–407.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Thermic responses to food in rats with lesions of the ventromedial hypothalamus. By H. J. CARLISLE, *Department of Psychology, University of California, Santa Barbara, USA* and N. J. ROTHWELL and M. J. STOCK, *Department of Physiology, St George's Hospital Medical School, Tooting, London SW17 0RE*

Stimulation of the ventromedial hypothalamus (VMH) results in activation of thermogenesis in brown adipose tissue (BAT) in experimental animals (Perkins *et al.* 1981). Conversely, obesity induced by destruction of the VMH is dependent on hyperphagia and impaired thermogenesis and is associated with reduced activity of BAT (Seydoux *et al.* 1981). Since the VMH is particularly sensitive to glucose and insulin, we have compared the thermogenic responses to meals of carbohydrate and fat in rats with lesions of the VMH.

Male, Sprague-Dawley rats (60 d old) received bilateral, electrolytic lesions of the VMH or sham operations under pentobarbitone anaesthesia, and after recovery were allowed free access to pelleted stock diet. Ten days after surgery, resting oxygen consumption (V_{O_2}, ml/min per kg body-weight$^{0.75}$) was similar for the two groups (lesioned 11·42 (SEM 0·55), sham 11·77 (SEM 0·13), n 7,8) and injection of noradrenaline (to test maximal thermogenic capacity) evoked peak increases in V_{O_2} of 38 (SEM 5) and 35 (SEM 4)% (not significant) in sham-operated and lesioned rats respectively. Subsequent measurements showed that gastric intubation with fat (40 kJ maize oil) also produced comparable postprandial increases in V_{O_2} in the two groups (sham 12·3 (SEM 1.0), lesioned 12·2 (SEM 1·5)%, not significant), whereas the response to intubation with carbohydrate (40 kJ maize starch) was lower in lesioned (6·5 (SEM 1·9)%) than in sham-operated rats (13·1 (SEM 1·9)%, $P<0·05$). This difference was still apparent when incremental increases in V_{O_2} were considered (sham 1·5 (SEM 0·2), lesioned 0·8 (SEM 0·2) ml/min per kg body-weight$^{0.75}$, $P<0·05$). Intubation with a mixed nutrient meal (40 kJ Complan®) resulted in an attenuated thermic response in lesioned rats (10·0 (SEM 1·5)%) compared with sham-operated animals (16·5 (SEM 1·5)%, $P<0·01$). Pretreatment of the animals with atropine sulphate (1 mg/kg, subcutaneously) did not affect preprandial V_{O_2} or the thermic response to carbohydrate in controls (16·9 (SEM 2·0)%), but greatly enhanced the response in lesioned rats, to a value (32·0 (SEM 5·2)%) that was significantly greater than that in sham-operated rats ($P<0·05$).

These results show that the thermic response to carbohydrate, but not to fat, is impaired in rats with lesions of the VMH. This results in a significant reduction in thermogenesis following ingestion of a mixed nutrient meal, and probably contributes to the development of obesity. The marked potentiation by atropine of the response to carbohydrate in lesioned rats is comparable to that seen in genetically obese Zucker rats (Rothwell *et al.* 1981), but its site of action remains unknown.

Perkins, M. N., Rothwell, N. J., Stock, M. J. & Stone, T. W. (1981). *Nature* **289**, 401–402.
Rothwell, N. J., Saville, M. E. & Stock, M. J. (1981). *Pflügers Archiv* **392**, 172–177.
Seydoux, J., Rohner-Jeanrenaud, F., Assimacopoulos-Jeannet, F., Jeanrenaud, B. & Girardier, L. (1981). *Pflügers Archiv* **390**, 1–4.

Assessment of daily physical activity using synchronous recording of heart rate and body acceleration. By G. A. L. MEIJER and K. WESTERTERP (introduced by A. M. PRENTICE), *Department of Human Biology, University of Limburg, 6200 Maastricht, The Netherlands*

The use of accelerometers for the assessment of physical activity is in a developmental stage. Evaluation of the different devices used so far has been merely based on testing in laboratory situations (Servais *et al.* 1984). Here, synchronous recordings of heart rate and body acceleration were made in adults under free-living conditions during the non-sleeping part of the day.

The sensor for the body accelerations consists of a piezo-electric element deformed by a lever. It is sensitive in three directions. The sensor is worn on the mid to lower part of the back, attached to a belt carrying the recorder. Full signal recording for both heart rate and acceleration was made on a two channel portable tape recorder. The electrocardiogram (ECG) signal was reduced by taking a sample of the heart rate (HR) every minute. The absolute value of the accelerations was integrated for each minute resulting in a rate (AR).

A striking result was the high correlation between both signals in each individual recording (r 0·60 to 0·88, number of recordings 28). This high correlation was unexpected because of the different sources of error in each method. The increase in HR with rising AR was different between individuals. Training seems to explain a great deal of these differences. A pilot study consisting of three untrained and three trained (more than 8 h/week) male subjects, showed the relation between HR and AR:

$$HR = a + b * AR$$

Trained				Untrained			
Subject	a	b	r	Subject	a	b	r
1	65·8	0·097	0·88	4	76·2	0·126	0·63
2	45·6	0·099	0·84	5	71·4	0·136	0·60
3	60·6	0·061	0·85	6	75·0	0·147	0·62

Both a and b were significantly higher in the untrained group (Mann-Whitney, one tailed, Q=9, $P<0·025$). Relating ECG to the recording of body acceleration, conclusions can be drawn on individual physical fitness and habitual activity level. Moreover the accelerometer probably gives a measure for energy expenditure (during the non-sleeping part of the day) without the need for individual calibration. Further validations on this new device are planned, including validation against the doubly-labelled water technique.

Servais, S. B., Webster, J. G. & Montoye, H. J. (1984). *Journal of Clinical Engineering* **9**, 159–170.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

The role of insulin resistance in impaired energy expenditure in the LA/N-*cp* (LA-corpulent) rat. By ORIEN L. TULP, SISTER THOMAS D. MCKEE and CAROLINE SANDLER, *Department of Nutrition and Food Sciences, Drexel University, Philadelphia, PA, USA*

Hyperinsulinaemia and insulin resistance (IR) are cardinal features of obesity in man and animals. Previous studies have shown that the obese phenotype of the LA/N-*cp* rat exhibits an impaired capacity for non-shivering thermogenesis (NST) following environmental and dietary challenge (Tulp, 1984; Tulp & Shields, 1984), associated with early-onset obesity and a decreased functional activity of the insulin-dependent enzyme thyroxine deiodinase (*EC* 3.8.1.4) in extrathyroidal peripheral tissues (Gavin *et al.* 1981; Tulp & McKee, 1986). This enzyme catalyses the deiodination of thyroxine to the metabolically more active 3,5,3′-triiodothyronine and would appear to be a prerequisite for the expression of NST.

IR and NST were assessed in lean and pre-obese LA/N-*cp* rats, maintained at 22 ± 1°, that were adrenalectomized (ADX) or left intact at 6 weeks of age. Sub-groups of ADX rats were given insulin (6U Ultralente/d subcutaneously (s.c.) for 6 weeks) following ADX to maintain IR in the absence of adrenalglucorticoid-mediated metabolic counter-regulation, and measurements of weight gain, the thermic response to noradrenaline (ml O_2/min per kg body weight$^{0.75}$ above resting metabolic rate at thermal neutrality (30°); 200 μg noradrenaline/kg body-weight, s.c.), adiposity (sum of epididymal, retroperitoneal and dorsal depots), and insulin:glucose ratios made. Rats were fed on Purina chow until 9 weeks of age and chow plus a 'cafeteria' supplement thereafter.

	Weight gain (g)		Adiposity	Thermic response to noradrenaline			Insulin:glucose
Group	6–9 weeks	9–12 weeks	(g)	6 weeks	9 weeks	12 weeks	
Lean	70	67	5·5	9·0	9·7	11·8	1·4
Obese	146	126	21·2	4·3	2·9	2·7	6·0
Obese+ADX	70	177	12·8	—	8·6	10·4	2·4
Obese+ADX+ insulin	167	133	28·2	—	0·9	5·7	8·7
ANOVA, $P<$	0·05	0·05	0·05	0·05	0·05	0·05	0·05

The results indicate that adiposity, IR, NST and the thermic response to diet became normalized following ADX, but IR and NST remained impaired in ADX plus insulin-treated rats when IR was maintained. These observations suggest that adrenal-mediated counter regulatory actions contribute to IR and impaired NST, and that IR is an important component of impaired NST in the obese phenotype of this strain.

Supported by the institutional resources of Drexel University.

Gavin, L. A., McMahon, F. A. & Moeller, M. (1981). *Diabetes* **30**, 694–699.
Tulp, O. L. (1984). *Life Sciences* **35**(16), 1699–1704.
Tulp, O. L. & McKee, S. T. D. (1986). *Biochemical and Biophysical Research Communications* **140**, 134–142.
Tulp, O. L. & Shields, S. J. (1984). *Nutrition Research* **4**, 325–332.

Analytical considerations in the doubly-labelled water ($^2H_2{}^{18}O$) method. By WILLIAM W. WONG, LUCINDA S. LEE and PETER D. KLEIN (Introduced by A. M. PRENTICE), *USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas 77030, USA*

Estimates of carbon dioxide production rates and energy expenditure using the doubly-labelled water ($^2H_2{}^{18}O$) method require accurate and precise measurements of the ^2H:^1H and ^{18}O:^{16}O ratios. These measurements must be made on a variety of physiological fluids at natural abundances and enriched levels of isotopes. Moreover, sample size requirements are an important consideration in studies of preterm infants, small mammals and birds. We have investigated the accuracy and precision of a zinc reduction technique for ^2H:^1H ratios and of a commercial H_2O–CO_2 equilibration system for ^{18}O:^{16}O isotope ratio measurements of physiological fluids (Wong *et al.* 1987*a*). We also have assessed the accuracy and precision of a guanidine hydrochloride method for the direct conversion of μl quantities of biological fluids to CO_2 for ^{18}O:^{16}O ratio measurements (Wong *et al.* 1987*b*). To make hydrogen isotope ratio measurements, 10 μl of a physiological fluid was reduced to hydrogen gas with 250 mg Analar Zn shot (≤ 1 mm diameter) at 475° for 30 min in a quartz reaction vessel. Enriched levels of ^2H (580‰) in urine, plasma, saliva and defatted human milk were measured with an accuracy of 4·6 (SE 4·4)‰ and a precision of 3·2‰. For oxygen isotope ratio measurements, a 100 μl sample was allowed to equilibrate with 300 mbar CO_2 at 25° for 10 h using a modified VG ISOPREP-18 system (VG Isogas Ltd, Cheshire, England). Enriched levels of ^{18}O (256‰) in physiological fluids were measured with an accuracy of 0·32 (SE 0·87)‰ and a precision of 0·97‰. With the guanidine hydrochloride technique, 10 μl of sample was converted to CO_2 with 100 mg guanidine hydrochloride at 260° in a sealed Pyrex tube for 16 h. At a 250‰ enrichment level of ^{18}O, the ^{18}O:^{16}O ratios of the biological fluids were accurate to 1·27 (SE 2·25)‰ and reproducible to within 0·95‰. Our results indicate that these improved methods provide accurate and precise ^2H:^1H and ^{18}O:^{16}O ratio measurements from μl quantities of biological fluids without previous distillation. As such, our methods should be valuable adjuncts to studies based on the doubly-labelled water methodology.

Wong, W. W., Lee, L. S. & Klein, P. D. (1987*a*). *American Journal of Clinical Nutrition* **45**, 905–913.
Wong, W. W., Lee, L. S. & Klein, P. D. (1987*b*). *Analytical Chemistry* **59**, 690–693.

Regulation of the purine nucleotide binding site of brown adipose tissue mitochondrial uncoupling protein. By T. PEACHEY, R. FRENCH and D. A. YORK, *Department of Nutrition, School of Biochemical and Physiological Sciences, Southampton University, Southampton SO9 3TU*

The ability of brown adipose tissue to increase thermogenesis in response to environmental cold and dietary stimuli has been related to the presence of a unique 32 kDa protein, the uncoupling protein (UP), in the inner mitochondrial membrane. This protein, which acts as a proton uniport to reduce the proton motive force and allow oxidation not coupled tightly to ATP synthesis, is inhibited by purine nucleotides. Brown adipose tissue thermogenesis responds very rapidly to changes in sympathetic stimulation but the mechanism of this acute response has been disputed.

We have studied the acute responses of 7-week-old Zucker rats to housing at 4°, to returning to a 27° environment after housing at 4° for 1 week and to exogenous noradrenaline administration (800 μg/kg). By measuring both [^{3}H] GDP binding and UP concentrations (by specific radioimmunoassay) we have been able to follow the time course of the appearance or disappearance of GDP binding sites and relate this to the changes in mitochondrial UP concentration. The Table shows the results for the acclimation of rats to a cold (4°) environment.

Period in cold (h) . . .	0		2		4		24		120	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
GDP binding (pmol/mg)	342	35	507	40	697	21	666	47	965	32
UP (μg/mg)	22	3	19·5	0·5	28	1	43	5·5	75	4
GDP binding/UP	0·55	0·06	0·83	0·05	0·80	0·03	0·5	0·1	0·42	0·05

The results show that the acute appearance of GDP binding sites was not accompanied by increased UP concentrations and supports the hypothesis that acute regulation of thermogenesis involves unmasking of UP. The acute response to exogenous noradrenaline was also associated with an increase in the molar binding ratio of GDP:UP. Conversely, remasking (reduction in the molar binding ratio) of GDP binding sites was demonstrated on returning cold-acclimated rats to the warm. The results also suggest that chronic changes in thermogenesis are associated with changes in UP concentrations and that the suggestion of only one GDP binding site per UP dimer (Lin & Klingenberg, 1983) is incorrect.

This work was supported by a grant from the Medical Research Council.

Lin, C. S. & Klingenberg, M. (1983). *Biochemistry* **21**, 2950–2956.

Validation of the doubly-labelled water technique at low and high activity levels. By K. WESTERTERP, J. HAMERS and F. BROUNS (introduced by A. M. PRENTICE), *Department of Human Biology, University of Limburg, 6200 Maastricht, The Netherlands.*

The doubly-labelled water ($^2H_2{}^{18}O_2$) technique for measuring energy expenditure in free living people has been validated against respirometry under sedentary conditions, yielding differences of less that 5%. Applying the technique under normal living conditions includes exercise bouts usually not performed in a respiration chamber. Comparisons with the intake-balance method under heavy sustained exercise resulted in differences higher than 10%.

We validated the technique at different activity levels by simultaneous measurements of energy expenditure with respirometry and doubly-labelled water in a respiration chamber. Five subjects were measured over 6 d at a low activity level, average daily metabolic rate 1·40 (SD 0·09) times sleeping metabolic rate. Four subjects were measured twice, with at least a 1 month interval, over three half days, including bicycle ergometer work resulting in an average daily metabolic rate of 2·61 (SD 0·25) times sleeping metabolic rate.

The isotopes were administered orally, 8–14 h before the start of the observation period in the respiration chamber, to allow for equilibration. The dose was calculated to give a minimal excess of 80 ppm for both isotopes in the final sample. Isotope abundances were measured in urine with an isotope ratio mass spectrometer (type Aqua Sira, VG Isogas). The carbon dioxide production was calculated using the equation of Schoeller *et al.* (1986).

The biological half lives of the isotopes were between 6 and 10 d at the low activity level and between 4 and 6 d at the high activity level, only just high enough to allow an accurate measurement over the observation period of 6 and 3·5 d respectively.

Low activity level				High activity level			
	Subject CO_2 production (l/d)				Subject CO_2 production (l/d)		
	Respiration chamber	$^2H_2{}^{18}O$	% Difference		Respiration chamber	$^2H_2{}^{18}O$	% Difference
1	508	531	+4·4	6A	894	894	0·0
2	479	511	+6·5	6B	905	936	+ 3·4
3	356	356	0·0	7A	818	916	+12·1
4	457	444	−2·9	7B	876	865	− 1·3
5	437	432	−1·0	8A	981	918	− 6·4
				8B	847	836	− 1·3
				9A	1104	970	−12·1
				9B	903	883	− 2·2
Mean			+1·4				− 1·0
SD			3·9				7·0

The results indicate that the two methods for measuring CO_2 production provide excellent agreement at both activity levels.

Schoeller, D. A., Ravussin, E., Schutz, Y., Acheson, K. J., Baertschi, P. & Jequier, E. (1986). *American Journal of Physiology* **250**, R823–R830.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Replacement of movement-induced thermogenesis with cold-defensive posture-induced thermogenesis in rats kept at 21° compared with 28°. By G. LIVESEY, P. PURDY and G. BROWNSEY, *AFRC Institute of Food Research, Norwich NR4 7UA* and D. BROWN and M. J. DAUNCEY, *AFRC Institute of Animal Physiology and Genetics Research, Cambridge CB2 4AT*

Environmental temperature (T_a), nutrition, endocrine status and genetic composition all influence energy expenditure (EE) and storage. The contribution of muscular work involved in movement and posture has received little attention. However, a reduction in activity has been found to play a role in the development and maintenance of obesity in *ob/ob* mice (Dauncey, 1986; Dauncey & Brown, 1987).

The effect of T_a (21 and 28°) on activity-induced thermogenesis (AIT) was examined in male Wistar rats (100–140 g body-weight) given a meal (170 kJ metabolizable energy) each day at 09.30 hours and caged singly, with lighting from 06.00 to 18.00 hours, for 15 d. Before definitive recordings of EE and activity, the animal was kept for 3 d in the measurement cage (185 mm diameter, 175 mm high) within the 43 litre respiration chamber. Values for EE were corrected for time lag, and movement was monitored with Doppler devices for 30 h, starting at 11.30 hours on day 14.

At 21°, EE was greater than that at 28° and at both temperatures it peaked episodically. Overt movement, an estimate of movement-induced thermogenesis (MIT) obtained by regression, and the correlation between EE and movement were all lower at 21°. Mean resting metabolic rate (RMR) was calculated from a line drawn through points of lowest EE. Posture-induced thermogenesis (PIT) was estimated as the difference between the intercept of the regression of EE on movement and RMR. This showed PIT to be greater in the cold than in the warm, both in absolute terms and as a proportion of EE.

		EE (kJ/24 h per g body-wt$^{0.67}$)		Overt movement (kcounts/24 h)		Correlation of EE with movement (r^2)		MIT (% EE)		PIT (% EE)		AIT (% EE)	
T_a(°)	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
28	9	4·75	0·11	17·2	2·1	0·71	0·07	15	1	4	1	19	2
21	7	5·93	0·13	9·9	1·4	0·38	0·03	6	1	15	1	21	1
P<		0·001		0·02		0·001		0·001		0·001		Not significant	

Video recordings showed that at 21° there was frequent adoption of a cold-defensive posture associated with rising EE which subsequently declined as the animal apparently went to sleep. The decline terminated coincident with a collapse of cold-defensive posture and awakening. This pattern was repeated frequently and interspersed with bouts of MIT. The proportion of 24 h EE due to AIT (= MIT + PIT) was similar at the two temperatures, indicating a replacement of MIT with PIT at 21°.

Dauncey, M. J. (1986). *Experiments* **42**, 547–549.
Dauncey, M. J. & Brown, D. (1987). *Quarterly Journal of Experimental Physiology* (In the Press).

Field validation of the doubly-labelled water method. By A. FERRO-LUZZI, C. SCACCINI and F. VIRGILI, *National Nutrition Institute, Via Ardeatina 546, Rome, Italy,* and P. HAGGARTY, B. A. MCGAW and W. P. T. JAMES, *Rowett Research Institute, Greenburn Road, Aberdeen AB2 9SB*

The doubly-labelled water ($^2H_2{}^{18}O$) method, a potentially promising innovation in the field of human energy expenditure (EE), has been validated only under ideal conditions of strictly regulated dietary intakes and activity schedules. It still remains to be validated under free-living conditions where several basic assumptions may be violated. Such validation, however, is hindered by the currently available methods for measuring EE under conditions of everyday life.

EE was measured over twenty-eight consecutive days by the standard and recognized method (minute-by-minute time allocation and measure of energy cost of activities by indirect calorimetry) and simultaneously estimated by the energy intake–balance technique (precise weighing of diet and measurement of changes of body mass and composition) on six young women. A dose of $^2H_2{}^{18}O$ was given orally at zero time, producing an initial body water enrichment of 300 ppm excess for ^2H, and of 900 ppm excess for ^{18}O. A second dose of $^2H_2{}^{18}O$ was administered on day 29 to measure final total body water. Isotopic enrichments of early morning urines, collected for 28 d, were measured by dual mass-spectrometry. Evaporative water losses were calculated as the difference between urine output and water input from all sources. Physically fractioned evaporative losses were calculated from lung ventilation and body surface area. Various models were developed to describe isotope fluxes, and carbon dioxide production rates were converted into energy by means of dietary respiratory quotients adjusted for body-weight changes. For the comparison of models, evaporative water loss was taken as 50% of water loss.

Compared with EE measured by the classical approaches, the best results (+2 (SD 5)%) were obtained by the monoexponential curve. Other models gave mean estimates that ranged from −10 (SD 13)% to +12 (SD 18)%. The two-point method proved highly unsatisfactory under our experimental conditions. The potential usefulness of the $^2H_2{}^{18}O$ method is confirmed, although, to produce reliable results, a large set of data is required and this is often hard to obtain under field conditions. Our results indicate that departure from the assumption of steady-state in free-living subjects requires further investigation.

Dietary fish oil supplementation modifies rat skeletal muscle fatty acid composition but does not influence the response of muscles to experimental damage. By M. J. JACKSON, J. ROBERTS and R. H. T. EDWARDS, *Department of Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX*

Damage to skeletal muscle occurs in a number of physiological and pathological conditions. The biochemical mechanisms by which loss of muscle cell viability occurs appears to involve an accumulation of intracellular calcium leading to activation of phospholipase enzymes (Jackson *et al.* 1984) and a consequent peroxidation of the liberated fatty acids by lipoxygenase enzymes (Jackson *et al.* 1987).

In an attempt to reduce muscle arachidonic acid content in order to modify the response of skeletal muscle to a specific damaging stress, groups of female Wistar rats were fed on casein-based semi-synthetic diets in which the lipid source was either 100 g maize oil or 100 g purified, deodorized fish oil (Pura Foods Ltd, Bootle, Merseyside) /kg diet. The diets were given to pregnant rats from day 20 to gestation until weaning; ten of the weaned female rats were then given the same diet until 60 d of age. Animals were killed, the fatty acid composition of the gastrocnemius muscle analysed, and soleus muscles were carefully dissected and the response to demage by treatment with Ca ionophore examined in vitro as previously described (Jones *et al.* 1984; Jackson *et al.* 1987).

Animals fed on the different diets were found to have similar growth rates, but the fish-oil diet induced substantial reduction of the gastrocnemius 20:4 and 18:2 fatty acids and replacement with other long chain fatty acids (mainly 22:6).

		Fatty acids (mg/g total fatty acids)						
		16·0	18:0	18:1	18:2	20:4	22:5	22:6
Maize oil	Mean	217	139	151	191	138	11	41
	SD	18	13	34	29	25	4	10
Fish oil	Mean	253	104	224	31	15	44	161
	SD	21	17	21	4	2	11	49

Fish oil supplementation also induced an exacerbation of the intracellular creatine kinase (*EC* 2.7.3.2) efflux following 30 min treatment with Ca ionophore in vitro (fish oil 154 (SE 14) *v.* maize oil 93 (SE 15) mU/30 min per muscle at 90 min post-ionophore treatment).

The results therefore show that reduction of 20:4 and 18:2 fatty acids in rat skeletal muscle can be achieved by dietary manipulation, but that this does not reduce the damaging response of muscle to intracellular Ca overload.

This work was supported by the Muscular Dystrophy Group of Great Britain and F. Hoffmann-La Roche and Co.

Jackson, M. J., Jones, D. A. & Edwards, R. H. T. (1984). *European Journal of Clinical Investigation* **14**, 369–374.

Jackson, M. J., Wagenmakers, A. J. M. & Edwards, R. H. T. (1987). *Biochemical Journal* **241**, 403–407.

Jones, D. A., Jackson, M. J., McPhail, G. & Edwards, R. H. T. (1984). *Clinical Science* **66**, 317–322.

Calculation of isotope flux rates in truly free-living subjects. By P. HAGGARTY[1], B. A. MCGAW[1], W. P. T. JAMES[1], A. FERRO-LUZZI[2], C. SCACCINI[2], F. VIRGILI[2] and M. FRANKLIN[1], [1]*Rowett Research Institute, Greenburn Road, Aberdeen AB2 9SB,* and [2]*National Nutrition Institute, Via Ardeatina 546, Rome, Italy*

Lifson & McClintock (1966) have described the simplifying assumptions used to derive their formula for calculation of carbon dioxide production rates from ^{2}H and ^{18}O flux rates. One of these assumptions is that 'all rates of intake and output remain constant' or the assumption that subjects are on a relatively uniform daily regimen of intake and output.

In contrast to the validation studies carried out to date, this assumption was investigated in six free-living human subjects where their intake was monitored but not controlled. In these subjects there was significant movement of isotope which was not described by single exponential decay. In some of the subjects a more sophisticated model was required to define the behaviour of isotope decay and in all six there were significant fluctuations about the fitted decay curve. Deviation of measured values from the fitted model can be ascribed to measurement error, biological variation and lack of fit. The measurement error, represented by within-sample variation (determined from six measurements of enrichments made on every urine sample), was consistently much lower than the between-sample variation. Thus the observed fluctuation about the fitted curve is mainly due to biological variation and lack of fit and not measurement error. In order to investigate the origin of this mainly biological variation in isotope decay, we used the information on water intake (as liquid and in ingested food and metabolic water) and changes in body water on a daily basis to calculate a dilution factor for body water (labelled with $^{2}H_{2}{}^{18}O$) each day. Daily values for CO_2 production (calculated from food intake and changes in body-weight) were used to calculate the additional water ^{18}O dilution factor. Daily isotope enrichments were then generated by computer assuming a starting enrichment of 100 ppm ^{2}H and 100 ppm ^{18}O and decay curves were fitted to these data. These simulated isotope decay curves showed variability which was similar to that actually observed. Furthermore, the simulated curves exhibited systematic deviation from the simple exponential which anticipated the pattern found in the actual decay curves.

In our free-living subjects, normal biological variability in isotope decay data resulted in gross inaccuracies when using the two-point method to calculate energy expenditure. The same variability made parameter estimation after log transformation unreliable. We suggest that rate constants and intercepts should be derived from untransformed data and that the two-point method should not be used in truly free-living subjects.

Lifson, N. & McClintock, R. (1966). *Journal of Theoretical Biology* **12**, 46–74.

Prevention of calcium-induced enzyme efflux from normal skeletal muscle by vitamin E.

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It has been proposed that intracellular enzyme efflux arising from skeletal muscle damage results from calcium activation of phospholipase, leading to liberation of fatty acids which then act as substrates for cyclooxygenase and lipoxygenase enzymes (Jackson *et al.* 1987). Since dietary vitamin E depletion exacerbates the efflux of intracellular enzymes from muscle following excessive contractile activity (Jackson *et al.* 1983) we have examined the effects of supplemental vitamin E on Ca-induced intracellular enzyme efflux from normal skeletal muscle in vitro.

Treatment of isolated rat soleus muscles with the Ca ionophore A23187 for 30 min induced a substantial rise in the efflux of creatine kinase (*EC* 2.7.3.2). Addition of α-tocopherol (0·23 mM) to the bathing medium throughout the experiment significantly inhibited the efflux of enzyme (27 (SE 11) *v.* 159 (SE 11) mU/30 min per muscle at 90 min post-ionophore treatment) and was also effective when added immediately following removal of the ionophore (90 (SE 15) *v.* 235 (SE 22) mU/30 min per muscle at 90 min post-ionophore treatment), indicating that the protective effect of the vitamin was not due to prevention of the ionophore-induced intracellular Ca accumulation. Similar studies with α-tocopherol acetate have revealed a less dramatic, but significant inhibition of creatine kinase efflux (210 (SE 21) *v.* 328 (SE 28) mU/30 min per muscle at 90 min post-ionophore treatment), but Trolox C, a derivative of α-tocopherol lacking the phytyl side chain but having similar antioxidant properties, was ineffective at reduction of intracellular enzyme efflux.

The results demonstrate an inhibition of Ca-induced pathological changes in muscle by supplemental vitamin E which may not be related to its role as an antioxidant.

This work was supported by the Muscular Dystrophy Group of Great Britain and F. Hoffmann-La Roche and Co.

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Energy expenditure of elite female athletes measured by the doubly-labelled water method. By P. HAGGARTY and B. A. MCGAW, *Rowett Research Institute, Greenburn Road, Aberdeen AB2 9SB* and R. J. MAUGHAN and C. FENN, *Department of Environmental and Occupational Medicine, University Medical School, Aberdeen AB9 2ZD*

Exercise acutely, and perhaps chronically (Maehlum *et al.* 1986) elevates metabolic rate. Athletes engaged in regular strenuous training would therefore be expected to balance their high rates of energy expenditure by an increased food intake. This seems to be true for male athletes but results from women generally show a lower than expected energy intake (Parizkova, 1985). If this is true it suggests a reduced energy cost of physical activity or a compensatory reduction in activity levels when not training. Other than dietary recall the only method of measuring energy expenditure in 'free-living' subjects is the doubly-labelled water ($^2H_2{}^{18}O$) technique. We have applied this technique to four elite female athletes over a 21 d period during a rigorous training regimen. The athletes all agreed to keep a record of their weighed food intake, and body composition was measured by densitometry at the beginning and end of the experiment. Because of the nature and intensity of the training we have assumed an evaporative loss equivalent to 70% of total water loss. The rate-constant for isotope decay was derived by fitting an exponential to the untransformed enrichment data. Calculation was otherwise as previously described (Coward *et al.* 1985).

The athletes had a mean body-weight of 50·6 (SEM 1·6) kg and, as expected, their body fat content was low (mean 143 (SEM 23) g/kg). The relatively high lean body mass was reflected in the high ratios of ^2H_2O:$H_2{}^{18}O$ space; mean 1·11 (SEM 0·02). This is due to the fact that the ^2H of water exchanges with tissue hydrogen and the extent of this process is mainly dependent on the proportion of lean tissue. The mean food quotient of these subjects (calculated from the composition of their diet) was 0·861 (SEM 0·007) and their purported energy intake was 9·7 (SEM 1·24) MJ/d. Energy expenditure determined by $^2H_2{}^{18}O$ was 14·61 (SEM 0·63) MJ/d. Using the Food and Agriculture Organization/World Health Organization/United Nations University (1985) equations for calculating basal metabolic rate (BMR), we found that these subjects were consistently expending 2·79 (SEM 0·07) times their BMR. Therefore, as has been found in other weight-conscious subjects (Prentice *et al.* 1986), values for energy expenditure based on the weighed intake technique underestimate true expenditure.

This study suggests that levels of energy expenditure in elite female athletes over a long period (21 d) are commensurate with their level of activity during high-intensity training and that such subjects do not reduce activity levels between training bouts nor become metabolically more efficient.

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Effects of dietary lactose and copper deficiency 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.*

A dietary copper deficiency and the consumption of unfermented milk or lactose have been identified (Strain, 1987) as potentially important factors in the aetiology of coronary heart disease (CHD). The present study investigated possible effects of dietary lactose and Cu deficiency which may be important in the pathogenesis of CHD.

Groups (*n*6) of male, weanling Wistar rats were housed individually and provided *ad lib.* for 77 d with deionized water and diets which contained sucrose (580 g/kg) or sucrose and lactose (387 g/kg and 193 g/kg respectively) with either (C) control (12 mg/kg) or (D) deficient (1·5 mg/kg) quantities of Cu. Although cytochrome *c* oxidase (*EC* 1.9.3.1) activity was not significantly lowered, hepatic Cu was reduced ($P<0·05$) in rats fed on the Cu-deficient diets. Cu deficiency also resulted in decreased copper–zinc superoxide dismutase (*EC* 1.15.1.1, CuZnSOD) activity but only in rats fed on sucrose.

Lactose feeding resulted in hypercholesterolaemia and increased hepatic manganese. Mn-superoxide dismutase (*EC* 1.15.1.1, MnSOD) activity was significantly elevated but catalase (*EC* 1.11.1.6, Cat), glutathione peroxidase (*EC* 1.11.1.9, GSH-Px), CuZnSOD and lactate dehydrogenase (*EC* 1.1.1.27, LDH) activities were all significantly reduced in rats fed on the control (C) lactose diet compared with those given the control sucrose diet.

	Sucrose				Lactose			
	C		D		C		D	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Serum total cholesterol (mM)	2·07	0·20	2·41	0·51	2·61*	0·46	3·02*	0·36
Liver:								
Mn (μg/g)	4·38	0·92	5·48	0·88	6·61**	0·81	7·54**	0·66
MnSOD (U/mg protein)	0·05	0·02	0·08	0·04	0·52*	0·13	0·08	0·06
CuZnSOD (U/mg protein)	1·29	0·20	0·70	0·25	0·73*	0·25	0·84	0·22
Cat (U/mg protein)	0·47	0·13	0·40	0·07	0·24**	0.11	0·37	0·09
GSH-Px (U/mg protein)	0·64	0·16	0·52	0·11	0·36**	0·12	0·48	0·05
LDH (U/mg protein) × 10^{-3}	3·25	0·50	3·29	0·71	1·72***	0·5	3·61	1·61

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

These results suggest that lactose consumption increases mitochondrial oxygen free radical production but also reduces the activities of extramitochondrial antioxidants. The elevated MnSOD activity seems to compensate for these effects since no significant increase in malondialdehyde content, a measure of lipid peroxidation, was found in the groups fed on lactose.

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

Strain, J. J. (1987). *Medical Hypotheses* (In the Press).

Energy expenditure of 4-month-old breast-fed and formula-fed infants. By NANCY F. BUTTE, WILLIAM W. WONG, LUCINDA S. LEE, CUTBERTO GARZA and PETER D. KLEIN (introduced by A. M. PRENTICE), *USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas 77030, USA*

Daily total energy expenditure (TEE) and basal metabolic rate (BMR) were measured in nine breast-fed (6·33 (SE 0·64) kg, 618 (SE 21) mm) and seven formula-fed (6·52 (SE 0·58) kg, 610 (SE 26) mm) infants at 4 months of age. Daily TEE was measured by the doubly-labelled water method using the Lifson equation corrected for linear growth (Lifson & McClintock, 1966). ^2H_2O (200 mg/kg body-weight) and ^{18}O (300 mg/kg body-weight) were administered orally after which urine samples were collected daily for the next 10–14 d. Enriched levels of ^2H and ^{18}O in urine were measured by gas-isotope-ratio mass spectrometry (Wong *et al.* 1987*b*). Isotope fractionation factors (f_1, f_2, f_3) in the Lifson equation were replaced with values derived at 37° from in vivo measurements in adults (Wong *et al.* 1987*a*). Evaporative water losses, expressed as a fraction of total water output (0·19 (SD 0·04)), were determined by continuous weighing of twelve sleeping infants. Daily TEE was calculated according to the Weir equation (Weir, 1949). BMR was measured 2–3 or 3–4 h postprandially in the sleeping state by indirect calorimetry.

TEE and BMR of breast-fed infants (325 (SE 22) and 208 (SE 19) kJ/kg per d) and formula-fed infants (309 (SE 44) and 223 (SE 14) kJ/kg per d) were not statistically different. The TEE:BMR ratio, however, differed significantly between breast-fed (1·57 (SE 0·12)) and formula-fed (1·38 (SE 0·13)) infants. These results suggest that a greater proportion of TEE is allocated to basal energy-requiring processes in formula-fed than in breast-fed infants. As measured, these processes may include homeostasis, synthesis, residual thermic effect of feeding and movement during sleep.

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Wong, W. W., Lee, L. S. & Klein, P. D. (1987*b*). *American Journal of Clinical Nutrition* **45**, 905–913.

The effect of riboflavin deficiency on absorption and distribution of iron. By HILARY J. POWERS and A. J. A. WRIGHT, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ* and SUSAN J. FAIRWEATHER-TAIT, *AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA*

Riboflavin status may influence iron economy via flavin-dependent release of ferritin Fe. This involvement may be important not only in the mobilization of Fe stores but also in the transport of Fe across the gastrointestinal mucosa (Adelekan & Thurnham, 1986; Powers, 1986). A pilot study was performed using a whole body counter (NE8112, Nuclear Enterprises) to determine whether or not Fe absorption was impaired in riboflavin-deficient rats.

Twenty-four young female Norwegian Hooded rats were paired and allocated to one of two groups, and for 6 weeks given one meal a day of either a diet deficient in riboflavin *ad lib.*, or a complete diet with intake restricted to maintain weight-matching. After a 24 h fast each animal was given a test meal of 3 g cooked starch:sucrose (1:1, w/w) paste containing 120 μg Fe, extrinsically labelled with 1·0 μCi ^{59}Fe ($FeCl_3$). On the day preceding the test meal, all rats received 10 g diet, in order to standardize Fe intake. The animals were counted immediately after consuming the meal, and 14 d later, and were then killed and blood samples collected for estimation of erythrocyte incorporation of ^{59}Fe. The distribution of ^{59}Fe in a number of tissues was also measured.

	Riboflavin-deficient		Weight-matched controls		
	Mean	SEM	Mean	SEM	$P<$
EGRAC* day 0	1·92	0·05	1·28	0·02	0·001
Whole body retention (% of dose) on day 14	26·0	2·5	39·6	2·6	0·001
% of body ^{59}Fe on day 14 in					
1·0 ml blood	6·14	0·15	6·05	0·14	NS
Total blood (estimated volume)	50·9	1·8	51·5	1·2	NS
Liver	22·4	1·8	32·9	1·4	0·001
Spleen	0·87	0·04	1·13	0·03	0·001
Small intestine	0·46	0·02	0·34	0·01	0·001

NS, not significant.
*Erythrocyte glutathione reductase (NAD(P)H) (*EC* 1.6.4.2) activation coefficient.

The results suggest that there was a marked reduction in absorption of Fe in riboflavin deficiency. The incorporation of absorbed Fe into erythrocytes appeared to be unaffected but there were lower levels of stored Fe in the liver. It is possible that Fe turnover is altered in riboflavin deficiency, and this is currently under investigation.

Adelekan, D. A. & Thurnham, D. I. (1986). *British Journal of Nutrition* **56**, 171–179.
Powers, H. J. (1986). *Annals of Nutrition and Metabolism* **30**, 308–315.

Day-to-day variations in the energy expenditure of rural Gambian women. By M. LAWRENCE, *Institute of Physiology, Glasgow University, Glasgow G12 8QQ*

The precision with which mean daily energy expenditure can be measured by the doubly-labelled water method depends partly on the variation in energy expenditure from day-to-day during the 14 d of study. Such variation will result largely from variation in the pattern of activity and the energy cost of individual activities. In the present study the duration of individual activities was assessed throughout the waking day (about 15 h) using teams of local observers working 3–4 hour shifts, and basal metabolic rate (BMR) and the energy costs of the various activities were measured using the Douglas-bag technique. Since mean values for BMR and for the energy costs of individual activities had to be used in the calculations, day-to-day variations in total energy expenditure (TEE) resulted from variations in activity pattern alone. Using this method of measurement, thirty-one pregnant or lactating rural Gambian women were studied on 1 d every 2 weeks for periods in excess of 1 year. Day-to-day variation in energy expenditure was calculated for six 2-monthly periods during the year. The results, expressed as the standard deviation of energy expenditure within subjects, are presented in the Table.

2 month period . . .	Dec–Jan	Feb–Mar	Apr–May	Jun–Jul	Aug–Sep	Oct–Nov
TEE:						
kJ/d	9540	9200	9500	10 500	9790	9620
kcal/d	2280	2200	2270	2520	2340	2300
Day-to-day variation:						
kJ/d	700	523	700	1377	1013	808
kcal-d	167	125	167	329	242	193
% of mean	7·3	5·7	7·4	13·1	10·3	8·4

Day-to-day variation in energy expenditure was relatively small in the months December–May, but tended to increase during the months of greater energy expenditure on agricultural work (June–September). Day-to-day variation during the 14 d over which the doubly-labelled water method measures energy expenditure may, however, be somewhat lower than these 2-month estimates suggest. For example, in six women studied for four to five consecutive days in September, day-to-day variation in energy expenditure was only 550 kJ/d (132 kcal/d) compared with 1000 kJ/d (242 kcal/d) estimated from the above analysis. In conclusion, day-to-day variations in energy expenditure caused by variations in activity pattern appear to be relatively small at all times of year in rural Gambian women, but tend to increase as the rate of energy expenditure increases. This has implications for the field use of the doubly-labelled water technique in the tropics.

Energy expenditure associated with placental Na^+, K^+-ATPase activity in chronically heat-stressed ewes. By B. W. MCBRIDE[1], A. W. BELL[2], I. VATNICK[2] and R. J. EARLY[1], [1]*Department of Animal and Poultry Science, University of Guelph, Ontario N1G 2W1 Canada* and 2*Cornell University, Ithaca, New York 14853, USA*

Previous work by Bell *et al.* (1987) showed that total uteroplacental oxygen consumption was reduced in heat-stressed ewes. The object of the present experiment was to further characterize the metabolic basis for this decline in uteroplacental O_2 consumption.

Pregnant ewes were fed to requirements and kept in an environmental chamber maintained at 20°, relative humidity (RH) 30% (thermoneutral (TN), *n* 7) or at 40° for 9 h and 30° for 15 h daily, RH 40% (heated (H), *n* 5) between days 60 and 136–140 of gestation. Animals were then slaughtered, the uterus was removed and dissected and fetal variables were measured. Samples of placental tissues and myoendometrium were taken for immediate measurement of total and ouabain-sensitive O_2 consumption in vitro. Tissue O_2 consumption was measured in a YSI 5300 O_2 electrode system for 6–10 min in the absence and then the presence of ouabain (10^{-4} M). Metabolic measurements were made from a single fetus from each ewe.

Means for TN conditions precede those for H. Fetal weight (4·46 *v.* 3·43 kg) and fetal brain size (49·0 *v.* 45·5 g) were not significantly affected, but fetal liver size (109·9 *v.* 76·6 g, $P<0·05$), total placental weight (425 *v.* 186 g, $P<0·001$) and mean weight of individual placentomes (8·0 *v.* 4·7 g, $P<0·05$) were reduced in H ewes. The disproportionate organ development of the fetuses from the H ewes complies with similar observations in other growth-retarded fetuses (Alexander, 1974). Total O_2 consumption (μl/mg wet weight per h) was decreased by heat in fetal placenta (0·31 *v.* 0·22, $P<0·05$) but not in maternal placenta (0·29 *v.* 0·27) or myoendometrium (0·18 *v.* 0·19). Ouabain-sensitive O_2 consumption was also decreased by heat in fetal placenta (0·09 *v.* 0·06, $P<0·05$) but not in maternal placenta (0·06 *v.* 0·05) or myoendometrium (0·05 *v.* 0·06).

It is apparent that Na^+,K^+-ATPase activity accounts for a substantial fraction (18·5–30·7%) of uteroplacental O_2 consumption. In the fetal placenta, maintenance of Na^+,K^+-ATPase activity accounts for 28·4–30·7% of the total tissue O_2 consumption. It is concluded that the decrease in placental O_2 consumption in heat-stressed ewes is due not only to a reduction in placental size but also to decreased respiratory activity in fetal placenta. A part of the reduction in fetal placenta O_2 consumption can be explained by the reduction in Na^+,K^+ATPase-dependent respiration.

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https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Doubly-labelled water measurements of energy expenditure in Gambian women during the agricultural season. By J. SINGH, W. A. COWARD, A. M. PRENTICE, J. ASHFORD, M. SAWYER, E. DIAZ* and R. G. WHITEHEAD, *Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ* and *Keneba, The Gambia*

Daily total energy expenditure (TEE) was measured by the doubly-labelled water method in twenty-nine Gambian women (weight 51·5 (SD 9·3) kg, fat-free mass 41·4 (SD 6·0) kg, fat 19·9 (SD 5·9)%) during a period of intense agricultural activity (August–September).

Results were calculated from isotope enrichments of daily urine samples over 12·7 (SE 2·4) d. The mean respiratory quotient (RQ) was estimated from food quotients to be 0·90. A vapour:liquid fractionation assumption of 0·15 was used since the tropical climate caused very high total water turnovers (Gambia 5·1 (SD 1·3), UK 3·0 (SD 0·6) litres/d, $P<0·001$). Resting metabolic rate (RMR) was measured by indirect calorimetry after an overnight fast. Food intakes were assessed by direct weighing over 13·3 (SE 1·7) consecutive days in thirteen of the subjects.

		Total energy expenditure (kJ/d)				Activity plus thermogenesis (kJ/d)*					
		Absolute		per kg BW		Absolute		per kg BW		TEE:RMR	
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NPNL	10	10 070	2607	204	45	5004	2351	101	43	1·97	0·40
Pregnant	6	10 826	1186	189	37	4868	833	87	28	1·84	0·19
Lactating	13	10 805	1638	221	33	5425	1748	111	34	2·03	0·36

BW, body weight; NPNL, non-pregnant non-lactating.
*Calculated as TEE minus RMR.

Isotopically measured TEE was not noticeably influenced by the stage of reproduction and was very high in all groups. The overall average TEE was 1·97 × RMR (range 1·33–2·59). These high levels of expenditure were consistent with an observed workload of approximately 8 h/d spent weeding rice fields, and with previous estimates from activity diaries (M. Lawrence, personal communication). However, the recorded food intakes in the thirteen women differed from TEE by an average of −5410 (SE 630) kJ/d. Although women in this community are usually in negative energy balance at this time of year, the oxidation of endogenous fat during the isotope measurements only contributed an estimated 1820 (SE 510) kJ/d to the deficiency between intake and expenditure. Potential errors in the RQ and fractionation corrections are unlikely to alter the TEE values by more than 5%, suggesting that the error lies in the food-intake measurements. The doubly-labelled water method appears to be an excellent technique for field applications of this type.

*E.D. is supported by the Nestlé Foundation.

Inhibition of fever but not tissue protein and zinc responses to *Escherichia coli* endotoxin by pregnancy in rats. By D. BIBBY, NYUK S. PHAN and R. F. GRIMBLE, *Human Nutrition Department, Southampton University Medical School, Southampton SO9 3TU*

Pregnant sheep and guinea-pigs, near term, fail to develop fever in response to endotoxin (Veale *et al.* 1981). The present study investigates whether the same phenomenon occurs in rats and whether other responses to endotoxin are also inhibited.

Pregnant Wistar rats fed on standard laboratory chow received subcutaneous injections of either sterile saline (9 g sodium chloride/l; S), or *Escherichia coli* endotoxin (E) (strain 0127:B8 butanol extract; Sigma, Poole, Dorset) in saline at 1·2 mg/kg bodyweight on the 6th, 13th and 15th days of pregnancy. Virgin littermates received similarly spaced injections. S animals were pair-fed with their E counterparts. Rectal temperatures were recorded at an ambient temperature of 23° before (t_0) and at hourly intervals (t_n) after injection. 24 h after injection rats were anaesthetized with chloroform, blood taken by cardiac puncture, and liver, tibialis muscle, spleen, fetuses and placentas removed. Analysis for tissue protein and zinc, and serum albumin was carried out as described elsewhere (Wan & Grimble, 1987).

	Pregnant		Non-pregnant	
Rectal temperature (°) at:	Saline	Endotoxin	Saline	Endotoxin
t_0	39·5	39·3	39·7	39·8
t_3	39·1	38·9^{a}	39·5	40·0^{a}
t_5	39·2	39·1^{a}	38·9^{b}	40·1^{ab}
t_6	39·2	39·1^{a}	38·9^{b}	39·9^{ab}
t_7	39·2	39·1	39·2	39·6
Serum albumin (g/l)	34·7^{a}	32·3^{a}	35·5^{b}	31·7^{b}
Liver protein (g)	2·36^{ac}	3·04^{ab}	1·86^{bc}	2·09^{b}
Tibialis protein (mg)	107	115	104	94
Spleen Zn (μg)	20·9^{a}	25·9^{a}	15·2^{ab}	21·2^{b}
Liver Zn (μg)	445^{ab}	555^{a}	323^{bc}	375^{ac}
Protein/fetus (mg)	36	28	—	—
Protein/placenta (mg)	44	37	—	—

Significance of difference analysed by either 1 or 2 way ANOVA and Tukey test.
$^{a-c}$ Values with common subscript letters are significantly different: $P<0·05$.

Responses to endotoxin were both suppressed and enhanced by pregnancy in rats. Fever and the decrease in serum albumin were suppressed whereas the gain in liver protein and Zn was enhanced. Fetal and placental protein contents were not significantly reduced by multiple treatments with endotoxin in mid-pregnancy.

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

Individual variation in the energy cost of pregnancy. 1. 24 h whole-body calorimetry. By G. R. GOLDBERG, A. M. PRENTICE, P. R. MURGATROYD, H. L. DAVIES and W. SCOTT, *Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

Most published studies on the energy cost of pregnancy have concentrated on establishing the average changes in metabolism and have ignored individual variation.

We have studied eight healthy, well-nourished women in the pre-pregnant state (mean age 29 (SD 4) years, height 1·65 (SD 0·09) m, weight 62·6 (SD 8·9) kg, parity 1·4 (SD 0·9)) and at 6-weekly intervals throughout pregnancy (birthweight 3·7 (SD 0·7) kg, gestation 40·6 (SD 1·5) weeks). Basal metabolic rate (BMR), 24 h energy expenditure (24 h EE) and the energy costs of weight-dependent exercise, weight-independent exercise and minor voluntary activity plus thermogenesis were measured under strictly standardized conditions during 36-h periods of continuous whole-body indirect calorimetry. The precision of both BMR and 24 h EE measurements was better than 2%. Fat-free mass (FFM) was calculated from ^2H_2O dilution space after correction for changes in hydration during pregnancy. The overall cost of maintaining the products of conception was calculated as the cumulative difference between the BMR curve and the pre-pregnant value.

Stage of pregnancy (weeks) . . .	12			24			36		
	Lowest	Mean	Highest	Lowest	Mean	Highest	Lowest	Mean	Highest
BMR*	−8·2	+0·5	+7·3	−7·1	+4·6	+14·9	+8·6	+20·0	+35·4
BMR/kg FFM*	−11·5	−1·1	+8·3	−11·4	−0·9	+12·2	−9·2	+5·3	+18·6
24 h EE*	−0·7	+2·4	+6·3	−4·9	+4·7	+9·5	+10·7	+19·6	+26·9
24 h EE/kg body-wt*	−4·2	+0·8	+1·5	−12·3	−6·8	−0·2	−12·5	−0·9	+11·2
Cumulative cost of maintenance (MJ)	−15·6	+8·1	+40·9	−55·1	+15·7	+76·3	−47·1	+81·4	+213·2

*Expressed as % difference from pre-pregnant value.

The mean changes in metabolism were consistent with published values. However, this longitudinal series demonstrated highly significant between-subject differences with some subjects showing depressed metabolism up until 24–30 weeks gestation (F ratio for changes in BMR = 4·8, df 7, 35; $P<0·001$). Ranges are shown in the Table. The cumulative cost of maintenance over the whole of pregnancy averaged 125 MJ with a between-subject coefficient of variation of 93%. These profound differences in the metabolic response to pregnancy have been obscured in most publications through the use of cross-sectional data analysis. Further studies to identify the factors controlling an individual's response are in progress.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Hypoalbuminaemia and oedema in protein-deficient rats infected with *Nippostrongylus brasiliensis*. C. A. NORTHROP and P. G. LUNN, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ* and C. P. J. ASH, *Department of Parasitology, Molteno Institute, University of Cambridge, Cambridge CB2 3EE*

It has proven extremely difficult to induce oedema in rats by giving diets of low protein content. However, in children consuming marginal diets, cases of severe malnutrition are invariably accompanied by infections of various types. Therefore we have investigated the effect of a parasitic nematode, *Nippostrongylus brasiliensis*, in exacerbating the hypoalbuminaemia caused by feeding rats on diets of low protein content.

Wistar rats (4 weeks old), fed on a diet containing 30 g protein/kg, were injected with 3rd stage larvae of *N. brasiliensis*. These animals and an uninfected control group were assessed for parasitic load, body-weight, plasma albumin, fractional catabolic rate (FCR) and gastrointestinal leakage of albumin at intervals, up to 21 d. The infection reached a peak between days 7 and 10 following which worm numbers declined rapidly. Plasma albumin concentrations, already low in response to the protein-deficient diet, fell sharply between days 3 and 7 post-infection (p.i.), but between days 14 and 21 p.i. returned to pre-infection values. Body-weight of the infected rats increased sharply by an average 7·7 g between days 7 and 10 p.i. One animal gained 17 g and five out of nine in the group were clearly oedematous. By day 14 body-weights were 4 g below the pre-infection value, 11·7 g below peak infection weight and no sign of the oedema remained. These weight changes did not correlate with food consumption. The reduction in plasma albumin concentration was closely associated with alterations in FCR of this protein (Table). A greater than four-fold increase in FCR occurred on day 10 p.i. but values had returned to near normal by day 18. The most likely explanation of the increased rate of albumin disappearance is that it has been lost through worm-induced lesions in the gut and this was confirmed by a substantial increase in the faecal loss of ^{51}Cr-albumin.

	Worm no.		Plasma albumin (g/l)		FCR of albumin	
Days p.i.	Mean	SE	Mean	SE	Mean	SE
3	676	117	24·4	0·6	0·56	0·13
7	1742	118	19·5	0·8	1·07	0·38
10	1040	161	18·2	1·7	2·10	0·18
18	312	159	26·1	0·8	0·56	0·02

Clearly, infections which cause intestinal leakage of plasma proteins can quickly precipitate hypoproteinaemic oedema in animals and presumably children who are already compromised by reasons of diet.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Individual variation in the energy cost of pregnancy. 2. Doubly-labelled water method. By H. L. DAVIES, A. M. PRENTICE, W. A. COWARD, G. R. GOLDBERG, A. E. BLACK, P. R. MURGATROYD, W. SCOTT, J. ASHFORD and M. SAWYER, *Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

The doubly-labelled water ($^2H_2{}^{18}O$) method was used to make serial measurements of total energy expenditure (TEE) at 6-weekly intervals throughout pregnancy in seven healthy, well-nourished women. Baseline measurements were obtained before pregnancy (age 28 (SD 4) years, weight 63·7 (SD 9·0) kg, height 1·65 (SD 0·1) m, fat 273 (SD 88) g/kg). TEE was calculated from daily urine samples over 14·6 (SE 1·4) d per run (*n* 47). Individual respiratory quotients were calculated from food quotients (7 d weighed intake) after adjustment for energy imbalance. Individual fractionation corrections (averaging 0·29 (SE 0·06) for vapour/total) were calculated from total water turnover, predicted lung losses (0·6 g/l carbon dioxide) and an estimate of 400 g/d for non-sweating insensible losses. Basal metabolic rate (BMR) was measured by whole-body indirect calorimetry at the beginning of each isotope period. Fat gains were calculated from ^2H dilution spaces after correction for changes in the hydration of fat-free tissue.

Subject no. . . .	1	2	3	4	5	6	7	Mean	SD
ΣTEE (MJ)									
0–12 weeks	–63	54	1	–1	74	65	0	19	49
0–24 weeks	–95	107	195	–42	283	204	17	96	140
0–term	–7	401	568	76	481	423	287	318	213
Cost of fat storage* (MJ)	220	137	100	–14	266	222	265	171	103
Total cost† (MJ)	256	581	710	104	790	687	594	532	254

*Computed as 46 kJ/g.
†Including 43 MJ for conceptus.

TEE values followed smooth but variable trends in each subject. The coefficient of variation about fitted quadratic curves averaged only 6·1%. This value includes deviation from the quadratic fit, individual between-run variability and measurement error. Most subjects showed a voluntary increase in activity during mid-pregnancy and the average cumulative change in TEE (ΣTEE) at term was much higher than the usual estimate of the maintenace cost of pregnancy (318 *v.* 150 MJ). ΣTEE was very variable (range −7 to +568 MJ) demonstrating that behavioural changes in energy expended as activity can be more important than the relatively minor stress of pregnancy in humans. The cost of fat deposition varied between −14 and +266 MJ. The total cost of pregnancy varied over a 7·5-fold range from 104 to 790 MJ. This high level of variability makes it impossible to prescribe incremental energy requirements for individual pregnant women and could usefully be emphasized in future recommendations by the Department of Health and Social Security.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Reduced faecal immune protein excretion in breast-fed Gambian infants by 3 months of age. By ANN PRENTICE, L. VASQUEZ-VELASQUEZ, D. M. STIRLING and S. M. CEESAY, *MRC Dunn Nutrition Unit, Keneba, The Gambia,* and *Milton Road, Cambridge CB4 1XJ*

The physiological roles of the breast-milk immune proteins lactoferrin and secretory-IgA are not fully understood. A recent study in Cambridge showed that although 83% of breast-milk IgA and 99% of lactoferrin are digested by 6 weeks of age, faecal immune protein excretion of breast-fed (BF) infants is many times higher than that of formula-fed (FF) infants (Prentice *et al.* 1987). This demonstrated that breast-milk IgA and lactoferrin have the potential to act as anti-infective factors in the intestine to compensate for immature mucosal immunity.

Faecal immune protein excretion has been measured in thirteen BF African infants at high risk of diarrhoeal disease. The subjects were healthy, 6 weeks or 3 months old and five were receiving complementary foods. IgA and lactoferrin excretion at 6 weeks was similar to that of Cambridge BF infants and greatly exceeded that of Cambridge FF infants (Table). By 3 months immune-protein excretion was significantly lower than that in Cambridge and approached endogenous levels. This result was not influenced by immune protein intakes (Table) nor by the ingestion of solid foods.

Age . . .		6 weeks						3 months					
		Cambridge†				Keneba		Cambridge†				Keneba	
		BF		FF		BF		BF		FF		BF	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Faecal output (mg/d)	IgA	160	28	14	2	111	20	94	17	25	5	26**	5
	LF	14	2	1	0·1	12	1	7	1	1	0·3	2***	0·2
Output/intake (%)	IgA	17	1	—		18	3	11	1	—		5**	1
	LF	1	0·2	—		1	0·1	0·6	0·1	—		0·2**	0 ·03
n		10		9		5		10		6		8	

LF, lactoferrin.
Significantly different from values for Cambridge BF (*t* test): **$P<0·01$, ***$P<0·001$.
†From Prentice *et al.* (1987).

Immune protein excretion by Gambian infants was strongly influenced by defaecation rate (lactoferrin, r 0·7, $P<0·01$; IgA, r 0·9, $P<0·01$), indicating that with increased transit time degradation of IgA and lactoferrin occurred in the large bowel. Low molecular weight fragments of both immune proteins were found in Gambian faeces, the quantity increasing at low stool frequency. The magnitude of the effect of defaecation rate on immune protein excretion was considerably greater in The Gambia than had been observed in Cambridge. These results suggest that differences in bacterial flora may influence the survival and, therefore, the protective capabilities of breast-milk immune proteins.

L.V.-V. is a UNU fellow; A.P. is supported by the Bristol-Myers Fund.

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Human skeletal muscle function following acute starvation. By S. A. WOOTTON, G. SUTTON and S. DAGUE, *Departments of Human Nutrition and Surgery, University of Southampton, Southampton SO9 3TU*

Whilst interest has recently been directed towards measurements of skeletal muscle contractility in the detection of malnourishment, the exact relation between muscle function and nutritional status remains unclear (Newham, 1986). The aim of the present study was to examine the influence of 48 h starvation and subsequent refeeding on muscle function in normal, healthy volunteers.

The adductor pollicis contractile characteristics over stimulation frequencies ranging from 10 to 100 Hz of seven subjects (four male, three female, aged 21–32 years) were assessed 3 h after consuming their normal breakfast (C) using a modification of the technique reported by Edwards *et al.* (1977). The tests were repeated after fasting for 24 h (24S) and 48 h (48S) and then after 6 h (6F), 24 h (24F) and 48 h (48F) of resuming their normal diet. The subjects' intake over the 48 h fast was restricted to low energy (<4 kJ (<1 kcal)) carbonated soft drinks. The hand was pre-warmed at 40° and electromyographic recordings were made to ensure supramaximal stimulation. The force generated at 10 Hz (F_{10}; Newtons), maximal force (F_{max}; Newtons), relative force (F_{10}/F_{max}; %) and maximal relaxation rate (MRR; % fall/10 ms) for each test are shown in the Table.

	Fed		24 h starved		48 h starved		6 h refed		24 h refed		48 h refed	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
F_{10}	17·9	3·1	22·9	3·0	21·6	3·4	19·2	3·7	17·4	2·6	16·9	2·3
F_{max}	60·3	4·1	61·7	4·5	59·3	3·7	59·2	4·3	59·6	4·6	62·8	4·1
F_{10}/F_{max}	29·3	3·8	37·3	3·9	36·7	5·0	32·2	5·5	28·9	3·9	26·9	3·4
MRR	12·0	0·3	10·5	0·5	11·4	0·3	13·5	0·6	12·6	0·6	12·3	0·3

Starvation resulted in significant increases in both absolute forces (F_{10}: 24S, 48S>C, 24F, 48F; ANOVA $P<0·05$) and relative forces (F_{10}/F_{max}: 24S, 48S> C, 24F, 48F; $P<0·05$) at low stimulation frequencies and a slowing of relaxation rate (24S<6F, 24F, 48F; 48S<6F; $P<0·05$) which all returned to control values within 24 h of consumption of the subjects' normal diet whilst maximal force remained unchanged throughout the study (not significant). These results suggest that alterations in functional characteristics of skeletal muscle comparable to those observed in chronic malnourishment can be achieved following acute food restriction and are rapidly reversible on refeeding.

The financial support of KabiVitrum (UK) is gratefully acknowledged.

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The protective effects of cheese against dental caries. By G. N. JENKINS*, M. F. A. SILVA and R. C. BURGESS, *Department of Preventive Dentistry, Dental Faculty, Toronto, Ontario, Canada*

Studies on dental plaque have shown that the usual drop in pH following sugar ingestion is reduced if cheese is eaten immediately after the sugar (Rugg Gunn *et al.* 1975). Experiments on rat caries indicated that cheese alternating or mixed with sugar-containing cariogenic diets reduces the caries scores (Harper *et al.* 1986).

The effect of cheese has been investigated in the human mouth (with ethical approval) by the Intra-oral Caries Test (ICT) of Koulourides *et al.* (1976). In this test, pieces of bovine enamel are attached to artificial dentures and worn for periods of 1 week during which, in the original test, the dentures were removed six times daily and dipped into solutions of the sugars being tested, returned to the mouth and the enamel demineralization quantified at the end of the week by a hardness test (Knoop *et al.* 1939). The present work consists of three experiments on cheese based on modified ICT.

Expt 1: In one subject, partial dentures were made for each side of the mouth and both were worn during meals and during six daily sucrose mouth rinses. After the rinse (1 min), one denture was removed for 5 min while 5 g cheese were eaten, thus enamel on both sides received the sugar but only one side the cheese. This was repeated four times, exposure to cheese alternating between the two sides of the mouth. Expt 2: In five subjects, sucrose (100 g/l) mouth rinses were taken six times a day for 6 weeks; in three of these weeks each rinse was immediately followed by eating 5 g of cheese. Expt 3: In five subjects, the dentures were dipped six times a day for 10 min into either distilled water or an aqueous extract of cheese and after returning to the mouth, sucrose rinses were taken.

In all the experiments, the enamel exposed to the cheese showed highly significant reduction in the demineralization produced by the sugar. These results suggest that cheese eaten after a sugary meal may be an effective measure to reduce dental caries.

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Effect of mixed meal ingestion on the exchange of glutamine, alanine and glutathione across human muscle. By M. ELIA and S. AUSTIN, *Dunn Clinical Nutrition Centre, Cambridge CB2 1QL,* A. SCHLATMANN and P. FOLMER, *Department of Human Nutrition, Agricultural University of Wageningen, The Netherlands*

The present study aimed to assess the effect of a single meal on (1) the overall amino acid balance across muscle; (2) the exchange across muscle of alanine and glutamine, which are the two major amino acids released by muscle (Elia & Livesey, 1983; Elia *et al.* 1985); and (3) the exchange across muscle of glutathione (GSH), which has been reported to be released rapidly by the gut and liver of dogs and to make major contributions to the overall nitrogen balance of these tissues (Elwyn *et al.* 1968).

A meal containing 3275 kJ (47·3% of which was carbohydrate, 39·4% fat and 13·2% protein) was ingested by six normal lean subjects, and the concentration of amino acids in arterialized and deep venous forearm blood was measured and related to forearm blood flow.

The blood concentration of most amino acids increased significantly after meal ingestion and remained elevated throughout the period of study. The amino acid balance across muscle changed from being negative before meal ingestion, to positive after meal ingestion (Table). However, glutamine continued to be released at a high rate in the post-prandial period accounting for a mean of 71% of the total amino acid released between 0 and 4 h after the meal. Alanine balance became significantly more positive during the first 3 h after the meal whilst GSH exchange (expressed in relation to haemoglobin (Hb) concentration because it is located almost exclusively in erythrocytes) could not be detected either before or after meal ingestion. The mean GSH concentration in arterialized blood (~80 μmol/g Hb) was usually within 1% of the concentration in deep venous blood.

Exchange (μmol/l forearm muscle per min) of alanine, glutamine, GSH and total free amino acids across forearm muscle

Period after meal (h) . . .	Basal	1	2	3	4
Total free amino acids	−46·1	+24·4**	+13·9**	+11·1**	+11·2**
Glutamine	−12·3	−13·3	−12·5	−14·2	−13.1
Alanine	−10·9	+7·6***	−2·5**	−7·6*	−10·4
Total GSH (GSH/g Hb)	+0·2	−0·2	+0·2	−0·2	−0·5

Positive signs indicate uptake and negative signs release.
Significantly different from basal values: *$P<0·05$, **$P<0·01$, ***$P<0·001$.

The results indicate that (1) glutamine is the single most important amino acid carrying N out of skeletal muscle both before and after meal ingestion, (2) alanine release is suppressed by meal ingestion with the result that the N carried out of skeletal muscle by alanine may be less than one-sixth of that carried out by glutamine (0–4 h), and (3) no significant exchange of GSH occurs between erythrocytes and resting human muscle.

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Effect of intraabomasal infusions of amino acids or of a mixed animal protein source on milk production in the dairy cow. By C. P. GIRDLER, P. C. THOMAS and D. G. CHAMBERLAIN, *Hannah Research Institute, Kirkhill, Ayr KA6 5HL*

In experiments with lactating dairy cows given grass-silage diets, Girdler *et al.* (1988) found that milk yield was increased by supplements of a mixed animal protein source of low rumen-degradability but not by supplements of 'rumen-protected' lysine and methionine. The following experiment was undertaken to investigate further these differences in response.

Six Friesian cows in the declining phase of lactation were used in a change-over experiment with four treatments and four 10-d periods. The animals were given a basal diet containing 22·4 g nitrogen/kg dry matter (DM) which consisted of silage and a barley–soya-bean meal concentrate (600:400 w/w on a DM basis). In addition they were given, via an intraabomasal catheter, infusions (6 litres/d) of water (control, C), a suspension of a mixed animal protein source (P; Girdler *et al.* 1987), a solution of amino acids (AA) or a solution of methionine and lysine (ML). P provided 432 g protein/d, containing 25 g L-lysine and 8·25 g L-methionine/d, AA provided a corresponding complete mixture of free amino acids, and ML provided corresponding amounts of methionine and lysine. Infusion treatments were balanced for energy supply by feeding 790, 740, 180 and 0 g barley/d for C, ML, AA and P respectively.

The results for DM intake and milk yield and composition are given in the Table. The infusion of protein and of the complete amino acid mixture led to an increase in milk yield and to a non-significant reduction in milk-fat content. In contrast infusion of methionine and lysine tended to increase milk fat content without affecting milk yield. The results indicate that the milk yield response observed with treatment P is related to the provision of an improved amino acid supply to the animal but the response cannot be accounted for only in terms of changes in the supply or methionine and lysine.

Treatment . . .	C	ML	AA	P	SED
DM intake (kg/d)	$14·07^{ab}$	$13·77^{a}$	$14·29^{bc}$	$14·24^{bc}$	0·20
Milk yield (kg/d)	$16·17^{b}$	$15·55^{b}$	$17·81^{a}$	$17·42^{a}$	0·47
Crude protein (g/kg)	$31·6^{b}$	$31·8^{b}$	$32·7^{a}$	$31·7^{b}$	0·04
Fat (g/kg)	$44·8^{ab}$	$47·4^{a}$	$42·5^{cb}$	$42·8^{cb}$	0·14

$^{a-c}$Mean values with unlike superscript letters are significantly different: $^{*}P<0·05$.

Girdler, C. P., Thomas, P. C. & Chamberlain, D. G. (1988). *Proceedings of the Nutrition Society* **47**, 82A.

Carbohydrate metabolism in muscle and in the whole body of man after mixed meal ingestion. By M. ELIA, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL and* P. FOLMER and A. SCHLATMANN, *Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands*

The present study aimed to assess the fate of carbohydrate in a mixed meal, when ingested by six normal male adult subjects. The meal contained 1550 kJ carbohydrate (about two-thirds in the form of simple sugars), 1290 kJ fat and 435 kJ protein (total metabolizable energy 3275 kJ). Net oxidation of carbohydrate and other nutrients was assessed by measuring gaseous exchange, urinary nitrogen in timed urine collections and changes in the plasma urea concentration Carbohydrate metabolism in muscle was assessed by measuring the arterialized–deep venous (A–V) concentration differences of glucose, lactate, pyruvate, alanine and oxygen in whole blood and relating them to the estimated blood flow to forearm muscle.

During the first 4 h after the meal there was an increase in whole body O_2 consumption and energy expenditure (about 14%, $P<0{\cdot}002$). There was also a non-significant increase in the estimated uptake of O_2 by forearm muscle (Table), which when extrapolated to the whole musculature of the body accounted for about 9% of dietary-induced thermogenesis.

Flux across muscle (μmol/l muscle per min) of O_2, glucose and 3-carbon glycolytic fragments

Period after meal (h) . . .	Basal	1	2	3	4
O_2	+62·5	+71·4	+62·5	+62·5	+63·5
Glucose	+4·4	+30·9**	+16·0*	+9·5*	+4·9
Lactate	−0·90	+8·29**	−0·57	−0·46	+0·21
Pyruvate	+0·25	+1·83**	+0·63*	+0·16	+0·40
Alanine	−1·09	+0·76***	−0·25**	−0·76*	−1·04

Significantly different from basal values: *$P<0{\cdot}05$, **$P<0{\cdot}01$, ***$P<0{\cdot}001$.

The total carbohydrate oxidized in the whole body during the first 4 h after the meal was equal to only about one-third of the carbohydrate provided in the meal (486 (SEM 63) kJ). The remaining two-thirds was presumably largely deposited in the tissues of the body although some may have still been present in the intestinal lumen 4 h after the meal. It is also estimated, on the basis of oxidative stochiometries and the rate of exchange of major nutrients (results not shown), that one-third to two-thirds of the glucose entering muscle between 0 and 4 h after the meal was oxidized and a similar amount stored in muscle. In the whole musculature of the body (estimated at 32 kg) this corresponds to an uptake of 21 g glucose and storage of 7–14 g glucose. The release of 3-carbon glycolytic fragments, which tended to decrease rather than increase after meal ingestion (Table), makes little difference to the above calculations. The results suggest that (1) resting muscle is not a major site of dietary-induced thermogenesis, (2) the increase in glucose uptake after mixed meal ingestion does not necessarily increase the release of 3-carbon glycolytic fragments by muscle or the recycling of these fragments between muscle and liver, (3) It is likely that non-muscular tissues took up more carbohydrate than skeletal muscle during the first 4 h after ingestion of this meal (even if it is assumed that only half the dietary carbohydrate had been absorbed at 4 h).

The use of in vitro fermentation heat to predict energy availability in ruminant feeds. By A. ARIELI and D. WERNER, *Faculty of Agriculture, Hebrew University, Rehovot, Israel*

The products of fermentation of feedstuffs in the rumen contribute a large portion of the useful energy to the ruminant. Thus qualification of fermentation end-products may predict energy availability in feeds. Under the anaerobic conditions of the rumen, stochiometric relations hold between the substrate and the fermentation products. Thus it can be hypothesized that fermentation end-products may be useful indicators of energy availability of substrates. Calorimetry enables rapid determination of heat dissipation by rumen microbes and net fermentation heat may be evaluated by difference. To test this hypothesis the correlation between fermentation heat and digestibility of feeds in an artificial rumen was evaluated. In addition, as heat dissipation of rumen microbes in the absence of substrate (blank) may be related to metabolic pathways that differ from those in the presence of food, the additivity of endogenous and substrate-heat dissipation was explored by comparing volatile fatty acid (VFA) production to heat dissipation in rumen fluid.

Feed samples (0·5 g) were incubated with rumen fluid samples (25 ml taken from sheep fed on 500 g concentrate and 500 g roughage/kg diet, mixed with 35 ml McDougall's buffer), for 1–30 h and performed on two different days. Heat dissipation of feed samples and blanks was measured in an adiabatic calorimeter (Arieli, 1986). Fermentation of wheat hay, wheat straw, wheat silage, *Pensilaria* hay, maize silage, oat hay, cellulose and starch was measured. In these feeds dry matter (DM) digestibility (Tilley & Terry, 1963) was also determined. Concentration of VFA was measured in feed and blanks of starch, cellulose and wheat straw.

Rate of heat dissipation in blank samples varied between days and averaged 4–7 mW. During each experiment the blank value was relatively stable with a coefficient of variation of 14%. Initial VFA content of blank vessels was 2–3 mmol and increased by 0·9 (SE 0·1) mmol during the experiments. Thus, the ratio of heat dissipation:VFA production in blanks varied between 250 J/mmol in short incubations (starch, 11 h) and 700 J/mmol in longer fermentations (up to 30 h). The ratio of heat dissipation of feeds:VFA production was similar in starch, cellulose and wheat straw (109 (SE 3) J/mmol). The similarity between these values and the theoretical value (100 J/mmol; Czerkawski, 1986), suggests that the by-difference measurement of cumulative net heat dissipation represents energy availability to rumen microbes in these substrates. Cumulative heat dissipation in feeds was highest in starch (753 J/g DM) and lowest in wheat straw (276 J/g DM). A linear relation was found between heat dissipation in feeds and their in vitro DM digestibilities ($P<0·001$; n 8; r 0·97).

The measurement of heat dissipation of feeds incubated in rumen fluid may provide a prediction of energy availability in ruminant feedstuffs.

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Muscle protein synthesis rate—a reappraisal of control values. By P. J. PACY[1], K. N. CHENG[1], M. READ[1], M. J. RENNIE[2] and D. HALLIDAY[1], [1]*Nutrition Research Group, Clinical Research Centre, Harrow HA1 3UJ,* [2]*Department of Physiology, The University, Dundee DD1 4HN*

Stable isotope tracers are extensively used to determine protein metabolism in the whole body and in individual tissues. L-[1–^{13}C]leucine, a branched-chain amino acid predominantly metabolized in muscle, is a suitable tracer to document muscle protein synthesis rate (MPSR, %/h). This variable is determined by a primed continuous infusion of L-[1–^{13}C]leucine (1 mg/kg per h), ideally with two muscle biopsies (quadriceps) taken 6 h apart after attainment of plasma isotope plateau enrichment of [^{13}C]leucine. During this time it is assumed that leucine incorporation into muscle protein is linear. It has been reported, using L-[1–^{13}C]leucine tracer, that MPSR is 0·098 and 0·198%/h in fasted and fed controls (Rennie *et al.* 1982). On purely theoretical grounds these values would appear high but are the only available published values for comparison with various pathological states to date. Since that original publication experience with this technique has increased and analytical methods to determine [^{13}C]leucine content of quadriceps muscle improved. The aim of the present paper is to report the value of MPSR we now obtain. The clinical details of the controls are as follows (mean and SD): fasting, eighteen males, age 28·8 (5·9) years, body mass index 23·1 (2·0); fed (protein intake 0·095 g/kg per h), seven males and three females, age 23·5 (2·0) years, body mass index 22·2 (1·7). The protocol employed was as described above with α-ketoisocaproic acid enrichment used as the precursor pool enrichment for leucine. The results are shown in the Table.

	Leucine from protein breakdown (μmol/kg per h)		Leucine oxidation (μmol/kg per h)		Leucine incorporated into protein (μmol/kg per h)		MPSR (%/h)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fasted	117·9	11·8	15·4	4·3	102·5	10·3	0·046	0·012
Fed	100·3	15·2	48·8	8·6	126·9	18·5	0·075	0·014

These MPSR results are compatible with a derived overall average whole body protein synthetic rate of approximately 0·09%/h—a figure based on the assumption of a 70 kg man containing 11·5 kg protein and synthesizing some 275 g protein/d (Halliday, 1981). We conclude that recently obtained values for MPSR in fed and fasted control subjects are more likely to be representative of the true values than those previously published.

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https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

A non-invasive technique for determining muscle protein synthesis. By K. N. CHENG, P. J. PACY, C. HICKS, G. C. FORD, H. MERRITT and D. HALLIDAY, *Nutrition Research Group, Clinical Research Centre, Harrow HA1 3UJ*

We have recently reported on the use of doubly-labelled leucine (^{13}C, ^{15}N) in combination with the forearm site to determine protein synthesis from leucine during feeding and fasting (Cheng *et al.* 1987).

The rates of protein synthesis and breakdown were calculated from blood flow and the arterio-venous difference of labelled leucine and its metabolites α-ketoisocaproate and carbon dioxide. Assuming the observed protein synthetic rates reflect predominantly that of muscle, that the forearm muscle content of males and females is 72% and 58% respectively (Maughan *et al.* 1984), that muscle contains 18% protein and that the leucine content of protein is 8% (Block & Weiss, 1956) it is possible to calculate muscle protein synthesis rate (MPSR). The aim of the present study was to compare values of MPSR from the forearm with a serial quadriceps biopsy technique. We have performed twenty-one separate studies of forearm leucine metabolism in fifteen fasted (>12 h) control subjects (fourteen males and one female). Their clinical features were as follows (mean and SD): age 31·8 (7·3) years, weight 73·8 (8·4) kg, body mass index 23·0 (2·1).

MPSR (%/h) determined by the forearm technique was (mean and SD) 0·0510 (0·008) which compares favourably with values from serial muscle biopsies (0·046 (0·012)). One female and one male were studied on three and five occasions respectively. In the female, mean MPSR (%/h) was 0·0454 (range 0·0421–0·0499; coefficient of variation 9%), while in the male it was 0·0507 (range 0·0423–0·0662; coefficient of variation 19%). We suggest that these results confirm the validity of the updated MPSR values and of the applicability of the forearm model in nutritional studies.

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Mathematical model of protein metabolism in growing lambs. By M. GILL and J. FRANCE, *AGRI, Hurley, Maidenhead, Berkshire SL6 5LR* and M. SUMMERS, B. W. MCBRIDE and L. P. MILLIGAN, *Department of Animal and Poultry Science, University of Guelph, Ontario N1G 2W1, Canada*

This model was developed to examine the relative importance of the energy costs associated with protein synthesis and ion transport in individual tissues, in response to varying levels of nutrient absorption. Simulations presented here are for a growing lamb (20 kg live weight), represented by ten tissue pools, namely adipose, the central nervous system (CNS), gut (including rumen, reticulum, omasum, abomasum and intestines), heart, kidney, liver, muscle, reticulo-endothelial system (RES), pancreatic and salivary glands (PSG) and skin. The synthesis and degradation of protein are described by two equations for each tissue, with a further two equations to calculate the exchange of amino acids with a central blood pool. The synthesis of liver export-proteins, the proteins secreted into the gut and wool protein are also considered. All oxidation of amino acids is assumed to take place in the liver, passing through a urea pool. The energy costs (in terms of moles ATP) associated with each of these equations are calculated using stoichiometric relations and the results are described in the following abstract (Milligan *et al.* 1988). The initial weights of tissues were based on data from a comparative slaughter experiment with lambs (Murray & Slezacek, 1976, 1980), while the fractional protein synthetic rates were estimated from a wide range of published data. The dynamics of the model are described by a series of differential equations (one for each pool) which are solved numerically.

Simulated growth rates ranged from 90–230 g/d or 9–25 g protein/d. The main tissues contributing to protein synthesis were the gut (25–27%), muscle (22–27%) and skin (22–24%), followed by liver (13–14%). Protein synthesis in the RES represented 6–7% of the total, PSG 3–5% and kidney 1·3%, while heart, CNS and adipose each accounted for less than 1%.

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Murray, D. M. & Slezacek, O. (1976). *Journal of Agricultural Science, Cambridge* **87**, 171–179.

Murray, D. M. & Slezacek, O. (1980). *Journal of Agricultural Science, Cambridge* **95**, 241–250.

Simulation of energy costs associated with protein metabolism in growing lambs. By L. P. MILLIGAN[1], M. SUMMERS[1], J. FRANCE[2], M. GILL[2] and B. W. MCBRIDE[1], [1]*Department of Animal and Poultry Science, University of Guelph, Ontario N1G 2W1, Canada* and [2]*AGRI, Hurley, Maidenhead, Berkshire SL6 5LR*

In the preceding abstract a mechanistic model of protein metabolism in the growing lamb was described (Gill *et al.* 1988). This model was used as the basis for estimating the energy expenditure associated both directly and indirectly with protein deposition. The costs of protein synthesis and degradation were accounted for individually while the corresponding Na^+, K^+ transport costs were separated into a basal service function, and expenditure on the uptake of substrates and removal of products linked with protein metabolism. The costs were based on assumed stoichiometric relations between ATP utilization and the rates of individual reactions. The model was used to examine the effect of changing these assumptions on the contribution of individual reactions or tissues to total ATP utilization over a range (9–25 g/d) of protein accretions. The total ATP equivalent of the heat production of each tissue group was calculated from in vivo oxygen uptakes reported in the literature. In the absence of such information, this was calculated assuming each of the tissues at low input expended the portion of total energy on protein synthesis as reported by Reeds *et al.* (1985) for muscle (0·17), kidney (0·07) and liver (0·15), and by Siems *et al.* (1984) for the reticulo-endothelial system (0·28). The increment in tissue expenditure resulting from increased input was estimated assuming applicability to all tissues of the whole-body relation of increased energy expenditure per unit increased protein synthesis described by Reeds *et al.* (1985). The sum of the values for the individual tissues could then be compared with total ATP utilization (TATP) calculated as the sum of maintenance expenditure and the energetic inefficiency associated with growth, fattening and wool deposition. This provided an internal check of the assumptions.

At the lowest rate of protein accretion simulated, the sum of ATP expended by the individual tissues accounted for 107% of TATP; at maximal accretion rates 100% of TATP were accounted for. At both levels of accretion, protein turnover accounted for 18% of TATP while ion transport accounted for 17% of TATP at the lowest level and 20% at the highest level of accretion. Absorption of amino acids across the gut and uptake by the tissue cells together with protein export accounted for approximately 3% of TATP or 15–20% of total ion transport costs.

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Bioimpedance analysis: is it a satisfactory method for assessment of body composition? By E. DIAZ[1,3], J. VILLAR[2], M. IMMINK[1] and T. GONZALEZ[1], [1]*Institute of Nutrition of Central America and Panama (INCAP), Guatemala,* [2]*Instituto Guatemalteco de Seguridad (IGSS), Guatemala* and [3]*Dunn Clinical Nutrition Unit, 100 Tennis Court Road, Cambridge CB2 1QL*

Bioimpedance analysis (BIA) is being promoted as a technique of body composition assessment. In order to test the reliability and accuracy of the method, we compared BIA with estimates derived from anthropometry and body density in ninety-nine Guatemalan women (mean and SD: age 26 (4·9) years, weight 51·9 (5·9) kg, height 1·52 (0·054) m, fat 265 (46) g/kg body-weight) and forty-nine men (age 29 (5·9) years, weight 54 (5·7) kg, height 1·61 (0·05) m, fat 102 (53) g/kg body-weight).

Anthropometry (A) included weight, height, skinfolds and body circumference measurements. BIA was measured with an RJL analyser (model 103) and body density (BD) by underwater weighing corrected for lung volume. Repeated body fat measurements in thirty-two subjects on two consecutive days by the three methods were highly reliable: A, r 0·99, standard error of the estimate (SEE) 1·1%; BIA, r 0·98, SEE 1·0%; BD, r 0·95, SEE 1·2%.

The estimates of body fat (BF) and fat-free mass (FFM) obtained by BD were used as reference values for stepwise multiple regression analysis.

	r^2 coefficients for BF				r^2 coefficients for FFM			
Group . . .	I	II	III	IV	I	II	III	IV
n . . .	32	18	49	49	32	18	49	49
Resistance (ohm)	0·10	0·12	0·01	0·16	0·17	0·00	0·18	0·19
Reactance + resistance (ohm)	0·13	0·19	0·01	0·19	0·17	0·01	0·21	0·19
Wt (kg)	0·75	0·81	0·38	0·34	0·78	0·75	0·56	0·69

*Women: I, INCAP staff; II, post-partum; III, labourers; men: IV, labourers.

Resistance and reactance readings alone, explained less than 21% of the variance in BF and FFM. In comparison body-weight explained from 34 to 81% of the variation in body composition. The inclusion of height improved the estimates based on weight alone. However, the further inclusion of resistance, reactance and height^2/resistance produced a maximum improvement in r^2 of only 7·7%.

In all groups, using only anthropometric variables produced better equations than those derived from weight, height and BIA; r^2 ranged from 0·65 to 0·99 for BF and 0·75 to 0·99 for FFM.

The present study demonstrated that BIA was not an appropriate predictor of BF and FFM in the population studied. It is unclear whether the better predictions claimed by other authors are due to BIA by itself or to the inclusion of anthropometric variables in the same equation.

Energy balance in women using oral contraceptives. By G. MCNEILL, A. C. BRUCE, E. ROSS and W. P. T. JAMES, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

It is often reported that women using oral contraceptive pills have a tendency to gain weight, but the mechanisms responsible have not been established. During a normal menstrual cycle women show cyclical changes in energy intake and energy expenditure which are related to changes in hormonal status, and which could be disturbed by the use of oral contraceptives. The present study was carried out to investigate differences in energy intake and expenditure in women between a normal menstrual cycle and a cycle under the control of a progesterone-only oral contraceptive.

Five, healthy, lean young women have completed the study protocol, which involves measurements on two menstrual cycles. In the first ('non-pill') cycle, energy intake was estimated both pre-ovulation and post-ovulation by 7-d weighed intake measurements: physical activity was estimated pre- and post-ovulation from 7-d activity diaries; basal metabolic rate (BMR) was measured weekly by ventilated hood indirect calorimetry, and body-weight was measured weekly with the subjects lightly clothed and after voiding but before breakfast. Ovulation was ascertained by measurements of urinary luteinizing hormone and temperature records. In the second ('pill') cycle, the subjects took Microval (30 μg levonergesterel/d), and energy intake, physical activity pattern, BMR and weight were measured as in the non-pill cycle.

The Table shows the average results for the two 7-d estimates of energy intake and physical activity index and the four measurements of BMR and weight in each subject in the two cycles. Energy intake was slightly higher and physical activity index was slightly lower in the pill cycle, although these differences were not statistically significant. BMR showed a significant drop in the pill cycle (paired *t* test, $P<0{\cdot}01$) and there was a small weight gain in all subjects.

Subject no.	Energy intake (kJ/d)		Physical activity index†		BMR (kJ/d)		Wt (kg)	
	Non-pill	Pill	Non-pill	Pill	Non-pill	Pill	Non-pill	Pill
1	9120	9556	1·48	1·52	6371	5705	58·8	59·2
2	9486	8449	1·44	1·38	6101	4848	58·6	59·0
3	7816	10638	1·34	1·38	6015	5183	63·4	65·0
4	9109	7651	1·45	1·31	6072	5614	52·9	53·3
5	8596	9737	1·42	1·41	5848	5402	54·0	54·8
Mean	8825	9206	1·43	1·40	6081	5350**	57·5	58·3
SD	647	1167	0·05	0·08	189	346	4·2	4·6

**$P<0{\cdot}01$ (paired *t* test).
†Multiple of BMR.

We suggest that a decline in BMR in response to progesterone, unaccompanied by a decline in energy intake, could explain the observed tendency to gain weight.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Body composition of healthy Swedish women during pregnancy and lactation. By ELISABET FORSUM[1], AIJA SADURSKIS[2] and JAN WAGER[2], [1]*Department of Medical Nutrition* and [2]*Department of Obstetrics and Gynecology, Karolinska Institute, Huddinge Hospital, S–141 86 Huddinge, Sweden*

Knowledge of energy metabolism during reproduction is essential for the understanding of how nutrition influences the growth and development of the offspring. The physiological accumulation and mobilization of body fat during reproduction have important impacts on the energy requirements of the mother and this has aroused interest in the study of body composition during reproduction of women living under different nutritional circumstances.

Body-weight, total body potassium (by whole-body counting), total body water (as described by Schoeller *et al.* 1980) and six skinfolds were measured in a longitudinal study on twenty-nine healthy Swedish women. Measurements were made before pregnancy (A), at gestational weeks 16–18 (B), 30 (C) and 36 (D), as well as 5–10 d (E) and 2 (F) and 6 (G) months post partum. Body fat was calculated from body-weight, body water and body K according to Pipe *et al.* (1979).

Body composition of pregnant (n 22) *and lactating* (n 23) *Swedish women*

	A		B		C		D		E	
Pregnant	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BW (kg)	61·0	9·9	63·7	9·7	70·2*	9·9	72·7*	10·3	67·6*	10·8
TBW (kg)	33·0	4·3	32·5	3·7	36·7*	3·7	38·7*	4·4	34·2	3·7
TBK (mol)	2·40	0·33	2·22*	0·30	2·29*	0·33	2·51	0·31	2·37	0·29
TBF (kg)	17·2	6·9	20·7*	6·0	22·6*	6·9	22·3*	7·1	22·9*	7·8

	A		E		F		G	
Lactating	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BW (kg)	61·5	9·4	67·6*	10·3	64·4*†	10·5	63·0*†	10·5
TBW (kg)	33·9	4·4	34·6	3·9	32·0*†	3·7	32·2*†	4·0
TBK (mol)	2·47	0·37	2·43	0·34	2·30*†	0·38	2·32*†	0·30
TBF (kg)	16·5	6·9	22·1*	7·0	22·1*	7·3	20·4*†	7·4

BW, body weight; TBW, total body water; TBK, total body potassium; TBF, total body fat.
*Significantly different from A ($P<0·05$).
†Significantly different from E ($P<0·05$).

Significant correlations ($r \geqq 0·9$) between various combinations of skinfolds and body fat content were established at all measurements. Changes in body fat estimated according to Pipe *et al.* (1979) were generally significantly correlated with changes in body fat calculated from skinfold measurements except during late pregnancy and early lactation. No correlation between changes in body fat and body-weight between A and E was obtained. However, correlations between the corresponding changes from A to G as well as from E to G were significant. The implication of these findings for the energy cost of human reproduction will be considered and discussed in relation to similar studies of other populations of women.

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The effects of dopamine at doses of 2–10 μg/kg body-weight per min on metabolic rate in normal man. By I. T. CAMPBELL, C. REGAN and R. DUCKWORTH, *University Department of Anaesthesia, Royal Liverpool Hospital, Liverpool* and J. FAIRHURST, P. MAYCOCK and K. N. FRAYN, *MRC Trauma Unit, Hope Hospital, Manchester*

Dopamine is a catecholamine and is the immediate biochemical precursor of noradrenaline. It is used pharmacologically at low doses to support renal function in critically ill patients, at higher doses to support myocardial function and is often given to intensive-care patients as a prophylactic measure against acute renal failure. It is well known that the catecholamines adrenaline and noradrenaline increase metabolic rate but there is no information on the effects of dopamine on metabolic rate in normal man.

Following an overnight fast, five male volunteers (median age 32 (range 29–40) years) were given dopamine in a glucose solution (50 g/l) intravenously at 2, 5 and 10 μg/kg body-weight per min for three consecutive periods of 45 min each. Oxygen consumption ($\dot{V}_{O_2}$) was measured continuously using a ventilated canopy. Blood was taken at 30 and 45 min after the start of each infusion period and analysed for dopamine, noradrenaline, adrenaline, glucose, free fatty acids, glycerol and lactate. The results were compared with the effects on the same subjects of the corresponding volume of glucose solution (50 g/l) alone.

By the end of the experiment $\dot{V}_{O_2}$ had increased by 20% in the group receiving dopamine and by 6% in the subjects receiving glucose solution only. $\dot{V}_{O_2}$ was significantly higher in the subjects receiving dopamine over the 15–30 ($P<0{\cdot}02$) and 30–45 ($P<0{\cdot}05$) min periods of the 10 μg dopamine/kg infusion period. Blood dopamine concentrations increased in proportion to the rate of infusion, rising to levels in excess of 1000 mmol/l at the 10 μg/kg infusion rate. Adrenaline was higher during dopamine infusion than when the subjects received the glucose solution alone only at the 30 min point of the 2 μg/kg infusion; noradrenaline was higher during the 5 μg/kg infusion ($P<0{\cdot}05$) and the 10 μg/kg infusion ($P<0{\cdot}01$). Blood glucose was higher during the 5 and 10 μg/kg infusion and glycerol and free fatty acids only during the 10 μg/kg infusion ($P<0{\cdot}05$). Lactate did not alter and there was no difference between the groups.

It is concluded that dopamine increases metabolic rate and promotes lipolysis and glycogenolysis. Using this protocol, however, it is not possible to say whether these effects are a direct effect of dopamine or are due to noradrenaline release, nor is it possible to say whether they are a function of the rate of dopamine infusion or the duration of its administration.

Summarizing growth standards by the LMS method. By T. J. COLE, *Dunn Nutrition Unit, Downhams Lane, Milton Road, Cambridge CB4 1XJ*

Cole (1987) describes a statistical method for fitting smooth centile charts to reference data. It assumes that, by applying a suitable power transformation to the data, the centiles at each age can be rendered non-skew and hence close to a normal distribution. The present study was carried out to see how close existing weight-for-age standards are to a normal distribution after their skewness has been removed.

The method works as follows: at each age, the power L appropriate to remove the skewness from the seven published centiles is derived, along with the adjusted mean M and the coefficient of variation S. Thus the seven centiles are summarized by the three values L, M and S. Because growth standards are carefully smoothed, L, M and S also change smoothly with age. For weight data, L lies typically in the range −1 to 1, corresponding to a range of transformations from the inverse, through the logarithmic, to the untransformed. The L, M and S values at each age are then used to derive a new set of weight centiles, on the assumption that, after transformation, they follow a normal distribution.

This exercise has been done on national weight-for-age standards from the UK, the USA and The Netherlands. The Table gives details of the three standards, and also summarizes the percentage differences between the seven published and calculated centiles at each age.

Growth standard	Age–sex points	Age range (years)	Centile differences (%)		
			Mean	SD	Range
UK (Tanner *et al.* 1966)	85	0–19	−0·002	0·65	−1·6,+2·2
USA (Hamill *et al.* 1979)	66	2–18	−0·006	0·98	−3·0,+3·3
Dutch (Roede *et al.* 1985)	76	1–19·5	−0·003	0·51	−1·6,+1·4

The agreement is remarkably close, particularly for the UK and Dutch standards. They were originally fitted by eye, whereas the US standard was fitted using cubic spline functions. It is concluded that all three standards are adequately summarized by their L, M and S curves, which represents a substantial saving over the seven centile curves as published.

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Tanner, J. M., Whitehouse, R. H. & Takaishi, M. (1966). *Archives of Disease in Childhood* **41**, 454–471.

The effect of physical training on basal metabolic rate. By S. A. BINGHAM[1], G. R. GOLDBERG[1], W. A. COWARD[1], A. M. PRENTICE[1], M. FITZPATRICK[2] and J. H. CUMMINGS[1], [1]*Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL* and [2]*Respiratory Physiology Department, Papworth Hospital, Cambridge CB3 8RE*

Six healthy but normally sedentary volunteers (three men, three women, 24–33 years) were maintained on the same constant diet during 3 weeks of inactivity (control) followed by 9 weeks of physical training. The energy content of the diet was 1·6 × basal metabolic rate (BMR) to avoid overall loss in body-weight. The maximum permitted activity in the control period was 15 min/d, and in the 9 week training period exercise was gradually increased so that by weeks 8–9 the subjects were capable of continuous jogging for 1 h/d, 5 d/week.

Body composition was assessed weekly by ^{40}potassium counting and by skinfold thicknesses, and in the control period and weeks 8–9 by oral doses of 0·05 g ^2H_2O/kg, when total energy expenditure was measured simultaneously by the addition of 0·15 g $H_2{}^{18}O$/kg. Maximum oxygen uptake ($\dot{V}_{O_{2max}}$) was determined on two occasions at the end of the control period and once at the end of the exercise period. BMR was measured under standard conditions (immediately upon wakening, 13 h post-absorptive, at thermoneutrality and at complete rest) by whole body indirect calorimetry in the control period, weeks 4–5, and weeks 8–9.

Over the course of the study, there were significant changes in body composition, energy expenditure and $\dot{V}_{O_{2max}}$, but no change in BMR (see Table). When diet is kept constant therefore, exercise and physical fitness do not appear to affect BMR.

	Control		Weeks 4–6		Weeks 7–9		Change	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body-weight (kg)	62·0	6·4	62·0	6·4	61·0	5·9	0·9	1·7
^{40}K (counts/4000 s)	4432	1033	4499	991	4557	1031	137***	54
Body fat (kg)	12·5	4·9	—		10·7	5·6	1·9**	2·1
$\dot{V}_{O_{2max}}$ (litres/min)	2·4	0·5	—		3·1	0·7	0·7*	0·6
Energy expenditure† (MJ/24 h)	9·6	1·4	—		12·4	3·0	2·8*	2·0
BMR (MJ/24 h)	5·8	1·0	5·8	0·8	5·9	0·8	0·1	0·5
Dietary intake† (MJ/24 h)	10·0	1·5	10·0	1·5	10·0	1·5	0	

*P<0·05, **P<0·02, ***P<0·001 (paired t test, weeks 7–9 v. control).

†Five subjects only. Energy expenditure for all six subjects in the control period was 9·1 (SD 1·7) MJ/24 h, and dietary intake throughout was 9·5 (SD 1·7) MJ/24 h.

Our thanks are due to Professor W. P. T. James for the loan of some stable isotope during the course of this study.

Contributions of low energy expenditure and excessive energy intake to the development of fatness during infancy. By S. B. ROBERTS*, J. SAVAGE, W. A. COWARD and A. LUCAS, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

The relative contributions of abnormally low energy expenditure and excessive energy intake to the development of fatness in the first year of life were investigated. The subjects were eighteen healthy infants (nine male, nine female), recruited within 3 d after birth, and initially of normal body-weight. They were born to lean mothers (<10th percentile of weight-for-height) or to fat mothers (>90th percentile) and were therefore at low risk (*n* 6) or high risk (*n* 12), respectively, of becoming obese. Open-circuit, indirect calorimetry was used to measure the sleeping or resting metabolic rate (MR) of each infant in the post-prandial state within 1 week of birth and at approximately 3 months of age. The doubly-labelled water method (Roberts *et al.* 1986) was used to determine total energy expenditure (TEE), metabolizable energy (ME) intake and total-body water at approximately 3 months of age. Standard anthropometric measurements were made at 3-monthly intervals from birth to 1 year, to distinguish between subjects who became overweight (defined as exceeding the 90th percentile of weight-for-age) and those who remained at a normal size.

None of the infants of lean mothers became overweight before 1 year of age, compared with 50% of the infants of fat mothers ($P<0{\cdot}05$). Four of the infants who became overweight were male and two were female. There was no significant difference at birth and 3 months of age between normal infants and infants who subsequently became overweight, with respect to weight, length, skinfold thickness and MR determined by calorimetry. There was also no significant difference between these two groups with respect to ME intake and total body water determined using the doubly-labelled water method at 3 months of age. However, infants who later became overweight were gaining weight significantly faster than normal infants by 3 months of age (5·0 (SE 0·2) *v.* 4·0 (SE 0·2) g/d per kg; $P<0{\cdot}01$), and had significantly greater skinfold thickness at 9 and 12 months of age ($P<0{\cdot}05$). The sum of triceps and subscapular skinfolds in the two groups at 12 months of age was 15·3 (SE 1·4) mm and 12·6 (SE 0·4) mm respectively. Infants who became overweight also had a substantially lower TEE (250 (SE 25·5) *v.* 310 (SE 15·5) kJ (60·0 (6·1) *v.* 74·4 (3·7) kcal)/d per kg) at 3 months of age ($P<0{\cdot}05$). TEE:theoretical basal MR estimated from body-weight (Schofield, 1985) was also significantly lower in infants who subsequently became overweight than in normal infants (1·17 (SE 0·17) *v.* 1·51 (SE 0·07); $P<0{\cdot}05$). These findings suggest that low energy expenditure, rather than excessive energy intake, is the initial cause of developing fatness in many infants who become overweight during the first year of life.

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New concepts in starch digestion in man. By H. N. ENGLYST, G. T. MACFARLANE and J. H. CUMMINGS, *MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

There is now much evidence to indicate that the long held belief that starchy foods are completely digested and absorbed from the human small intestine is wrong. In a series of studies using human ileostomists as a model we have shown that non-starch polysaccharides (NSP; dietary fibre) pass through the stomach and small intestine and can be totally recovered in ileostomy effluent. In similar studies we have also shown that a substantial amount of starch is not digested (Table). 2–5% of ingested cereal starch is recovered in ileostomy effluent, 3–12% of potato (Englyst & Cummings, 1987*a*) and more than 90% of banana starch pass undigested through the small intestine.

Banana contains starch granules that are highly resistant to pancreatic amylase. Potatoes and other moist-heated starchy foods are incompletely digested when cooled because of retrogradation of the starch dispersed during cooking. The formation by food processing of starch with reduced digestibility depends on the amylose:amylopectin ratio, water content, pH, heating temperature and time, reheating, cooking, freezing and drying. It is therefore to a very large extent in the hands of the food industry to control the site of digestion of starchy foods.

In a controlled dietary study in four healthy volunteers we have given banana and cooled potatoes and have been able to recover only very small amounts of starch in faeces (Table). This suggests that starch escaping digestion in the small intestine is effectively fermented in the large bowel.

Diet	Starch given (g/d)	% starch recovered in ileostomy effluent	% starch recovered in faeces from healthy volunteers
White bread	61·9	2·4	
Oats	57·8	1·6	
Cornflakes	74·2	4·6	
Banana	19·3	89·5	
Banana	31·0		1·6
Freshly cooked potatoes	45·4	3·3	
Cooled potatoes	47·2	12·3	
Cooled potatoes	105·0		0·1
Reheated potatoes	47·2	7·6	

In vitro fermentation studies using a series of starch and NSP showed that different end-products were produced from the fermentation of these different polysaccharides and that more butyric acid resulted from starch than from NSP (Englyst *et al.* 1987).

Based on these studies and the in vitro susceptibility of starch to pancreatic amylase we have proposed a new nutritional classification of starch (Englyst & Cummings, 1987*b*).

Starch and NSP are very different, both chemically and in their effect in the small and large intestine. Starch escaping digestion in the small intestine may of its own right be an important food component and should not be included with NSP as dietary fibre.

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Achieving energy balance in clinical studies. By M. E. J. LEAN, *Diabetic Clinic, Aberdeen Royal Infirmary, Aberdeen AB9 1GS*

Changes in body-weight affect the interpretation of many clinical studies, and in short metabolic experiments failure to maintain energy balance may have profound effects even before body-weight changes. Four strategies for diet prescription in clinical studies are compared here.

Seven free-living male volunteers completed 7-d weighed diet inventories to assess individual energy intakes on a programme of light activity plus a standard period of moderate exercise on a bicycle ergometer. The estimated amount was fed and 24 h energy expenditure (EE) was measured by indirect whole body calorimetry. Energy intake was 12 000 (SD 1155) kJ/d for the group, and EE 12 484 (SD 1290) kJ/d, however, for individuals there was an average error of 9·9% (range −20 to +10%) and estimates failed to correlate significantly with measured EE (r 0·19). Feeding a standard 12 000 kJ/d to these same subjects would have resulted in an average difference from EE of 7% (−25 to +10%). By contrast, estimation of 24 h energy requirement from body-weight using basal metabolic rate (BMR) (Food and Agriculture Organization/World Health Organization/United Nations University, 1985) and a 'light activity' factor of BMR × 1·55, plus 720 kJ for the standard exercise period, gives a mean of 12 065 kJ/d with average error from the measured EE of 4.6% (−6·8 to +8·6%; r 0·87).

Energy balance in metabolic studies is thus more closely achieved using estimated EE than by dietary assessment. For most accurate results, the activity factor relating BMR to 24 h EE can be assessed by previous measurement: in another study of twenty-three lean and obese women on a sedentary regimen, energy requirement was estimated from a previous measurement of fasting EE using the same protocol. This resulted in mean food energy within 1·2% of requirement for energy balance for the group and average individual errors between EE and food provided of only 2·6% (−4·2 to +9·9%; r 0·95).

Food and Agriculture Organization/World Health Organization/United Nations University (1985). *Technical Report Series no.* 724. Geneva: WHO.

Within-day variations in the digestive enzyme activity of breast-milk from five Cambridge mothers. By O. DEWIT and ANN PRENTICE, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

The human digestive system is immature at birth and slowly develops with age. Breast-milk contains a number of digestive enzymes which may compensate for the infant's low digestive capacity. The aim of the present study was to investigate within-day variations in the activity of three breast-milk enzymes: amylase (*EC* 3.2.1.1), bile-salt-stimulated esterase (*EC* 3.1.1.1, BSSE) and bile-salt-stimulated lipase (*EC* 3.1.1.3, BSSL).

Five Cambridge mothers at 1–3 months of lactation took part in the study. Small samples of breast-milk (1–2 ml) were collected from both breasts before and after each feed during a single 24 h period. Published assay methods for the three enzymes were investigated for use with breast-milk. BSSL activity was measured by the liberation of free fatty acids from tritiated trioleylglycerol in the presence of sodium taurocholate. Substantial inhibition of BSSL activity by breast-milk was demonstrated. This led to an apparent rise in enzyme activity with increasing sample dilution. The inhibition was most marked at the 1·7 mM substrate concentration commonly used in this assay. The factor responsible for the inhibition was not removed from milk by acetone treatment but was absent in enzyme purified from breast-milk. The effect of the inhibitor was minimized by increasing the substrate concentration to 10·2 mM and by expressing the results of all milk samples relative to a reference milk. No modification of published amylase and BSSE methods was required.

The amylase activity of Cambridge breast-milk remained constant during feeds and throughout the day. Analysis of variance demonstrated that the mothers had characteristic levels of amylase activity ($F = 281$, $P<0·001$); a tenfold range of values was observed. Both BSSE and BSSL showed a significant decrease in activity between the beginning and end of each feed, the mean decreases observed were −25% and −16% respectively. The mean activity measured at each feed for either enzyme did not vary with the time of day. Significant differences between mothers were observed in the breast-milk levels of both enzymes (BSSE, $F = 7·06$, $P<0·001$; BSSL, $F = 9·31$, $P<0·001$), the values varying by 1·5-fold.

The results of this detailed study will enable the development of rational sampling procedures for future studies of digestive enzymes in breast-milk.

O.D. is a research fellow funded by the Ministry of Research, France; A.P. is supported by the Bristol-Myers Fund.

Metabolic effects of isoenergetic nutrient exchange over 24 h in relation to obesity in women. By M. E. J. LEAN, *Dunn Clinical Nutrition Centre, Addenbrookes Hospital, Cambridge CB2 1QE* and W. P. T. JAMES, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

It has often been suggested that fat people eat the 'wrong' foods and dietary fat is increasingly incriminated (Flatt *et al.* 1985). The present study used a previously described 24 h whole body indirect calorimetry method (Dallosso *et al.* 1982) to study the effects of feeding subjects, during a sedentary test day, on isoenergetic diets which varied in fat (3 or 40% of total energy) and carbohydrate (82 or 45% of total energy) content. Three groups of women were studied (mean and SD): lean, 42 (15) years, 56 (11) kg, 1·66 (0·08) m; obese, 41 (10) years, 96 (15) kg, 1·61 (0·04) m; 'post-obese' after slimming, 47 (10) years, 67 (7) kg, 1·65 (0·09) m. Results were analysed by split-plot analysis of variance.

Energy expenditure (EE) was greater than that of controls in absolute terms in the obese women, but lower in both obese and post-obese when expressed per kg fat-free mass (FFM) as assessed from skinfold measurements (Durnin & Womersley, 1974). 24 h EE was lower by only 3–7% when fasting compared with that when fed to achieve energy balance. There were thus no large differences in EE between the two diets or between the groups, but overall the thermogenic effect of the high-carbohydrate diet was significantly greater than that of the high-fat diet (5·8 *v.* 3·5% of EE for all subjects; $P<0·01$). The post-obese tended to have particularly lower EE per kg FFM than controls when fasting and when fed on a high-fat diet: 24 h EE was significantly lower when fed on the high-fat diet compared with the isoenergetic low-fat diet ($P<0·05$), but this pattern was not shown by the obese. Sleeping EE was particularly low in the post-obese group when fed on the high-fat diet.

The present study provides further evidence that dietary fat content may be an important environmental factor in revealing a tendency to conserve energy in subjects prone to obesity.

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Onset of menstruation in different ethnic groups in the UK. By S. J. ULIJASZEK, *Department of Physical Anthropology, University of Cambridge, Downing Street, Cambridge CB2 3DZ,* and ELIZABETH EVANS and D. S. MILLER, *Department of Food and Nutritional Sciences, King's College, Campden Hill Road, London W8 7AH*

Although mean age at menarche has been well documented in the UK (Scott, 1961; Roberts *et al.* 1975), no values have been reported for British ethnic minority populations.

A total of 2177 girls aged 9–17 years were asked whether or not they had started menstruating, and if so, when. Mean ages at menarche were calculated for each ethnic group using probit analysis, and comparisons between the groups were made using Student's *t* tests.

Age (years) at menarche of three ethnic groups

	European			Afro-Caribbean			Indo-Pakistani		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
By social class:									
1+2+3	374	13·35	1·19	155	12·69	1·55	150	13·00	1·85
4+5	854	13·50	1·33	328	13·10	1·39	132	13·21	1·83
Unemployed+single parent	137	13·73	1·25	(Sample size too small for analysis)					
Family size (no. of children):									
1+2+3	945	13·36	1·30	297	13·09	1·10	153	13·07	1·96
4+	420	13·76	1·54	233	13·20	1·38	129	13·44	1·56
Birth order:									
1+2+3	1152	13·52	1·24	359	13·10	1·18	219	13·01	1·83
4+	213	13·48	1·85	171	13·22	1·59	63	13·16	1·99
Total:	1365	13·53	1·27	530	13·18	1·16	282	13·06	1·35

European girls began menstruation significantly later than the Afro-Caribbean and Indo-Pakistani girls ($P<0·05$) as well as contemporary London European populations (13·0 years; Tanner, 1973). Age at menarche in European girls was related to family size, but not birth order and social class. Amongst the Afro-Caribbeans and Indo-Pakistanis, there was a non-significant trend towards later onset of menarche in those girls of lower social class, or from larger families, or those who were later-born offspring. Afro-Caribbean girls had similar timing of onset of menarche to that of other European populations, but had a delayed onset, in comparison with Afro-Americans (12·5 years; MacMahon, 1973) and Jamaicans of high socio-economic status in Jamaica (12·0 years; Alleyne *et al.* 1980). The Indo-Pakistani girls had a similar mean age at menarche to well-off populations in India (12·9 years; Roberts *et al.* 1977).

Future studies should reveal whether there is a trend towards earlier maturation amongst these immigrant groups, due to improved environmental and nutritional conditions.

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Temperature changes and tissue zinc effects induced by *Escherichia coli* endotoxin in rats fed on maize oil- and coconut oil-enriched diets. By D. BIBBY, SALLY COLEGATE and R. F. GRIMBLE, *Human Nutrition Department, Southampton University Medical School, Southampton SO9 3TU*

The metabolic responses to bacterial endotoxin may be modified by feeding animals on fats of varying linoleate content (Wan & Grimble, 1987). Linoleate via arachidonate acts as a precursor for prostaglandins which are implicated in fever. The present study examines the influence which a diet low in linoleate has on endotoxin-induced fever and subsequent changes in tissue zinc.

Male Wistar rats (68 (SE 2) g) were fed *ad lib.* on standard laboratory chow enriched with either 120 g maize oil (MO) or coconut oil (HCO)/kg for 6 weeks. Diets contained 69·2 and 11·4 g linoleic acid/kg respectively. Rats grew equally on either diet (final body-weights MO 345 (SE 8) g; HCO 342 (SE 8) g). Half of each group received 2·4 mg *Escherichia coli* endotoxin/kg body-weight subcutaneously (strain 0217:B8, butanol extract; Sigma, Poole, Dorset) (n 6) at 09.00 hours on the day before killing. The other half received sterile saline (9 g sodium chloride/l). Rectal temperatures were measured before (t_0) and at hourly intervals after injection for 7 h (t_n). All rats were given 60% of their *ad lib.* intake and maintained at an ambient temperature of 20°. Rats were killed with chloroform 24 h after injection, blood sampled by cardiac puncture, and liver, muscle, kidney, spleen, thymus and ≃ 100 mm small intestine proximate to the caecum rapidly removed, cleaned and weighed. Tissues were 'wet ashed' for Zn assay as described elsewhere (Wan & Grimble, 1987).

	MO		HCO	
Rectal temperatures (°) at:	Saline	Endotoxin	Saline	Endotoxin
t_0	39·2	39·0	39·1	38·7
t_2	38·4	38·3	38·4[a]	37·5[a]
t_5	38·0[a]	39·1[a]	38·3	38·8
t_6	38·3[a]	39·2[a]	38·2	38·8
t_7	38·1[a]	38·9[a]	38·4	38·9
Zn (μg)/liver	440	497	410[a]	528[a]††
Zn (μg)/spleen	24·8	25·7	20·4[a]	25·7[a]††
Zn (μg)/thymus	19·5	23·8	17·9	15·7*
Zn (μg)/tibialis	11·4[a]	10·1	8·7[a]	9·8*

Differences between groups determined by 2 way ANOVA. Values with a common superscript letter are significantly different.

Significant difference due to fat (*P<0·05) and *E. coli* (††P<0·01).

HCO affected some responses to endotoxin. While the increase in liver and spleen Zn was enhanced the rise in rectal temperature above that of controls was blunted and a transient hypothermia developed at t_2. Kidney and intestinal Zn were unaffected by diet or endotoxin. Changes in prostaglandin metabolism may underly the suppressive effects of HCO on body temperature in response to endotoxin.

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What the British public spread on bread. By MARGARET J. WHICHELOW and JUDITH NICKSON, *Office of the Regius Professor of Physics, Cambridge University School of Clinical Medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ*

In the context of the emphasis, in current campaigns aimed at reducing the prevalence of coronary heart disease, on persuading people to reduce their fat, particularly saturated fat, intake, the reported dietary habits of the 3905 men and 5098 women in the Health and Lifestyle Survey (Cox, 1987) in respect of the fats spread on bread are relevant. Butter was the most popular form of spread (40·8%) followed by margarine (hard or soft) (30·0%), polyunsaturated margarine (15·6%) and low-fat spread (9·7%). Despite its cost butter was used by similar proportions in all socio-economic groups, but polyunsaturated margarine and low-fat spread was used more frequently by those in the non-manual groups. Although the coronary prevention programmes have been directed *prima*rily at men, polyunsaturated margarine was chosen equally by men and women, but low-fat spread was used by more women (10·8% compared with 8·1% for men). Respondents who reported being on diets for obesity, hypertension or heart disease were more likely than others to use polyunsaturated margarine or low-fat spreads (see Table). Women, but not men, consuming low-fat or low-energy diets were more likely than others to use low-fat spreads. Nevertheless only a minority of these respondents were using the spread which might be considered appropriate to their condition. Furthermore, subjects who gave 'too much fat' in reply to a question about the cause of heart disease were only slightly more likely than others to report choosing polyunsaturated margarine or low-fat spread.

Choice of spread in relation to diet

	Reports dieting for:						Type of diet reported:					
	Obesity		Hypertension/ heart disease		All other respondents		Low energy		Low fat		All other respondents	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
n	42	112	59	55			33	92	133	179		
Per cent using:												
Polyunsaturated fat	24	19	37	38	15	15	36	17	34	24	15	15
Low-fat spread	21	21	17	11	8	11	6	20	10	20	8	11

There were marked differences in the choice of spreads between the smokers and non-smokers in each age (18–39, 40–59, 60+ years), sex and socio-economic group. The smokers were less likely to eat polyunsaturated margarine ($\chi^2 = 95{\cdot}56$ for men and 29·98 for women; $P<0{\cdot}001$ for both) or low-fat spread (χ^2 49.86 for men and 48·89 for women; $P<0{\cdot}001$ for both). This is in accord with the poorer dietary habits of smokers reported elsewhere (Whichelow *et al.* 1986).

Overall, 50% of men and 27% of women consumed over 30 g fat/d as spread, reflecting the higher consumption of bread by men. In both sexes consumption (g/d) increased with decreasing socio-economic group, and with increasing age in women. In each group the mean intake of spread by smokers was higher than that by non-smokers. Of those reporting to be on a low-energy diet, 15% men and 14% women were consuming over 30 g spread/d. However, of those reporting to be on low-fat diets 31% men and 19% women were consuming over 30 g/d.

The results suggest that the choice of spread and level of intake by subjects is not always appropriate. The choice of spread and high level of intake by cigarette smokers, a high risk group for coronary heart disease, is of concern.

This study was supported by the Health Promotion Research Trust. M.J.W. was in receipt of a grant from NAPP Laboratories.

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Mechanisms of energy exchange in a rat model of endotoxaemia. By G. JENNINGS and M. ELIA, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

Injury, whether it be accidental, surgical or septic, is typically associated with an increase in energy expenditure. The mechanism of this increase is uncertain, although brown adipose tissue (BAT) may play a role, especially in small animals. In circumstances where energy expenditure is elevated, BAT activity is either increased (e.g. cold acclimation, overfeeding) or decreased (e.g. lactation). However, there is little information about the effect of trauma or sepsis (endotoxaemia) on BAT activity. To investigate this, an assessment was made of the activity of the proton conductance pathway in the BAT (by GDP binding) of rats that were given a single intraperitoneal injection of butanol-extracted *Escherichia coli* endotoxin type 0127 B8 (Sigma, Poole, Dorset) in saline (9 g sodium chloride/l) at a dose of 3 mg/kg body-weight. The rats (300 g) were maintained at 26° both before the study (3 weeks) and during the study. Resting oxygen consumption (0–6 h), tail and rectal temperatures (0–6 h) and indices of BAT thermogenesis (4 h post-treatment) were measured in three separate experiments. Control animals given saline were included in all experiments.

Treatment with endotoxin produced a significant increase in O_2 consumption, rectal temperature and GDP binding (Table). Tail temperature, BAT mitochondrial mass (assessed by cytochrome *c* oxidase (*EC* 1.9.3.1) activity) and BAT uncoupling protein were not significantly affected by endotoxin administration.

Effect of E. coli *endotoxin on O_2 consumption, rectal temperature and BAT indices*

	Control (*n* 6)		Endotoxin (*n* 6)	
	Mean	SE	Mean	SE
O_2 (ml/min per kg)	19·0	0·6	22·6***	0·9
BAT wt (mg)	430	21	355*	18
Rectal temperature (°)	38·3	0·2	39·3***	0·1
GDP bound (μmol/mg mitochondrial protein)	182	32	304**	28
Cytochrome *c* oxidase (μmol enzyme oxidized/min)	83·9	8·0	93·0	10·2
Uncoupling protein (μg/mg mitochondrial protein)	24·6	2·0	31·8	3·2
Total BAT protein (mg)	23·3	1·0	23·2	1·2

*$P<0·05$, **$P<0·02$, ***$P<0·01$.

All measurements 4 h post-treatment, except O_2 consumption which is the mean for 3–4 h post-treatment.

It is concluded that *E. coli* endotoxin 0127 B8 produces an increase in total energy expenditure and an increase in the activity of BAT. In addition, since tail temperature did not increase despite a rise in rectal temperature, it is concluded that endotoxin reduces the proportion of heat loss through the tail.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Differential effects of endotoxin on the production of fever, diarrhoea and loss of body-weight in mice. By G. JENNINGS and M. ELIA, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

Endotoxin administration to animals has been used as an animal model of sepsis/trauma for studying changes in body temperature, protein turnover, trace element metabolism and changes in the function of various tissues. However, little attention has been paid to the possibility that different endotoxins or different preparations (extractions) of the same endotoxin may produce a different spectrum of effects within the body. The present preliminary study aimed to assess this possibility with respect to the development of fever, diarrhoea and changes in body-weight.

Six groups of four male mice (Aston strain, 3–4 months of age) were injected intraperitoneally with either saline (9 g sodium chloride/l, control) or specific endotoxins (see Table) at a dose of 3 mg/kg body-weight at 0, 8 and 24 h. Mice were transferred from 22 to 32° (thermoneutral) 24 h before the start of the study. They were also acclimatized to the procedures during the day before the study.

Effect of various endotoxins on body-weight, rectal temperature and the incidence of diarrhoea in mice

(Mean values for groups of four mice)

Endotoxin	Extraction procedure	Maximum temperature rise (°) 0–8 h	Maximum temperature rise (°) 24–32 h	% change in body-weight 24 h	% change in body-weight 48 h	Diarrhoea 0–8 h	Diarrhoea 24–36 h
Escherichia coli 0127 B8	Butanol	0·7	0·9**	−1·1	−3·2	0/4	0/4
E. coli 0111 B4	TCA	0·9**	1·0**	−3·3	−4·8	0/4	4/4
E. coli 0111 B4	Phenol	0·7*	1·0**	−8·4	−13·6	2/4	4/4
Salmonella typhosa	TCA	0·3	0·3	−1·2	−2·4	2/4	4/4
S. typhosa	Phenol	0·3	1·0†	−7·2	−13·4	4/4	4/4
Saline controls	—	—	—	+1·4	+1·7	0/4	0/4

Values given are for temperature increase $P>0·05$ when compared with controls.
Significantly different from control values: *$P<0·05$, †$P<0·02$, **$P<0·01$.

The Table shows the various combinations of end-effects that were produced by various endotoxins, e.g. fever with and without weight loss, or with and without diarrhoea. Different effects were also produced by the same endotoxin extracted by different procedures (Table). Furthermore, administration to different species of animals of the same endotoxin at the same dose per kg body-weight was found to produce a different spectrum of effects, e.g. salmonella endotoxin types, which produce diarrhoea in mice (Table) do not do so in either young or old rats (result not shown). The results suggest that care should be taken in choosing the appropriate endotoxin for studying specific responses and end-organ effects.

Appropriate equations of calculation of carbon dioxide production in doubly-labelled water studies. By J. R. SPEAKMAN and P. A. RACEY (introduced by A. M. PRENTICE), *Department of Zoology, University of Aberdeen, Aberdeen AB9 2TN*

Two methods have been proposed for calculation of carbon dioxide production in doubly-labelled water ($^2H_2{}^{18}O$) studies. Originally, Lifson & McClintock (1966) suggested the apparent turnover rates of the two isotopes should be multiplied by the volume of the body water pool which can be evaluated from the dilution space of ^{18}O. More recently, Coward *et al.* (1985) suggested the turnover of the two isotopes should be multiplied by their respective dilution spaces, which typically differ by about 3%.

In theory the equation of Coward *et al.* (1985) is superior when flux of the small pool penetrated exclusively by hydrogen is zero. The Lifson & McClintock (1966) equation, however, is theoretically superior when turnover of the subsidiary pool is significant and occurs at the same rate as the body water pool (Speakman, 1987). Neither equation is intrinsically superior but they are simply more or less appropriate as the assumptions about the system become more or less relevant to the situation under consideration.

Validation studies suggest that the Coward *et al.* (1985) equation is more appropriate in humans. Is this equation also more valid in smaller mammals? We have validated the $^2H_2{}^{18}O$ technique by comparison with indirect calorimetry over a fivefold range of metabolism in small insectivorous bats (seven *Pipistrellus pipistrellus*, two *Plecotus auritus*) which at 6–10 g are amongst the smallest living mammals. The original equation of Lifson & McClintock (1966) resulted in mean algebraic error of +9·5%, the Coward *et al.* (1985) equation +5·1% and a modified version of the latter equation, after Schoeller *et al.* (1986), of +3·4%. Although these means suggest an improved accuracy using the Coward *et al.* (1985) type equations, the range of errors was large and calculation method was not a significant factor influencing error in an analysis of variance ($F = 0{,}3$; $P>0{\cdot}05$). Re-examination of previous validation studies suggest this may also be the case in humans. Neither of the equations would appear to be theoretically or empirically superior.

This work was supported by NERC grant GR3/5155.

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The effect of exercise on isotope fractionation in elite female athletes. By P. HAGGARTY[1], B. A. MCGAW[1], R. J. MAUGHAN[2], E. MILNE[1] and M. GLEESON[2], [1]*Rowett Research Institute, Greenburn Road, Aberdeen AB2 9SB* and [2]*Department of Environmental and Occupational Medicine, Aberdeen University Medical School, Aberdeen AB9 2ZD*

It is established that fractionation of ^{2}H and ^{18}O in water occurs during evaporative water loss in human subjects (Schoeller *et al.* 1986). However, the general applicability of the fractionation factors to all physiological situations has not been tested. The present study investigates the effects of different sweating and breathing rates on fractionation factors. We have measured isotope fractionation by measuring the $^{2}H_{2}{}^{18}O$ enrichment in saliva, water lost from the skin of the forearm, and breath water and the ^{18}O enrichment in expired carbon dioxide. These variables were measured in four elite female athletes at rest and whilst exercising for 20 min at 75% of their maximal oxygen uptake ($\dot{V}_{O_{2max}}$).

Effects between athletes and between exercise and rest were evaluated. There was a significant effect between athletes on the $^{2}H_{2}O_{2}$ of saliva relative to urine. There was no significant subject effect on fractionation in water lost from the skin or in breath. Exercise had a significant effect on fractionation of water lost from skin; ^{2}H fractionation in water lost from the forearm was 0·966 (SEM 0·005) at rest and 0·911 (SEM 0·007) during exercise; ^{18}O fractionation in water lost from the forearm was 0·992 (SEM 0·001) at rest and 0·983 (SEM 0·001) during exercise. Exercise did not, however, affect the fractionation of isotopes in saliva or breath water or in breath CO_{2}.

We conclude that it may not be appropriate to apply one value for water fractionation during evaporative loss under all physiological conditions. However, even if fractionation were measured for all routes of loss it would still be necessary to quantify the proportion of total water lost by these routes. This problem requires further investigation.

Schoeller, D.A., Leitch, C. A. & Brown, C. (1986). *American Journal of Physiology* **251**, R1137–R1143.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

The effect of diet restriction on the development of prenatal and postnatal brown adipose tissue thermogenesis in the guinea-pig. By DOROTHY M. STIRLING and MARGARET ASHWELL*, *MRC Dunn Nutrition Unit, Downhams Lane, Milton Road, Cambridge CB4 1XJ*

The ability of the neonate to cope with thermal or dietary stress, or both, is related to the amount and activity of its brown adipose tissue (BAT). In the present study we have investigated the effect of diet restriction during pregnancy on the amount and activity of BAT in guinea-pig fetuses and pups.

Pregnant guinea-pigs (Dunkin Hartley strain) were divided into three groups: control group (AL) fed *ad lib.*, diet-restricted (DR) groups receiving 50% reduction of *ad lib.* intake from days 0 to 30 gestation (early DR) or from day 30 to parturition (late DR). The interscapular BAT pads of fetuses and pups were analysed for cytochrome *c* oxidase (*EC* 1.9.3.1) activity (assessment of mitochondrial mass), GDP-binding (measure of thermogenic activity) and uncoupling protein (UCP, measure of thermogenic capacity) at ages ranging from 63 d gestation to 5 d old (Trayhurn *et al.* 1987).

		Pre-partum†			0–1 d			2–5 d		
	Group	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*
GDP-binding	AL	119	21	10	423	30		260	15	12
(pmol/mg mitochondrial	Early DR	80	23	7	384	22	14	326	34	8
protein)	Late DR	288***	6	4	653*	125	7	290	33	11
Cytochrome *c* oxidase	AL	103	9	10	129	13	9	61	3	12
(μmol enzyme oxidized/min	Early DR	90	14	7	84*	4	14	28***	3	8
per tissue)	Late DR	115	12	4	140	10	7	43*	6	11
UCP	AL	24	6	10	50	4	16	35	3	18
(μg/mg mitochondrial	Early DR	20	4	7	47	3	14	38	2	8
protein)	Late DR	26	2	4	40	4	7	28	4	10

Significantly different from AL value: *$P<0.05$, ***$P<0.001$.
†63 d gestation to parturition.

GDP-binding and UCP concentration peaked significantly at 0–1 d in all three groups. Diet restriction in early pregnancy had no significant effect on pup weight, GDP-binding and UCP concentration but resulted in a substantial reduction ($P<0.001$) in post-partum cytochrome *c* oxidase activity. In contrast, diet restriction during late pregnancy resulted in significantly reduced pup weights at birth (AL 89·8 (SEM 15·8) g, late DR 75·5 (SEM 13·0) g; $P<0.05$). This was associated with a marked increase in GDP-binding both pre-partum ($P<0.001$) and at 0–1 d ($P<0.05$) compared with AL controls. Cytochrome *c* oxidase activity was also significantly reduced at 2–5 d post-partum.

The results of the study showed an increase in the BAT activity at birth of the growth-retarded pups from late DR sows, suggesting that at this critical stage the thermal stress could be overriding the need to grow.

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The importance of sedentary activity in the 24 h energy budget. By P. R. MURGATROYD, H. L. DAVIES, G. R. GOLDBERG and A. M. PRENTICE, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

To investigate the variability between individuals in energy expended during sedentary activities data have been analysed from two whole-body calorimetry studies.

In study 1 the energy expenditure of six women was measured on five to seven occasions throughout pregnancy. In study 2, five men and three women were studied at different levels of imposed exercise on each of five occasions. In each 24 h analysis period 9 h were spent asleep followed by a 1 h basal metabolic rate (BMR) measurement. Of the 14 h spent out of bed, 2 h were taken up by programmed exercise in study 1 and 3 h in study 2. The residual 12 and 11 h were spent sitting except for short periods (<10 min) before and after exercise and before meals. The portion of energy expended in the sedentary periods which was in excess of BMR was calculated, scaled to represent 14 h and expressed as a percentage of BMR to normalize for metabolic size. We have defined this as the sedentary expenditure index (SEI). SEI represents the sum of all sedentary activity and diet-induced thermogenesis. No thermoregulatory thermogenesis is included since the studies were carried out at 26°.

Analyses of variance of the complete data sets showed that each subject had a characteristic level of SEI throughout the series of measurements in a study (study 1, $F(5,32) = 10{\cdot}3$, $P<<0{\cdot}001$; study 2, $F(8,32) = 9{\cdot}6$, $P<<0{\cdot}001$). The geometric mean SEI for study 1 was 21·7% (95% confidence limits 14·9–31·8) and for study 2 was 28·5% (95% confidence limits 19·8–41·0). The mean range between 95% confidence limits was 19·1%.

We conclude that energy expended in sedentary activity is an individual characteristic which can vary between individuals over a range of about 20% of BMR. In a hypothetical group of subjects spending 12 h/d in sedentary activity and having a 24 h energy expenditure of 1·5 × BMR, differences in SEI would contribute 11·4% to the inter-individual variability in total energy expenditure.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Characterization of brown adipose tissue in diabetic SHR/N-*cp* rats. By SUSAN P. DEBOLT[1], O. E. MICHAELIS IV[2] and ORIEN L. TULP[1], [1]*Department of Nutrition and Food Sciences, Drexel University, Philadelphia, PA, USA,* and [2]*Beltsville Human Nutrition Research Center, USDA, Beltsville, MD, USA*

The obese phenotype of the adult male SHR/N-*cp* rat has been reported to exhibit an impaired capacity for non-shivering thermogenesis (NST) following nutritional and environmental manipulation, in association with insulin resistance, non-insulin-dependent diabetes (NIDDM), and early-onset obesity (Tulp *et al.* 1986). In other studies, the obese phenotype of similar-aged adult LA/N-*cp* rats, which share the same trait for obesity in a non-diabetic background, also demonstrated similar impairments in NST, in spite of brown adipose tissue (BAT) that was typically quite normal in appearance, lipid locularity and structure (Tulp *et al.* 1982). Because of the requirement for insulin in the expression of NST (Rothwell *et al.* 1981), it was of interest to characterize BAT in the obese-NIDDM animals to determine whether the impaired NST was associated with abnormal development of BAT. Groups of lean and obese SHR/N-*cp* rats were fed on diets containing (g/kg): 540 carbohydrate (maize starch), 200 protein (equal parts lactalbumin and casein), 160 fat (equal parts lard, maize oil, beef tallow, and coconut oil), plus essential vitamins, minerals and cellulose, from weaning until 9 months of age. Rats were maintained in hanging wire-bottomed steel cages at 22°, with free access to food and water. Rats were killed by decapitation and the interscapular BAT (IBAT) excised in its entirety for lipid content, adipose cellularity, lipoprotein lipase activity (LPLA), and protein determinations.

			IBAT											
	BW (g)		(g/pad)		IBAT: BW $\times 10^{-3}$		Cells $\times 10^6$		μg lipid/cell		Lipid (mg/g)		mg protein	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Lean	455	8	0·65	0·1	1·4	0·1	2·4	0·1	0·23	0·06	520	20	130	11
Obese	820	32	5·93	0·6	7·2	1·0	13·8	3·7	0·38	0·13	770	30	110	22
$P<$	0·001		0·001		0·05		0·001		NS		0·001		NS	

BW, body-weight; NS, not significant.

LPLA/g tissue per h was greater in lean than in obese animals (10·24 (SE 2·20) *v.* 2·20 (SE 0·40); $P<0·05$) but LPLA was similar in the two groups when expressed per 10^6 cells per hour (2·77 (SE 0·60) *v.* 1·91 (SE 0·35), not significant).

These results indicate that obese animals had marked increases in IBAT mass, lipid content, adipocyte size and adipocyte number compared with their lean littermates, and that the greater IBAT was due to a combination of hypertrophy and hyperplasia of BAT. These observations are consistent with an impaired capacity for the activation or expression of NST, in spite of greater mass and cellularity, and indicate that the superimposition of the diabetic stigmata in an already obese animal may further compromise its capacity for the development of BAT thermogenesis.

Supported by institutional resources of BHNRC-USDA and Drexel University.

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Dietary intakes and weekly expenditures in pregnant diabetic and non-diabetic women.

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The present study assessed the habitual daily dietary intakes, using the 7-d weighed food inventory method, of sixteen women in the second half of pregnancy. Prices of food items at the time of the study were calculated according to a standard retail index (Anon, 1986) and costs of fresh fruit, vegetable, bakery and butchery items were obtained from the largest local supplier, ASDA supermarkets. Eight subjects were diabetics, six insulin-dependent, two diet-controlled (mean age 29 (range 25–33) years, pre-pregnant body mass index 24·6 (range 21–30)), who had received regular individual advice based on current British Diabetic Association (BDA) (1982) recommendations. Eight were non-diabetics (mean age 26 (range 19–30) years, pre-pregnant body mass index 21·3 (range 19–30)), who had not received dietary advice. Subjects matched for gestational age (mean 29 weeks) were recruited consecutively from combined diabetic/antenatal and routine antenatal clinics.

	Diabetic				Non-diabetic			
			% energy				% energy	
Intake (/d)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy: (kJ)	8232	1520	—	—	8282	1229	—	—
(kcal)	1960	332	—	—	1972	295	—	—
Carbohydrate (g)	215	49	42	6	232	48	45	5
Fat (g)	93	18	42	6	89	16	40	5
Protein (g)	72	17	15	1	72	13	15	2
Dietary fibre (g)	22	12	—	—	19	7	—	—

Carbohydrate intakes were close to the individually prescribed amounts (the only specified nutrient) in the diabetic women (221 (SD 15) g/d), but as a proportion of total energy fell below BDA recommendations. All the insulin-dependent diabetics had originally been instructed on low-carbohydrate diets before publication of the current recommendations, which possibly accounts for the relatively low proportion of carbohydrate: energy requirement was made up without increasing carbohydrate above the prescribed amount.

Mean weekly food costs were £12.91 (range £9.20–19.40) for the diabetic women and £11.95 (range £10.30–14.50) for the non-diabetics, but the difference was not statistically significant. Dietary fibre content correlated with total cost (r 0·81, $P<0·01$ in diabetics; r 0·73, $P<0·05$ in non-diabetics). There were no other correlations between cost of the diet and macronutrient intakes.

If the diabetic diet is prescribed in terms of carbohydrate then at least 250 g/d is required to achieve current BDA recommendations. Improvements would result from greater emphasis on breakfast cereals, potatoes, bread and fruit, with compensatory reductions in full-fat milk, spreading fats and meat which comprised 39·6% of total fat intake.

Anon (1986). *Shaw's Guide to Fair Retail Prices* (November). Abingdon: Shaw's Price Guides Ltd.
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Diet-induced thermogenesis may be mediated by tryptophan and serotonin. By N. J. FULLER, MARGARET ASHWELL* and D. M. STIRLING, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ* and S. DUNNETT, *Department of Experimental Psychology, Downing Street, Cambridge CB2 2EB* and G. REYNOLDS, *Department of Pathology, University of Nottingham Medical School, Nottingham NG7 2UH*

The involvement of insulin in diet-induced thermogenesis (DIT) may be implicated but the mechanism of its action is not yet fully understood. Insulin acts peripherally leading to increased brain concentrations of tryptophan and serotonin (5-HT). Serotonergic fibres run to the ventromedial hypothalamus, the origin of the sympathetic innervation of brown adipose tissue (BAT).

Male hooded rats (240–300 g) received injections of the tryptophan hydroxylase inhibitor parachlorophenyl alanine (PCPA) into the lateral ventricles of the brain (Breisch *et al.* 1976). They were then fed *ad lib.* or pair-fed with controls. Six days post-operatively the neurotransmitters 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA), noradrenaline (NA) and dopamine (DA) were measured. BAT was removed for measurement of biochemical indices of thermogenesis and morphological assessment. Fat deposition was measured in epididymal white fat pads (WAT).

In both groups of PCPA-treated animals, whole brain levels of 5-HT and 5-HIAA were significantly decreased, to 80–90% of control values, whereas NA and DA showed no significant changes. The Table shows that central administration of PCPA reduces brown fat thermogenesis in both pair-fed and *ad lib.*-fed groups. Deposition of lipid (not shown) in WAT and BAT was significantly increased in both PCPA-treated groups. These results indicate that a decrease in brain 5-HT concentration is associated not only with a decrease in BAT thermogenesis, but also with an increase in fat deposition. It is suggested that (1) the effects of PCPA in decreasing BAT thermogenesis and increasing lipid deposition in WAT and BAT are not due to hyperphagia alone; (2) tryptophan and 5-HT may be mediators in a chain of events linking insulin to DIT.

	PCPA-treated					
	Ad lib.-fed (*n* 7)		Pair-fed (*n* 11)		Control (*n* 11)	
Interscapular BAT	Mean	SEM	Mean	SEM	Mean	SEM
Protein (mg/g)	48***	3	68***	3	91	3
Total cytochrome *c* oxidase (*EC* 1.9.3.1; μmol enzyme oxidized/min)	131*	17	130*	4	195	21
GDP binding (pmol/mg mitochondrial protein)	164*	22	178*	34	335	39
Uncoupling protein (μg/mg mitochondrial protein)	14*	3	17*	3	27	3
Fat-cell diameter† (μm)	37·1***	2·3	32·6***	1·0	28·2	0·6
Fat-cell weight† (ng)	29**	6	23*	3	13	1
% Multilocular cells	34***	5	56	5	77	3

*$P<0.05$, **$P<0.01$, ***$P<0.001$.
†Dorso-cervical BAT.

Breisch, S. T., Zemlan, F. P. & Hoebel, B. G. (1976). *Science* **192**, 382–385.

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Intestinal permeability in man: effect of total and partial short-term starvation. By M. ELIA and C. A. NORTHROP, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ* and R. BEHRENS, *London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT* and G. NEALE, *Addenbrooke's Hospital, Cambridge CB2 1QE*

Intestinal permeability tests with non-metabolizable markers such as mannitol, lactulose and [^{51}Cr]EDTA (5μCi), have been used in clinical practice to screen for the presence of enteropathies and to monitor the effectiveness of treatment of small-intestinal diseases such as coeliac disease and Crohn's disease. Since intestinal diseases are often associated with anorexia we have assessed the effects of total short-term starvation and of very low-energy diets on intestinal permeability.

An oral dosing solution containing a mixture of mannitol (5 g and 0·5 μCi ^{14}C), lactulose (10 g) and ^{51}Cr-labelled EDTA was given to three groups of subjects and urine collected over the next 6 h. The groups were (1) lean subjects (*n* 5) undergoing total starvation for 4–5 d, (2) obese subjects (*n* 4) undergoing total starvation for 5 d, and (3) obese subjects (*n* 5) on a very low-energy diet (VLED; 1255 kJ (300 kcal)/d) for 1 week, and then total starvation for 5 d. Creatinine clearance and the time taken for the fastest component of the test solution to pass from mouth to caecum (measured by the increase of hydrogen in breath) were assessed in all subjects. Plasma and renal clearance of the markers and oxidation of [^{14}C]mannitol to carbon dioxide was assessed in normal and group 2 subjects by intravenous administration of the markers. The passage of markers through various segments of the gastrointestinal tract was monitored by a radioactive scanning technique in group 2 subjects only.

There was no significant change in the uptake of lactulose or [^{51}Cr]EDTA in any of the groups (Table). This is in contrast to increased uptake of these markers observed in diseases of the small intestine and after administration of prostaglandin inhibitors. However, total starvation in both lean and obese subjects produced a reduction in mannitol absorption and excretion (Table). This change in mannitol absorption was not due to an alteration in intestinal transit time, nor to a change in the distribution, volume and clearance of mannitol by the kidney. It was also not due to a change in the oxidation of mannitol which was estimated to account for about 1% of the dose. This selective decrease in mannitol absorption observed during short-term total starvation, but not in subjects receiving a VLED for 1 week, may be due to a reduction in mucosal mass. The abnormal permeability tests associated with decreases of the small intestine are unlikely to be due to the short-term effects of partial starvation.

Effect of total starvation and of very low-energy diets on the 6 h urinary excretion of mannitol, lactulose and [^{51}Cr]EDTA

	Lactulose (% dose)	Mannitol (% dose)		[^{51}Cr]EDTA (% dose)
Group 1 (lean subjects):				
Before starvation	0·293	9·88	$P<0·025$	0·34
After 4 d of total starvation	0·250	5·21		0·33
Group 2 (obese subjects):				
Before starvation	0·180	11·56	$P<0·05$	0·311
After 5 d of total starvation	0·245	7·67		0·366
Group 3 (obese subjects):				
Before starvation	0·323	17·2		0·42
After 7 d on VLED	0·249	16·0	$P>0·05$	0·33
After 5 d of total starvation	0·290	10·2		0·46

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Folate–zinc interaction in pregnant and lactating rats, fetuses and pups. By N. J. FULLER, P. H. EVANS, M. HOWLETT and C. J. BATES, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

Inhibition of zinc absorption by pteroylmonoglutamic acid (PGA) has been reported (Milne *et al.* 1984; Ghishan *et al.* 1986; Simmer *et al.* 1987). In the present study, changes in tissue Zn or fetal growth were sought in rats fed on two levels of PGA and Zn during reproduction.

Before mating (3 weeks), two groups of female rats were fed on diets containing 6·6 μg Zn/g and two groups on diets containing 20·2 μg Zn/g. One low- and one high-Zn group had no supplementary PGA; one low- and one high-Zn group received 100 μg PGA/g diet. Half of each group were killed on day 20 of gestation; the others (dams and pups) on day 20 post-partum. Selected gestational indices are shown in the Table.

	Pregnant dams (*n* 9)							
	Blood PGA (ng/ml)		Liver Zn (μg/g)		Kidney Zn (μg/g)		20 day fetal wt (g)	
Group	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
−PGA−Zn (A)	97[a]	8	18·1	0·6	15·9	1·8	2·66	0·10
+PGA−Zn (B)	412[b]	24	18·1	0·7	20·8	2·7	3·31	0·27
−PGA+Zn (C)	165[c]	8	19·1	0·4	19·7	0·8	2·78	0·07
+PGA+Zn (D)	446[b]	10	20·2	1·0	30·6	2·1	3·32	0·27

[a–c] Mean blood PGA values with unlike superscript letters were significantly different: $P<0·001$.

Liver Zn: A+B *v.* C+D, ($P<0·05$); kidney Zn: A+B *v.* C+D, ($P<0·005$); A+C *v.* B+D, ($P<0·001$); fetal wt: A+C *v.* B+D, ($P<0·005$).

As expected, supplementary PGA produced a large increase in whole blood and plasma PGA but in addition, supplementary Zn raised blood PGA levels in the absence of extra PGA. Supplementary PGA did not affect plasma or liver Zn in dams or pups, but it increased Zn in kidneys of pregnant dams. Zn *per se* had a small but significant enhancing effect on Zn in livers of pregnant dams and pups, in kidneys of pregnant and lactating dams, and in whole fetuses. PGA *per se* had a significant enhancing effect on fetal mean weight; Zn did not.

In conclusion, PGA supplementation did not result in a detrimental effect on tissue Zn, or on other indices, in the present study.

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Effect of rumen-protected methionine and lysine on milk production from cows given grass silage diets. By C. P. GIRDLER, P. C. THOMAS and D. G. CHAMBERLAIN, *Hannah Research Institute, Kirkhill, Ayr KA6 5HL*

Two experiments were conducted to investigate the effects of 'rumen-protected' L-lysine (LYS) and DL-methionine (MET) supplements (Papas *et al.* 1984) on milk production from Friesian cows given diets containing approximately 650–700 g silage dry matter (DM)/kg DM. In Expt 1, eight cows were used in a duplicated 4 × 4 Latin square design with 4-week periods. Cows were given a basal diet (24 g nitrogen/kg DM) *ad lib.* and approximately 1·1 kg DM/d of supplements containing barley (B; 17 g N/d), a low rumen-degradability protein source containing fish meal, blood meal and meat and bone meal (P; 128 g N/d; 700 g undegraded N/kg total N) or one of two mixtures of barley and rumen-protected amino acids (A1 providing 12 g MET and 18 g LYS/d; A2 providing 12 g MET and 36 g LYS/d). In Expt 2, twelve cows were used in a cyclic change-over experiment with six treatments and four 3-week periods. They were given a basal diet (23·2 g N/kg DM) *ad lib.* and approximately 1·7 kg DM/d of supplements of barley (B; 27 g N/d), soya-bean meal (S; 138 g N/d) and barley and protein P (P; 138 g N/d). Each supplement was given with or without A2 rumen-protected amino acids.

As shown in the Table, treatment P increased milk yield and reduced milk fat content relative to treatment B, and similar though smaller effects on milk yield were evident relative to treatment S in Expt 2. A1 and A2 supplements had no significant effect on milk yield but depending on the basal diet tended to increase milk protein content or fat content or both, and for some diets these effects were significant ($P<0·05$).

	Treatment						
Expt 1:	B	A1	A2	P			SED
DM intake (kg/d)	17·01	16·71	16·32	17·17			0·43
Milk yield (kg/d)	$19·84^{b}$	$19·02^{b}$	$19·41^{b}$	$23·04^{a}$			0·66
Fat (g/kg)	$43·0^{ab}$	$46·3^{bc}$	$48·6^{c}$	$41·2^{a}$			1·70
Protein (g/kg)	32·9	33·5	34·0	32·9			0·40
Expt 2:	B	BA2	S	SA2	P	PA2	SED
DM intake (kg/d)	$15·40^{a}$	$15·50^{ab}$	$16·35^{bc}$	$16·57^{c}$	$16·23^{abc}$	$17·06^{c}$	0·45
Milk yield (kg/d)	$19·35^{a}$	$19·66^{a}$	$22·32^{bc}$	$21·53^{b}$	$23·44^{c}$	$23·25^{c}$	0·59
Fat (g/kg)	43·8	45·0	41·5	43·2	42·1	41·4	1·70
Protein (g/kg)	$29·3^{ab}$	$28·8^{a}$	$30·8^{cd}$	$31·7^{de}$	$30·1^{bc}$	$32·1^{e}$	0·57

Means with unlike superscript letters are significantly different: $P<0·05$.

Papas, A. M., Sniffen, C. J. & Muscato, T. V. (1984). *Journal of Dairy Science* **67**, 545–552.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press

Tolerance to the thermogenic effect of caffeine in active adult rats. By J. DÉCOMBAZ and H. G. ANATHARAMAN-BARR, *Nestlé Research Centre, Nestec Ltd, Verschez-les-Blanc, CH-1000 Lausanne 26, Switzerland*

Caffeine (C) has long been known to increase metabolic rate, therefore it has been thought to be of value in stimulating the loss of body energy in the long-term control of energy balance, although evidence for this is lacking. In a previous study using rats, no benefit of repeated C administration on overall energy balance was found (Décombaz *et al.* 1985). To understand this, the present work investigates the influence of repeated, single daily administration of C on energy expenditure (EE) over a full day.

Male Sprague-Dawley rats (420 g), six per group, were housed in cages with a wheel-running facility. C (10 mg/kg) or water (W) was given by mouth at 10.00 hours for twenty-four consecutive days. EE was measured by indirect calorimetry on days 2 and 24.

The animals ate similar amounts of food. At the end of the experiment, there was no significant difference between W and C in either body-weight or live weight fat content (88 and 89 g fat/kg body-weight). The immediate rise in EE following C lasted 6 h (Table) and was equal to 22 kJ, theoretically equivalent to 13·4 g body fat loss had this rise been reproduced daily over the entire experiment. On day 24, the immediate thermogenic effect was only half as much (+ 23%) and significant ($P<0·05$) only for 2 h after administration. In addition, wheel running late at night was reduced in C rats ($P<0·05$), contributing to lower EE, so that overall 24 h EE was not different from that of the controls. A theoretical estimate of fat loss over the experiment based on two intermediate 24 h EE measurements amounted to 2·2 g/24 d (observed difference: 1·3 g, not significant).

Average oxygen consumption (ml/min per kg body-weight $^{0·75}$)

	Water			Caffeine			Difference	Statistical significance:
	Mean	SEM	*n*	Mean	SEM	*n*	(%)	*P*<
Day 2								
First 6 h	15·7	0·5	5	21·3	1·7	6	+36	0·01
Last 6 h	16·1	0·7	5	15·3	0·6	6	−5	NS
Full day	15·5	0·6	5	16·5	0·6	6	+6	NS
Day 24								
First 6 h	15·8	0·5	5	18·6	1·4	6	+18	NS
Last 6 h	17·9	0·5	5	15·5	0·5	6	−13	0·01
Full day	17·2	0·4	5	17·4	1·0	6	+1	NS

NS, not significant.

These results provide evidence that, in the long term, the thermogenic effect of C is negated by two processes: (1) tolerance, and (2) a behavioural change in the distribution of EE within a day. These might limit the potential benefits of C in weight-reducing regimens.

Décombaz, J., Vallotton, F. & Ballenegger, B. (1985). In *Die Verwertung der Nahrungsenergie durch Mensch und Tier*, pp. 90–91 [H. Bickel, editor]. Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH.

https://doi.org/10.1079/PNS19880013 Published online by Cambridge University Press