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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Sixty-first Meeting of the Nutrition Society was held in the Liverpool Medical Institution, 116 Mount Pleasant, Liverpool on Thursday and Friday, 6/7 April 1989, when the following papers were read:

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Failure to detect hypermetabolism in elderly mental patients suffering acute wasting. By A. M. PRENTICE, KAY LEAVESLEY, P. R. MURGATROYD and W. A. COWARD, *Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*, and C. J. SCHORAH, *Department of Chemical Pathology, Old Medical School, Leeds* and P. BLAYDON and R. P. HULLIN, *Regional Metabolic Research Unit, High Royds Hospital, Menston, Yorkshire*

Many chronically ill mental patients, particularly those with Alzheimer's disease, show rapid and severe weight loss which can lead to extreme nutritional debilitation. A number of studies have claimed that food intake was adequate in such patients and there was no evidence of malabsorption in the only study which has tested for it (Singh *et al.* 1986). The present study used the doubly-labelled water ($^2H_2{}^{18}O$) method to test the hypothesis that the wasting was caused by excessive energy requirements secondary to hypermetabolism or hyperactivity.

Total energy expenditure (TEE) over 18–20 d and energy intake (EI) over 7 d were simultaneously measured in fourteen female long-stay mental patients suffering from advanced dementia (five probable Alzheimer's disease) or depression (age 79 (SE 9) years, weight 45·3 (SE 5·5) kg, height 1·54 (SE 0·10) m, body mass index 19 (SE 2) kg/m^2, lean body mass (^{2}H dilution) 33·8 (SE 4·0) kg, fat 11·6 (SE 4·2) kg or 250 (SE 8) g/kg). Recent weight loss or a clinical assessment of deteriorating nutritional status were the major selection criteria. All patients were free from acute infections at the time of study. Food intake, by the 7 d weighed intake method, was measured by a dietitian with careful assessment of all wastage. Resting metabolic rate (RMR) was measured using a ventilated hood when the patients were asleep.

	kJ/d				kJ/kg per d		
	Mean	SD	Range		Mean	SD	Range
RMR	4058	460	3400	4760	118*	13	98–138*
TEE	6095	1353	4485	9627	135	30	98–196
TEE−RMR	2033	1303	328	5347	45	28	7–109
EI	6300	1278	4180	9167	141	31	89–186
Balance (EI−TEE)	205	892	−1042	1945	6	21	−22– 51

*Expressed per kg lean body mass.

The Table shows that in spite of the very low food intake (6·3 MJ/d) the group as a whole were in slight positive energy balance since energy expenditure averaged only 6·1 MJ/d. RMR was significantly lower than predicted from standard tables (−0·4 MJ/d, $P<0·01$). Energy expended as activity and thermogenesis (TEE−RMR) was also low. None of the subjects showed significant negative energy balance after allowing for the known imprecision (±1·2 MJ) in calculating EI minus TEE.

We conclude that these patients were in satisfactory energy equilibrium at the time of study and did not display hypermetabolism. Previous weight loss may therefore have occurred either as an adaptive response to an energy intake which was inadequate for their higher body-weight or during episodes of infection.

Singh, S., Johnson, A. W., Mulley, G. P. & Losowsky, M. S. (1986). *Proceedings of the Nutrition Society* **45**, 85A.

The metabolic rate of oxen after work. By P. R. LAWRENCE, S. F. BUCK and I. CAMPBELL, *Centre for Tropical Veterinary Medicine, Roslin, Midlothian EH25 9RG*

Three well trained Brahman × Friesian oxen (5 years old, live weights 615–750 kg) were given a straw-based pelleted diet at levels calculated to provide either 1·4 × maintenance (high level) or 0·7 × maintenance (low level).

After a 2 week pre-feeding and acclimatization period, the energy expenditure, calculated from gaseous exchange, of each ox at both levels of feeding was determined using an open circuit respiration chamber. Measurements were taken for 48 h except for 15-min breaks after 7, 24 and 31 h while the animal was fed and the chamber cleaned. During the first 7 h of the 3rd day, the ox worked for 6 h pulling a loading device round a circular track. Average energy expenditure of the oxen during work was 3·0 times the expenditure during the same time of the day when they were not working, i.e. about 3·4 times their calculated maintenance metabolic rate. After work the ox was led back to the respiration chamber where its energy expenditure was monitored for a further 65 h.

	High-level feeding						Low-level feeding					
Ox no. . . .	1	1	2	2	3	3	1	1	2	2	3	3
17 h MR on work days (watts)	906	881	958	959	958	910	743	724	759	725	776	732
17 h MR on other days (watts)												
Mean	882	884	982	958	940	892	684	653	717	684	692	693
SD	34	34	32	36	4	38	8	18	10	8	8	6
Difference (watts)	24	–3	–29	1	18	18	59	71	42	41	84	39
Difference (%)	2·8	–0·3	–2·4	0·1	1·9	2·0	8·6	10·9	5·9	6·6	12·1	5.7

At the high level of feeding, the metabolic rate (MR) in watts during the 17 h after work (17 h MR) was much the same as the average value during the same period of the 2 d before and after the work day. However, at the low level of feeding, the MR was on average 8·2% higher. A two-way analysis of variance showed this difference to be significant ($P<0·01$). Similar calculations for the 8 h after work showed a difference of 9·1%.

A possible explanation for the difference between the two diets could be that at the low level of feeding oxen have to use up more of their energy reserves during work than they do at the high level. Resynthesis of these reserves after work may account for the extra energy expenditure. This theory is also consistent with the findings of Goldberg *et al.* (1989) whose human subjects fed at maintenance showed an increase in resting metabolic rate after work which depended on the amount of work done during the day.

Goldberg, G. R., Murgatroyd, P. R., Davies, H. L. & Prentice, A. M. (1989). *Proceedings of the Nutrition Society* **48**, 129A.

The underlying 'resting' energy consumption of oxen during work. By P. R. LAWRENCE, R. SOSA and I. CAMPBELL, *Centre for Tropical Veterinary Medicine, Roslin, Midlothian EH25 9RG*

Attempts have been made to calculate the energy oxen use for work according to the amount and type of work done (Lawrence, 1985). In order to calculate the total energy expenditure of such animals, estimates are also needed of the underlying 'resting' rate of energy expenditure. One way to do this is simply to measure energy expenditure while the ox is standing still between bouts of work, another is to measure energy expenditure at various levels of work and extrapolate back to 'zero work'.

Three well trained Brahman × Friesian oxen (5 years old, live weight 710–855 kg) were fed on a straw-based pelleted diet calculated to meet maintenance energy requirements. On two occasions the rate of energy consumption of each ox, as calculated from its gaseous exchange, was determined for 5 d while the animal was in a closed circuit respiration chamber except for the period from 09.00 to 16.00 hours on the 3rd day. During this time the ox pulled a load (mean value 327 (SD 21·7) N) round a circular track at different speeds in the range 0·6–1·3 m/s. Each speed was maintained for 20 min and after four or five sessions, the ox stood still for 20 min. Rates of energy consumption were calculated from the gaseous exchange during the last 10 min of both the working and standing periods and expressed as a multiple of the average rates during the corresponding times of day on the 4 d when the animal was in the respiration chamber.

Ox no.	Regression equation	r	'Standing' energy consumption	
			Mean	SD
1	$y = 2{\cdot}43x + 1{\cdot}14$	+0·96	1·28	0·04
2	$y = 2{\cdot}08x + 1{\cdot}08$	+0·97	1·30	0·10
3	$y = 2{\cdot}75x + 0{\cdot}91$	+0·92	1·21	0·06

For each equation, the number of results is 27, y is the rate of energy consumption as a multiple of the resting rate on non-working days and x is speed (m/s). The 'standing' energy consumption is expressed in the same units as y and each value is the average of six separate determinations.

The regressions of speed on energy consumption for the three oxen were not significantly different and combined gave $y = 2{\cdot}42x + 1{\cdot}04$ (n 81, r +0·90). This implies that the underlying 'resting' metabolic rate of working oxen during work is the same or slightly higher (+4%) than the rate during the same time of day on non-working days and the average 'standing' value was 26% higher. Since it is often taken as the baseline for experimental determinations of the energy costs of walking and working in animals, this latter value has important implications for calculations of total energy expenditure.

Lawrence, P. R. (1985). In *Draught Animal Power for Production, ACIAR Proceedings series* no. 10. Queensland, Australia: James Cook University.

Glucose utilization rates and the thermogenic effect of infused glucose in critical illness. By CERI J. GREEN, CATHY J. REGAN, ELLEN O'SULLIVAN, S. UNDERHILL, ALISON M. CLEGG, D. P. M. MACLAREN and I. T. CAMPBELL, *Intensive Therapy Unit and University Department of Anaesthesia, Royal Liverpool Hospital, Liverpool L69 3BX*

There is evidence that the utilization of energy substrate in trauma and sepsis is abnormal. The hyperglycaemic glucose clamp is a useful technique for observing the metabolic response to glucose infusion under controlled conditions (DeFronzo *et al.* 1979). We have applied this technique to a group of critically ill, ventilated patients with failure of one or more organ systems to determine whether glucose utilization rates bore any relation to severity of illness.

Fifteen ventilated, intensive care patients (seven male, eight female) with an age range of 18–76 (median 66) years, sepsis scores (Elebute & Stoner, 1983) of between 8 and 21 (median 15) and APACHE 2 scores (Knaus *et al.* 1985) of 11–32 (median 16) were studied. Seven healthy volunteers, (five male, two female), aged 23–55 (median 51) years were also studied resting in bed. All patients were fed intravenously and this was discontinued at 18.00 hours of the evening before the experiment. Patients were permitted a glucose solution (50 g/l) as part of their fluid requirements until 06.00 hours on the morning of study. The volunteers were also fasted overnight. All patients had *in situ* arterial lines and central venous catheters which were used for blood sampling and glucose infusion. For the control subjects, a cannula was placed retrogradely in a dorsal hand vein and the heated hand-box technique used to arterialize the blood. An additional cannula was placed in the other arm for infusion of glucose.

A 30 min control period was followed by a priming infusion of glucose (200 g/l) to acutely raise blood glucose concentration to 12 mmol/l. Blood glucose was maintained at 12 mmol/l by measuring blood glucose levels at 5-min intervals and altering the infusion rate in accordance with the algorithm of DeFronzo *et al.* (1979). Oxygen consumption ($\dot{V}_{O_2}$) measurements were made continuously for the duration of the experiment. An Engstrom metabolic computer (Gambro Engstrom AB, Bromma, Sweden) was used to measure $\dot{V}_{O_2}$ of the patients, and a ventilated hood technique for the controls.

During the clamp procedure, glucose was maintained close to 12 mmol/l in both groups: in patients 12·1 (SD 0·5) mmol/l, and in controls 12·2 (SD 0·4) mmol/l. Glucose utilization rates were calculated over 20-min periods.

	Baseline blood glucose (mmol/l)		Glucose utilization rates (μmol/kg per min)				Energy expenditure (kJ/m² per h)			
			40–60 min infusion		160–180 min infusion		Pre-infusion		180 min infusion	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Patients	5·0	0·3	35·0	3·8	30·8	4·8	168	7·9	171	8·2
Controls	4·4	0·2	28·3	3·7	60·1*	6·5	157	4·2	179†	4·2

*Significant difference compared with patients: $P<0·01$.
†Significant difference compared with pre-infusion: $P<0·01$.

It appears that critically ill patients demonstrate a similar response to infused glucose as that previously described in septic surgical patients (White *et al.* 1987), with no increase in rate of glucose utilization and absence of glucose-induced thermogenesis.

DeFronzo, R. A., Tobin, J. D. & Andres, R. (1979). *American Journal of Physiology* **237**, E214–E223.
Elebute, E. A. & Stoner, H. H. (1983). *British Journal of Surgery* **70**, 29–31.
Knaus, W. A., Draper, E. Q., Wagner, D. P. & Zimmerman, J. E. (1985). *Critical Care Medicine* **13**, 818–829.
White, R. H., Frayn, K. N., Threlfall, C. J., Stoner, H. B. & Irving, M. H. (1987). *Journal of Parenteral and Enteral Nutrition* **11**, 345–353.

High-energy feeding in infants with congenital heart disease. By M. JACKSON and E. M. E. POSKITT, *Institute of Child Health, University of Liverpool, Royal Liverpool Children's Hospital, Liverpool L12 2AP*

Infants with congenital heart disease (CHD) who fail to thrive have both low energy intake and elevated energy expenditure compared with control infants (Menon & Poskitt, 1985). We have studied the effects of increased energy-dense feeds on energy balance, resting oxygen consumption ($\dot{V}_{O_2}$) and weight gain of CHD infants with failure to thrive.

Fourteen infants with CHD (three male, eleven female; mean age 0·23 (SE 0·2) years) were studied by 3 d energy balance on standard feeds and feeds supplemented to approximately 125% standard energy content with glucose polymer (Caloreen; Roussel). Energy contents of feeds, vomit, stools and urine were determined by bomb calorimetry. Respiratory gas exchange was measured over 7·5 h during each balance period by an open circuit system using a paramagnetic O_2 analyser (Servomex) and infra-red carbon dioxide analyser (PK Morgan).

Energy balance, respiratory gas exchange and weight gain in infants fed on normal and supplemented formulas

	Age (years)		Body-wt (kg)		Gross energy intake (kJ/kg per d)		Losses in stools, urine, vomit (kJ/d)		Resting $\dot{V}_{O_2}$ (ml/kg per min)		Respiratory quotient		Spare energy (kJ/d)		Wt gain (g/d)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Controls*	0·17	0·02	3·55	0·55	563	39	296	55	9·3	0·4	0·83	0·01	634	163	34·8	6·4
Normal formula	0·21	0·03	3·18	0·14	440	23	229	33	10·5	0·5	0·79	0·02	62	74	4·3	2·5
Supplemented formula	0·24	0·03	3·34	0·15	580	29	231	36	10·5	0·4	0·88	0·02	520	92	18·7	3·6

*Menon & Poskitt (1985).

The Table shows that energy intakes of CHD infants on normal feeds were low compared with those expected for age, weight and other values in control infants without CHD studied previously (Menon & Poskitt, 1985). Energy losses and mean resting $\dot{V}_{O_2}$ did not increase on high-energy feeds, although respiratory quotient rose with the increased dietary carbohydrate. Energy estimated available for growth ('spare') was increased on supplementation and correlated with weight gain (r 0·84, $P \leq 0{\cdot}01$).

Energy deficiency resulting from low-energy intakes and high-energy expenditure can explain poor growth in CHD infants. These infants tolerate energy-dense feeds with preservation of metabolic efficiency. Weight gain is improved but not enough for catch up growth. CHD infants need dietary supplementation before failure to thrive is established.

Menon, G. & Poskitt, E. M. E. (1985). *Archives of Disease in Childhood* **60**, 1134–1139.

Effects of dietary lactose or sucrose on tissue oxidative metabolic capacity in male and female rats. By HEATHER E. BRISTOW, J. J. STRAIN and R. W. WELCH, *Biomedical Sciences Research Centre, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB*

Ilback *et al.* (1988) have shown that different carbohydrate sources can influence myocardial metabolism in chicks, with fructose-containing diets resulting in lower activities of lactate dehydrogenase (LDH, *EC* 1.1.1.27) and cytochrome *c* oxidase (CCO, *EC* 1.9.3.1) compared with diets containing sucrose or glucose. Recent work has suggested that lactose combined with sucrose might result in increased mitochondrial oxidative metabolic capacity in the rat compared with sucrose consumption alone (Lynch & Strain, 1989). Since there is some evidence to indicate that females may be more exposed to disorders due to galactose consumption than males (Gona & Fu, 1982), the current study investigated the effects of lactose- or sucrose-containing diets on oxidative metabolic capacity in body tissues of male and female rats.

Two groups (*n* 6) of female weanling Sprague-Dawley rats were housed individually and were pair-fed on diets which contained (1) lactose (290 g/kg) and starch (290 g/kg) or (2) sucrose (290 g/kg) and starch (290 g/kg) as the carbohydrate source. Two groups (*n* 6) of male weanlings were treated similarly. After 42 d all rats were anaesthetized and killed by exsanguination, whereupon the livers, kidneys and hearts were removed and weighed. Tissue homogenates were assayed for the activities of CCO, LDH, isocitrate dehydrogenase (ICDH, *EC* 1.1.1.41) and citrate synthase (CS, *EC* 4.1.3.7).

A two-way analysis of variance was carried out, using sex and carbohydrate as main effects. Only kidney CS was significantly affected by carbohydrate source, with lactose-fed rats exhibiting a greater activity than their sucrose-fed counterparts. Cardiac CS and ICDH were the only enzymes which were significantly different with respect to sex, these enzyme activities being significantly lower in the cardiac tissue of females. Sex × carbohydrate source interactions were all non-significant.

Apart from kidney CS activity there was little evidence that lactose compared with sucrose consumption resulted in greater tissue oxidative metabolic capacity. The lower CS and ICDH levels, however, would suggest a decreased oxidative metabolic capacity in female cardiac tissue compared with males.

	Enzyme activity (units/mg protein)	Lactose				Sucrose			
		Female		Male		Female		Male	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver	CCO	9·8	3·4	15	5·1	34	18	18	7·8
	CS	27	2·9	20	2·6	32	5·1	28	3·8
	ICDH	2·3	0·26	1·7	0·26	2·2	0·24	2·0	0·23
	LDH	1·7	0·25	1·8	0·18	2·0	0·16	2·1	0·18
Heart	CCO	0·93	0·20	0·49	0·09	0·54	0·10	0·45	0·07
	CS*	6·2	0·76	13	2·2	11	3·7	21	6·3
	ICDH*	0·73	0·07	1·3	0·19	1·1	0·17	1·6	0·35
	LDH	0·93	0·04	1·1	0·05	0·96	0·07	0·88	0·09
Kidney	CCO	0·64	0·02	0·54	0·08	0·69	0·09	0·61	0·06
	CS†	30	5·9	24	5·4	14	3·8	16	2·8
	ICDH	1·4	0·31	1·7	0·24	1·5	0·19	1·2	0·13
	LDH	0·33	0·02	0·29	0·03	0·27	0·02	0·29	0·03

*Significant difference between sexes ($P<0·05$).

†Significant difference between carbohydrate source ($P<0·05$).

Gona, O. & Fu, S. C. J. (1982). *Proceedings of the Society of Experimental and Biological Medicine* **171**, 285–288.

Ilback, N.-G., Squibb, R. L. & Friman, G. (1988). *Nutrition Research* **8**, 539–547.

Lynch, S. M. & Strain, J. J. (1989). *British Journal of Nutrition* **61**, 345–354.

Enhancement of biliary copper excretion in sheep. By H. W. SYMONDS and S. C. CORLETT, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Ammonium tetrathiomolybdate (TTM) can be used systemically to treat copper toxicity in sheep because it increases the rate of loss of Cu from the liver and increases excretion into bile. Biliary excretion is enchanced further if the TTM is given in combination with the α_2 agonist xylazine (Rompun; Bayer Agrochemicals). The effect is blocked by the α_2 antagonist idazoxan (Symonds & Ke, 1989). The effectiveness of TTM is also enhanced by pentobarbitone or halothane general anaesthesia (Ke & Symonds, 1989). We report preliminary observations made to determine whether other α_2 agonists, in particular clonidine, also enhance biliary Cu excretion and whether the anaesthetic-induced enhancement is also an α_2 agonistic effect.

Four male, mule sheep weighing 46 to 50 kg, surgically prepared to allow bile collection and fed on approximately 1·4 kg Green Keil nuts and 250 g hay daily were used. Liver Cu content ranged between 571 and 1469 mg/kg dry matter. Biliary Cu excretion was measured during consecutive 30 min periods, while maintaining the enterohepatic circulation of bile salts, for a control period of 1·5 h before, and for 4·5 h after, intravenous dosing with 50 mg TTM, 0·75 mg clonidine or a combination of both. General anaesthesia was induced in two sheep with thiopentone and maintained with halothane, and the effect of 40 mg idazoxan on the TTM-induced Cu excretion measured. The treatment schedules were in the order given in the Table, alternating between sheep. A minimum of 3 d were allowed between any treatment. Values for Cu excretion in bile are given in the Table. Sheep were conscious during the anaesthetic control periods.

		Control (μg/h)		Cu above control value during 4·5 h post-treatment (μg/h)		
	n	Mean	SE	Mean	SE	(%)
0·75 mg Clonidine	4	11·8	4·9	33·6	8·9	285
50 mg TTM	4	24·0	6·7	54·1*	5·8	225
TTM + clonidine	4	17·3	6·7	88·9†	16·4	514
50 mg TTM conscious	2	23·7		48·6		205
Anaesthesia alone	2	21·5		79·0		367
+ 50 mg TTM	2	25·3		154·5		611
+ TTM + 40 mg idazoxan	2	22·8		117·1		513

Significantly different from clonidine: *$P<0·001$.
Significantly different from TTM: †$P<0·001$.

Clonidine significantly enhanced both biliary Cu excretion and the effectiveness of TTM. Idazoxan produced a partial reduction in the response to TTM by anaesthetized sheep. The increase in excretion was still 2·4-fold that due to TTM in the conscious animals, suggesting that either the enhancement was not due only to stimulation of α_2 receptors or that the dose of idazoxan was insufficient in the face of a continuous, high concentration of anaesthetic.

The idazoxan was a generous gift from Reckitt & Colman, Hull.

Ke, Y. & Symonds, H. W. (1989). *Research in Veterinary Science* (In the Press).
Symonds, H. W. & Ke, Y. (1989). *Research in Veterinary Science* (In the Press).

The effect of D-penicillamine with and without xylazine on copper excretion by sheep. By S. C. CORLETT and H. W. SYMONDS, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

The copper chelator D-penicillamine is used orally to alleviate Cu toxicity associated with Wilson's disease in human patients. Cu removed from the liver is excreted in urine. In sheep ammonium tetrathiomolybdate (TTM) is used to remove Cu from the liver in cases of Cu toxicity. Much of the Cu removed is excreted in bile, urinary excretion being little changed (Ke & Symonds, 1989). Combining treatment with the α_2 agonist xylazine can double the response to TTM, raising the maximum effective dose (Symonds & Ke, 1989). We report preliminary experiments to determine whether D-penicillamine causes an increase in biliary Cu excretion in sheep and whether xylazine has any enhancing effect on its chelating ability.

Six Mule and Suffolk cross sheep (weighing between 45 and 55 kg) were surgically prepared to enable bile collection with minimum interruption of the enterohepatic circulation of the bile salts. They were retained in metabolism crates and fed on a diet of approximately 1·4 kg Green Keil nuts and 250 g hay daily. Hepatic Cu concentrations ranged between 571 and 1469 mg/kg dry weight. Bile flow was measured for consecutive 30 min periods. Each measurement period lasted 6 h consisting of a 1·5 h predosing control period and a 4·5 h period after dosing. The doses used were 5 ml saline (9 g sodium chloride/l) for control, 500 mg D-penicillamine in 5 ml saline and 20 mg xylazine all given intravenously. Urine was collected for 24 h after dosing at 11.00 hours. The Cu excreted in bile and urine (μg) are given in the Table.

	Biliary Cu excretion					
	Control 1·5 h (μg/h)		Post-treatment 4·5 h (μg/h)		Cu excretion in urine (μg/24 h)	
	Mean	SE	Mean	SE	Mean	SE
Control	15·0	3·0	12·7	2·2	146	21
500 mg D-penicillamine	27·2	4·5	19·5**	4·7	807***	87
20 mg Xylazine + D-penicillamine	24·2	4·0	32·1***	3·5	878***	119

Significantly different from control: $^{**}P<0.01$, $^{***}P<0.001$.

Unlike TTM, D-penicillamine produced a small decrease in biliary Cu excretion. The increase, when D-penicillamine was given in combination with xylazine, was no greater than that which would be produced by xylazine alone (Symonds & Ke, 1989). Xylazine had no significant effect on the D-penicillamine-enhanced excretion of Cu into urine. It is proposed that the α_2 agonist acts primarily on the pathways associated with Cu excretion into bile and not at sites where D-penicillamine acts.

The D-penicillamine was a generous gift from the Lilly Research Centre Ltd, Windlesham, Surrey.

Ke, Y. & Symonds, H. W. (1989). *Research in Veterinary Science* (In the Press).
Symonds, H. W. & Ke, Y. (1989). *Research in Veterinary Science* (In the Press).

Influence of homocysteine on copper status in the rat. By J. C. W. BROWN and J. J. STRAIN, *Biomedical Sciences Research Centre, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB*

Sulphur amino acids have been shown to ameliorate copper toxicity in various animals (Baker & Czarnecki-Maulden, 1987) and dietary methionine supplementation can exacerbate Cu deficiency symptoms in rats (Lynch & Strain, 1989). Homocysteine (HCy) is a transulphuration metabolite of methionine catabolism and has been implicated in atherogenesis (Kang *et al.* 1986). In the present study the effect of HCy on Cu status was investigated in the rat.

Two groups (*n* 6) of male weanling Sprague-Dawley rats were provided with deionized water and given diets containing DL-HCy (10 g/kg) and either adequate in Cu (14·0 mg/kg) or low in Cu (1·3 mg/kg). Two control groups (*n* 6) of rats were pair-fed against the respective HCy groups for 42 d.

Activities of the Cu-dependent enzymes cytochrome *c* oxidase (CCO, *EC* 1.9.3.1) and superoxide dismutase (Cu/Zn-SOD, *EC* 1.15.1.1) were measured in the liver and heart. The latter enzyme was also measured in erythrocytes (RBC) while Cu content in the various tissues, haemoglobin (Hb) levels and plasma caeruloplasmin (Cp, *EC* 1.16.3.1) provided further indices of Cu status.

	Controls				HCy				Two-way analysis of variance	
	Cu-adequate		Cu-deficient		Cu-adequate		Cu-deficient		Main effects: *P*<	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Cu	HCy
Blood										
RBC Cu/Zn-SOD (U/mg protein)	1·18	0·10	0·78	0·07	0·63	0·08	0·52	0·07	0·01	0·001
RBC Hb (U/l)	126	5·4	117	14·5	114	3·3	79	11·5	0·05	0·05
Plasma Cu (μg/ml)	1·09	0·10	0·22	0·03	0·31	0·03	0·29	0·04	0·001	0·001
Plasma Cp (U/l)	178·1	18·31	<0·01		11·46	2·98	<0·01		0·001	0·001
Liver										
Cu (μg/g dry wt)	18·36	1·02	10·67	1·45	8·90	1·83	5·90	0·54	0·001	0·001
CCO (U/mg protein)	3·06	0·46	1·75	0·60	1·73	0·29	0·53	0·19	0·01	0·01
Cu/Zn-SOD (U/mg protein)	128·3	13·14	84·2	4·73	81·7	7·38	61·7	5·43	0·01	0·001
Heart										
Cu (μg/g dry wt)	29·18	3·17	13·33	2·23	17·93	3·25	12·66	1·01	0·001	0·05
CCO (U/mg protein)	1·75	0·35	1·05	0·17	1·02	0·17	0·67	0·05	0·05	0·05
Cu/Zn-SOD (U/mg protein)	176·7	14·06	85·0	4·28	85·0	3·87	61·7	4·59	0·001	0·001

These results indicate that both HCy feeding and low Cu diets resulted in a marked lowering of Cu status.

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The effect of in vitro zinc deficiency on Zn and copper ion concentration and intracellular enzyme activity in Molt-3 cells. By G. MAZDAI and B. M. HANNIGAN, *Biomedical Sciences Research Centre, University of Ulster, Coleraine, Co. Londonderry BT52 1SA* and J. J. STRAIN, *Biomedical Sciences Research Centre, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB*

It is well known that zinc is an essential trace element for normal cell growth and function. The metabolism of any one trace element may be closely related to the metabolism of many others. Zn and copper absorption, transport, intracellular binding, uptake and efflux are often inversely related (Cousins, 1985), Zn generally being antagonistic to Cu.

The present study investigated the in vitro relation between intracellular levels of Zn and Cu together with the Cu-dependent enzyme superoxide dismutase (SOD, *EC* 1.15.1.1), and thymidine kinase (TK, *EC* 2.7.1.21) which has Zn as an essential part of its structure, in a human T-lymphoid cell line, Molt-3. Cells were maintained for 30 h in medium (RPMI–1640 + 10% fetal calf serum) with normal (0·2–0·3 μg Zn/ml) or deficient levels of Zn, or with the Zn-deficient medium repleted with Zn (50 μM–$ZnCl_2$). Zn-deficient medium, at 11·2% of the control value, was obtained by overnight dialysis against 0·1 M–2,6-pyridinedicarboxylic acid in Tris buffer, pH 7 at 4°. Cu was not significantly depleted (4% decrease).

Cell growth was comparable in control and $ZnCl_2$-repleted media but was significantly diminished under Zn deficiency. Replacement of Zn ($ZnCl_2$) after 30 h slightly restored cell growth capacity, but very slowly. Intracellular levels of Zn and Cu were measured using atomic absorption spectrometry of lysed cells. Intracellular activities of TK and SOD were also measured.

	per 10^6 cells						
	Zn (ng)	Cu (ng)	Mg (μg)	SOD (IU)	TK*	Zn:Cu	Cell count†
Control							
Mean	17·7	0·77	0·33	0·012	35600	23	2·24
SD					2000		0·046
Zn deficient							
Mean	5·6	0·83	0·05	0·018	31200	6·7	1·63
SD					5000		0·072
Zn repleted							
Mean	12·5	0·27	0·22	0·011	40300	47	2·13
SD					2000		0·031

*Total counts/min after 1 h of incubation with [^{3}H]thymidine as substrate.
†Cell counts (× 10^5/ml) after incubation for 30 h. Initial cell count 1 × 10^5/ml.

The results suggest that extracellular Zn deficiency may perturb intracellular Cu and Zn status. Activity of the Cu-dependent enzyme, SOD, was increased (48%) by a reduced availability of Zn. TK activity was slightly but not significantly decreased with Zn deficiency. Both enzymes showed normal activities when $ZnCl_2$ was present. It is clear that alteration in extracellular levels of Zn alone profoundly influenced uptake of Cu and Mg. Competition for uptake may occur between Zn and Cu, with preferential uptake of Zn. Zn deficiency, with a concomitant decrease in intracellular Zn:Cu ratios may have enhanced SOD activity either by the induction of SOD or by permitting Cu to bind to the apoenzyme more easily.

Any decrease in TK activity may also relate to decreased intracellular Mg levels as this ion is a co-factor for the enzyme. Thus impaired cell growth in Zn deficiency may reflect alterations of a number of ions and dependent enzymes.

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Effect of tumour necrosis factor α on temperature and zinc metabolism in weanling, young, adult and elderly rats. By D. C. BIBBY and R. F. GRIMBLE, *Human Nutrition Department, Southampton University Medical School, Southampton SO9 3TU*

Febrile effects of endotoxins are reduced in both young and elderly animals (Ford & Klugman, 1980; Tocco-Bradley *et al.* 1985). Tumour necrosis factor (TNF) is one of a range of cytokines produced in response to endotoxin and brings about changes in body temperature and zinc metabolism (Bibby & Grimble, 1989). Differences in cytokine production may therefore account for differences in the pyrogenic response. We examined whether age affects the pyrogenic response to TNF in a similar manner as that to endotoxins and whether Zn metabolism is affected in parallel.

Male Wistar rats aged 4, 7, 20 and 80 weeks were caged separately. Rats at each age received intravenous injections of either sterile non-pyrogenic saline (9 g sodium chloride/l) or recombinant human TNF (50 μg/kg, endotoxin content <0·137 ng/mg protein, BASF/Knoll A.G., Ludwigshaven). Temperatures were measured for 8 h after injections, whereupon rats were decapitated and blood and liver collected. Serum and liver Zn were measured by atomic absorption spectroscopy and liver protein by the Lowry method.

Age (weeks) . . .	4		7		20		80	
Injection . . .	Saline	TNF	Saline	TNF	Saline	TNF	Saline	TNF
No. of rats per group	6	6	4	4	6	6	4	4
Temperature°								
Δt_0–t_3 h	−0·1	−0·4	−0·4	0·9**	0	0·5**	−0·3	−0·3
Δt_0–t_6 h	−0·2	−0·4	−0·5	0·2**	−0·3	−0·2	−0·4	−0·3
Serum Zn (μg/ml)	1·82	0·98***	1·94	0·86***	1·94	0·83***	1·69	0·52***
Total liver protein (g)	0·47	0·53	1·06	1·21*	2·35	2·30	3·50	3·18
Liver Zn (μg/g)	41·5	51·9*	39·0	49·5*	35·6	45·7***	33·5	39·3*

t, time after injection.
Significantly different from saline control (ANOVA): *P<0·05, **P<0·01, ***P<0·001.

Although serum and liver Zn contents responded to TNF equally well in all age groups, the pyrogenic effects were not apparent in weanling or elderly rats. Reduced pyrogenic responses of young and elderly animals to endotoxins may therefore not be due to decreased cytokine production but to differences in the sensitivity of pyrogenic mechanisms to cytokines.

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Unidirectional calcium fluxes across the isolated rumen mucosa of goats as affected by 1,25-$(OH)_2D_3$. By G. BREVES[1], G. GÄBEL[2], E. PFEFFER[3] and H. MARTENS[2], [1]*Institute of Animal Nutrition, Federal Agricultural Research Station, D-3300 Braunschweig,* [2]*Department of Physiology, School of Veterinary Medicine, D-3000 Hannover* and [3]*Institute of Animal Nutrition, University of Bonn, D-5300 Bonn, West Germany*

Recent in vitro experiments using isolated ruminal and omasal mucosa of sheep have shown that calcium may be absorbed actively from both organs and that the active transport depends on an intact Na^+,K^+-ATPase and the presence of Na ions (Höller *et al.* 1988*a*,*b*).

It was the aim of the present study to characterize regulatory mechanisms and to detect species differences of active Ca transport. Unidirectional Ca flux rates were measured across the isolated ruminal mucosa of goats under short circuit conditions in Ussing chambers.

Pieces of ruminal mucosa from the ventral sac were mounted between the two halves of Ussing chambers, identical buffer solutions were given to both sides of the tissues and the chambers were run under short circuit conditions. When the initial electrical indices (conductance (G_t) and short circuit current (I_{sc})) differed by less than 20%, two corresponding chambers were paired and 8 μCi $^{45}CaCl_2$ were added either to the mucosal or to the serosal side of the tissue for calculation of unidirectional flux rates. In each chamber two flux periods of 30 min each were run as controls. When they were finished 1,25-$(OH)_2D_3$ was added to the serosal side (115 pg/ml), 20 min were allowed for equilibration and two further flux periods were performed.

In control periods the mean Ca flux rate from mucosal to serosal side (J_{ms}) was about three times as high as in the opposite direction (J_{sm}). Since the resulting Ca net flux (J_{net}) did not differ from corresponding data from the sheep rumen (Höller *et al.* 1988*b*), these results clearly indicate that an active Ca transport system is present in both species.

After addition of 1,25-$(OH)_2D_3$, J_{ms} increased significantly (Table). This resulted in an increased J_{net} since J_{sm} was unaffected. These results suggest a possible regulatory function of 1,25-$(OH)_2D_3$ for active Ca transport across the rumen wall.

*Ca flux rates (nmol/cm² per h), G_t (mS/cm²) and I_{sc} (μEq/cm² per h) from control periods and after addition of 1,25-(OH) $_2D_3$**

	J^{Ca}_{ms}		J^{Ca}_{sm}		J^{Ca}_{net}		G_t		I_{sc}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	15·8[a]	2·0	4·8	1·5	10·9[a]	2·2	2·7	0·6	0·96	0·15
1,25-$(OH)_2D_3$[b]	20·3[b]	2·3	4·5	1·2	15·7[b]	2·6	2·4	0·4	0·84	0·16

[a,b]Values in vertical columns with unlike superscript letters are significantly different (paired *t* test): $P<0·001$.
*Five goats and twenty-five tissues for each flux direction.

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Effect of a specific α_2-antagonist on weight gain of genetically obese Zucker rats. By H. D. MCCARTHY and J. A. CARNIE, *Department of Biochemistry and Applied Molecular Biology, UMIST, Manchester M60 1QD*

In an earlier study, Dulloo & Miller (1984) reported that yohimbine, a noradrenergic α_2-receptor antagonist, when added to the diet of obese Zucker rats, caused a significant reduction in body-weight. A thermogenic effect of the drug was responsible for the weight loss.

Efaroxan (2-[2-(2-ethyl-2,3-dihydrobenzofuranyl)]-2-imidazoline hydrochloride) is a highly specific α_2-antagonist (Chapleo *et al.* 1984). We tested its effect on weight gain, food intake and brown adipose tissue (BAT) thermogenic activity in obese Zucker rats.

Two weight-matched groups (*n* 9 per group) of male obese Zucker rats, aged 6–7 weeks, fed on a standard laboratory chow diet *ad lib.*, were injected subcutaneously twice daily with either efaroxan at 2 mg/kg for 14 d and then 4 mg/kg for 21 d, or with saline (9 g sodium chloride/l). Food intake and body-weight were monitored, and at the end of the experiment animals were killed and the thermogenic activity of the interscapular BAT was assessed.

Mean weight gain was similar for treated (210 (SEM 6·2) g) and control animals (228 (SEM 6·2) g) as was food intake (treated 1154 (SEM 19·4), controls 1181 (SEM 15·1) g). Epididymal fat pad mean weights were identical for both groups (treated 11·35 (SEM 0·39), controls 11·31 (SEM 0·40) g) and BAT mitochondrial GDP binding was unchanged (treated 49·9 (SEM 3·3), controls 47·2 (SEM 6·3) pmol/mg protein). Efaroxan had no significant effect on any of the above indices (Student's *t* test).

These results indicate that efaroxan at the doses used does not stimulate thermogenesis or promote weight loss in obese Zucker rats. The reported effects of yohimbine in this species are therefore unlikely to be mediated via an α_2-adrenoceptor mechanism but are probably related to the fact that, in addition to its α_2-antagonist properties, it exerts marked effects on the central release of 5-hydroxytryptamine and dopamine (Pettibone *et al.* 1985).

We are grateful to Reckitt & Colman, Hull, UK for a kind donation of efaroxan.

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Effects of treatment with 3,5,3′-triiodothyronine and clenbuterol on skeletal and cardiac muscle mass in the rat. By PETER A. MACLENNAN and RICHARD H. T. EDWARDS, *Department of Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX*

Hyperthyroidism induces skeletal muscle wasting (Ramsay, 1966) and cardiac hypertrophy (Holness & Sugden, 1987). Clenbuterol, a β_2 agonist, partially reverses the muscle wasting observed in some pathological conditions (Rothwell & Stock, 1987). We have investigated whether clenbuterol has the ability to oppose the muscle atrophy induced by hyperthyroidism and have examined the effects of the β_2 agonist on cardiac mass in the hyperthyroid state.

Female Wistar rats (114–129 g, four per group) were sham injected or treated daily for 7 d with 3,5,3′-triiodothyronine (T_3) (1 mg/kg), clenbuterol (0·25 mg/kg), or both T_3 and clenbuterol at the above doses. Animals were fed on CRM rat diet (Biosure, Cambs.) *ad lib.* throughout the experimental period. Similar amounts of food were consumed by animals from all four experimental groups.

	Gastrocnemius muscle				Heart			
	Wet wt (mg)		Lactate (μmol/g wet wt)		Wet wt (mg)		Lactate (μmol/g wet wt)	
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sham	759[a]	37	1·82[a]	0·45	531[a]	35	6·79[a]	1·24
T_3	633[b]	51	9·19[c]	1·11	770[c]	46	9·19[a]	1·11
Clenbuterol	978[c]	92	5·20[b]	0·65	593[a]	51	7·52[a]	1·12
T_3 + clenbuterol	661[b]	45	9·00[c]	2·24	876[b]	58	7·35[a]	1·39

[a,b,c] Values in vertical columns with unlike superscript letters are significantly different ($P<0·05$).

Clenbuterol alone caused skeletal muscle hypertrophy and elevated muscle lactate concentrations. However, the β_2 agonist did not oppose the muscle atrophy induced by T_3 and, although muscle lactate concentrations were elevated by hyperthyroidism, no additional increase was induced by clenbuterol.

The lactate concentration of skeletal muscle is an index of the degree of β-adrenoceptor stimulation following clenbuterol treatment (MacLennan & Edwards, 1989). It is thus possible that, in the hyperthyroid state, skeletal muscle was rendered insensitive to both the hypertrophic and β-adrenergic effects of clenbuterol. In contrast, clenbuterol potentiated the cardiac hypertrophy induced by T_3.

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Effects of vitamin E on prostaglandin E_2 production from skeletal muscle. By JOANNE PHOENIX, R. H. T. EDWARDS and M. J. JACKSON, *Department of Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX*

Calcium activation of phospholipase A_2 (PLA_2; *EC* 3.1.1.4) leading to the production of prostaglandins and other metabolites of arachidonic acid has been implicated in the mechanisms of pathological degradation of skeletal muscle (Rodemann *et al.* 1981; Jackson *et al.* 1986). Vitamin E has been shown to inhibit the cytosolic enzyme efflux from muscles treated with Ca ionophore (Phoenix *et al.* 1989) and also reported to inhibit the purified PLA_2 enzyme (Douglas *et al.* 1986). In order to examine whether vitamin E acts by inhibition of phospholipase-mediated prostaglandin E_2 (PGE_2) production, we have studied the effect of α-tocopherol on Ca ionophore (A23187)-stimulated PGE_2 release from control muscles and muscles from animals of differing vitamin E status.

Isolated soleus muscles from female Wistar rats were incubated in oxygenated Ringer solution at 37° for periods of up to 3 h. After 30 min, muscles were treated with A23187 (20 μM), and creatinine kinase (*EC* 2.7.3.2) and PGE_2 release measured. Addition of A23187 induced a significant increase in PGE_2 release from control muscles (51·3 (SE 11·5) pg/30 min per mg wet weight at 30 min post-A23187 treatment *v.* 16·2 (SE 2·7) pre-A23187) and this was unaffected by the addition of 0·23 mM-α-tocopherol to the incubation medium (43·7 (SE 13·6) pg/30 min per mg wet weight 30 min post-A23187 *v.* 16·4 (SE 9) pre-A23187). Vitamin E deficiency was induced in groups of rats (*n* 10) by giving a casein-based tocopherol-deficient diet (Hoffman La-Roche diet no. 814 obtained from Dyets Inc., Pennsylvania); a control group was fed on the same diet supplemented with α-tocopherol acetate (100 μg/g). Plasma PGE_2 concentrations in vitamin E-depleted (E–) rats (258 (SE 36·9) pg/ml) were not significantly different from vitamin E-supplemented (E+) rats (E+: 229 (SE 32·6) pg/ml) although plasma α-tocopherol concentrations were significantly lower (E+: 14·9 (SE 0·9) μg α-tocopherol/ml *v.* E–: not detectable). Tissue α-tocopherol contents were significantly reduced in the E− rats (liver, E+: 198 (SE 16) μg α-tocopherol/g wet wt *v.* E–: 28 (SE 6); muscle, E+: 25·6 (SE 1·7) *v.* E–: 1·3 (SE 0·7)), but isolated soleus muscles from these groups of animals did not show significantly different PGE_2 release (E–: 44·7 (SE 4·3) *v.* E+: 49 (SE 3·0) pg/30 min per mg wet weight muscle 30 min post-A23187).

These results do not support the hypothesis that vitamin E exerts a physiological regulation of PLA_2-mediated release of PGE_2 from skeletal muscle.

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The effect of acute food deprivation on contractile characteristics of rat skeletal muscles.

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Acute food restriction has been shown to result in slowing of relaxation rate and shifts in the force-frequency characteristics of rat gastrocnemius muscle, a muscle of mixed fibre types (Russell *et al.* 1984). The aim of the present study was to examine the influence of acute food deprivation on the contractile characteristics of skeletal muscles selected to reflect the differing muscle fibre types: soleus (type I), extensor digitorum longus (EDL: type IIb) and tibialis anterior (TA: Type IIa/b).

Muscle function was assessed *in situ* under Sagatal anaesthesia in male Wistar rats (initial mean weight: 184 (SE 3) g) after an overnight fast (12 h) and following 5 d of food deprivation with free access to water. Contractile characteristics, elicited via supra-maximal stimulation of the sciatic nerve, were assessed during single twitches (1 Hz) and during stimuli of increasing frequency up to 200 Hz using a microcomputer.

Significantly lower body-weight (20%; $P<0.01$), EDL weight (26%; $P<0.01$) and TA weight (11%; $P<0.05$) were observed following the 5 d fast, whilst the weight of the soleus muscle was similar in both groups. No changes in muscle protein concentration were detected. The changes in muscle weight, time to peak tension (TPT), half relaxation time ($RT_{1/2}$), peak twitch tension (PT) and maximal tetanic force (F_{max}) are shown in the Table.

			Muscle wt (mg)		TPT (ms)		$RT_{1/2}$ (ms)		PT (N/g muscle)		F_{max} (N/g muscle)	
Muscle	Period of fast (d)	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Soleus	0	10	71·8	4·3	31·6	1·9	53·4	4·3	72·6	32·4	665·4	129·3
	5	10	70·9	5·1	27·6	1·9	46·3*	3·6	190·3	34·4	830·4	161·9
EDL	0	10	78·2	4·6	14·4	0·5	13·3	0·6	258·3	37·6	846·8	56·7
	5	10	57·6**	6·3	14·0	0·7	12·0	0·4	561·1**	71·6	1567·9**	201·0
TA	0	7	324·6	9·8	13·4	0·8	13·6	0·7	192·2	36·2	910·3	96·4
	5	9	292·3*	8·1	12·3	0·3	92·6	0·6	203·5	18·2	1020·9	76·9

*$P<0.05$, **$P<0.01$ by unpaired Student's *t* test.

In general, no impairments in twitch characteristics, absolute maximal force generation or force-frequency relations were evident. However, the half relaxation time of the soleus was approximately 13% faster ($P<0.05$) and peak twitch tension of the EDL muscles was 49% greater ($P<0.05$) following the 5 d fast. The absolute maximal tetanic force of the EDL was maintained despite a reduction in muscle weight, effectively representing an 85% increase in the force generation per unit muscle mass ($P<0.01$).

Thus, in contrast to the changes observed in a muscle of mixed fibre types (Russell *et al.* 1984), the contractile characteristics of the skeletal muscles selected in the present study appear to be preserved, or even enhanced, in the face of acute food deprivation.

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Breakdown of 3-(OH)-4-(1H)-pyridone by bacteria from the rumen of sheep in Venezuela.

By M. GLORIA DOMINGUEZ-BELLO, *Central University of Venezuela, Maracay, Venezuela* and C. S. STEWART, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* (introduced by M. F. FULLER)

The tropical forage legume *Leucaena leucocephala* contains a non-protein amino acid, mimosine, which is converted to 3-(OH)-4-(1H)-pyridone (3,4-DHP) by enzymes present in rumen micro-organisms. Both mimosine and 3,4-DHP are potentially toxic to ruminants. It has been suggested that the ability of ruminants to consume *Leucaena* without manifesting toxic symptoms is dependent on the presence in the rumen of micro-organisms that degrade mimosine and 3,4-DHP. These micro-organisms are thought to be limited in their geographical distribution (Allison *et al.* 1983; Jones & Megarrity, 1986).

In Venezuela, West African sheep (initial weight 30 kg), fitted with rumen cannulas, were fed on diets containing different amounts of sun-dried leaves and stems of *Leucaena*. The control diet contained (g/kg): 500 lucerne (*Medicago sativa*) hay; diet 1 contained 250 *Leucaena* and 250 lucerne hay and diet 2 contained 500 *Leucaena*. All three diets contained 480 rice straw and 20 mineral mix, and contained similar amounts of crude protein (111 to 113 g/kg) and metabolizable energy (7·1 to 8·3 MJ/kg).

Although 3,4-DHP was detected (by high-performance liquid chromatography) in samples (*n* 4) from the rumen (0·34 (SD 0·16) μmol/ml), plasma (0·11, (SD 0·01) μmol/ml) and urine (0·43 (SD 0·03) μmol/ml) of the animals fed on diet 2, the animals showed no signs of toxic symptoms. Giving *Leucaena* did not significantly affect any of the following: body-weight, feed intake, rate of passage of solids through the rumen, rumen pH, concentrations of rumen volatile fatty acids, digestion of rice straw in porous synthetic fibre bags, and numbers of saccharolytic and cellulolytic rumen bacteria and fungi. The concentration of ammonia in the rumen of the animals fed on the control diet was on average 188 (SD 32) mg/l. Dietary inclusion of *Leucaena* increased rumen ammonia concentration to 271 (SD 37) mg/l for diet 2.

Cultivation of bacteria from the rumen of the sheep resulted in the isolation of six cultures capable of degrading 3,4-DHP. Two isolates were Gram variable coccobacilli, three were Gram negative rods and one isolate was a Gram variable spore-forming rod. When 3,4-DHP was added to anaerobic nutrient media at a final concentration of 2 μmol/ml, and then incubated for 54 h at 39°, these cultures degraded more than 50% of the toxin. The presence of these bacteria may contribute to the apparent tolerance of *Leucaena* toxins shown by Venezuelan sheep in these experiments.

The authors gratefully acknowledge the help and guidance of the late Professor Rodrigo Parra, and Dr J. Combellas, of the Central University of Venezuela.

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Voluntary food intake and digestion of hay and straw diets by donkeys and ponies. By JANE B. MERRITT* and R. ANNE PEARSON, *Centre for Tropical Veterinary Medicine, Easter Bush, Roslin, Midlothian EH25 9RG*

Donkeys are used for work mainly in the semi-arid and mountainous areas of Africa and Asia, where they originate. In addition to their traditional role as pack and riding animals, donkeys are used for light cultivation tasks, threshing, drawing water and carting loads. Despite this, donkeys have a low status in many of the countries where they are now found. They are an underrated, often neglected source of power. However, their low value makes them accessible to poorer farmers who cannot afford other forms of power. Although donkeys are usually left to forage for themselves and generally maintain good body condition in doing so, little quantitive information is available on their nutrition. The ability of donkeys to consume and digest roughage diets was investigated and compared with that of ponies.

Four adult gelding donkeys (160–190 kg) and four ponies (170–260 kg), maintained in climate rooms at 20°, were provided *ad lib.* with a ration of meadow hay or barley straw plus 2 g vitamin/mineral supplement (Colborn Dawes Nutrition Ltd, Derbyshire), given with 0·25 kg rolled barley/d. The animals were given each diet in periods lasting 21 d with measurements made during the last 7 d. At the end of 6 weeks the donkeys repeated the trial for a further 6 weeks, during which they walked 14 km and ascended 260 m on 5 d/week. Statistically significant differences between treatments were determined using analysis of variance.

Species . . .	Ponies		Donkeys				SE of difference	
Treatment . . .	Resting		Resting		Working			
Diet . . .	Hay	Straw	Hay	Straw	Hay	Straw	Between species	Between diets
DM intake (g/kg body-wt$^{0.75}$ per d)	99·1	59·8	80·9	36·8	87·6	37·3	4·98	6·4
DE intake (MJ/d)	54	24	43	15	49	16	3·5	5·0
Apparent digestibility								
DM	0·49	0·43	0·54	0·47	0·57	0·47	0·016	0·021
ADF	0·40	0·46	0·47	0·52	0·50	0·53	0·023	0·032
Live-wt gain (g/d)	181	–657	238	–714	476	–833	129	285

At rest, ponies ate significantly ($P<0·01$) more dry matter (DM) and digestible energy (DE) than the donkeys. However, the donkeys showed a significantly ($P<0·05$) higher digestibility of DM and acid detergent-fibre (ADF) than the ponies on both diets.

Both species lost weight ($P<0·05$), ate less ($P<0·01$) and had a lower ($P<0·001$) digestibility of DM on the straw than on the hay ration. A greater proportion of cellulose in the straw (45%) than in the hay (35%) may have accounted for the higher ADF digestibilities seen on the straw diet.

Work had no significant effect on food intake, digestibility of food or live-weight change in the donkeys.

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Dietary energy and carbohydrate intakes of runners in relation to training load. By R. J. MAUGHAN, *University Medical School, Foresterhill, Aberdeen AB9 2ZD* and J. D. ROBERTSON and A. C. BRUCE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

During prolonged strenuous exercises, oxidation of carbohydrate (CHO) in the form of muscle and liver glycogen makes a major contribution to energy provision: if exercise is repeated on a daily basis, an adequate dietary CHO intake is essential. In spite of this, several studies have shown that athletes engaged in regular endurance training do not have markedly higher CHO intakes than sedentary individuals (Wootton, 1988).

We have measured dietary energy and CHO intakes calculated from 7 d food intake records in twenty-nine healthy young (21–39 years) male subjects; six of these were sedentary and the other twenty-three were recreational or competitive runners. All active subjects had been running regularly for at least 2 years and at the time of the study had run an approximately constant weekly training distance (16–147 km) for at least 10 weeks. All subjects were in approximately steady state with respect to body-weight.

Daily energy intake and substrate intakes (expressed in g/d and as a percentage of total energy intake) for three groups of subjects: sedentary (Sed, n *6), low mileage runners (16–64 km/week,* n *10) (Low) and high mileage runners (69–147 km/week,* n *13) (High)*

	Sed		Low		High	
	Mean	SE	Mean	SE	Mean	SE
Energy (MJ)	12·1	0·8	13·6	0·7	15·5	0·6
Protein (g)	92	6	113	5	109	5
Protein (%)	13	1	14	1	12	0
Fat (g)	124	15	130	13	149	5
Fat (%)	38	3	35	2	36	1
CHO (g)	352	41	402	24	481	26
CHO (%)	46	4	48	3	49	1
Alcohol (g)	12	4	13	5	18	5
Alcohol (%)	3	1	3	1	3	1

Mean weekly training distance, over the 10 weeks before the survey and taking the sedentary subjects as 0, was correlated with total energy intake (r 0·52, $P<0·01$) and CHO intake (r 0·52, $P<0·01$) but not with protein (r 0·15), fat (r 0·36) or alcohol (r –0·10) intakes. There was a negative relation between body-weight and weekly training distance (r −0·56, $P<0·01$), and when energy intake and CHO intake were expressed relative to body-weight, both were related to the weekly training distance (r 0·73, $P<0·001$ and r 0·70, $P<0·001$ respectively). The proportion of total energy intake derived from protein decreased as the training load increased (r −0·50, $P<0·01$). There was a tendency for an increased proportion of energy to be derived from CHO as the training load increased (r 0·21) but this did not reach statistical significance. The pattern remains the same if the sedentary subjects are excluded from the statistical analysis.

These results suggest that runners in steady state conditions of training and body-weight increase energy intake to balance the increased energy output of training. It also appears that the increased energy intake is accounted for primarily by an increased CHO intake.

Wootton, S. (1988). *Nutrition for Sport.* London: Simon and Schuster.

The acute effects of two fruit-based soft drinks on blood acid-base status. By J. McBRINE, J. B. LEIPER and R. J. MAUGHAN, *Department of Environmental and Occupational Medicine, University Medical School, Foresterhill, Aberdeen AB9 2ZD*

We have previously shown that acute changes in dietary composition can induce changes in blood acid-base status which persist for several days (Greenhaff *et al.* 1988). We were therefore interested by recent claims that a commercial fruit-based soft drink (Aqua Libra; Callitheke UK Ltd, London) can act to 'restore alkaline balance', and have investigated this effect.

Ten healthy young adult males acted as subjects. On each of two occasions, subjects reported to the laboratory after an overnight fast and an arterialized venous blood sample was obtained from a dorsal vein of a heated hand as described by Forster *et al.* (1972). Subjects then drank, in randomized order, 500 ml of either Aqua Libra or a still-orange squash drink (Quosh; Beechham Products, Brentford) diluted according to the manufacturer's instructions; both drinks were lightly carbonated. Subjects then rested for 3 h and further blood samples were obtained at hourly intervals. Plasma pH and blood carbon dioxide tension were measured using a Radiometer BMS3 Mk2 blood gas analyser; plasma bicarbonate (HCO_3^-) and blood base excess values were calculated (Siggaard-Andersen, 1963). Serum concentrations of Na^+, K^+, Cl^- and total protein and serum osmolality were measured on all samples. Haemoglobin concentration and spun packed cell volume were also measured. Statistical analysis of the data was by repeated measures analysis of variance, with subsequent use of the Student's *t* test for paired data where appropriate.

Small, but statistically significant, changes were observed in plasma pH and HCO_3^- concentration and in blood base excess after ingestion of Aqua Libra (Table); no change in these variables was observed after Quosh ingestion. There were no changes in serum concentrations of Na^+, Cl^- or total protein after either drink; a small ($P<0.05$) fall in serum K^+ concentration was observed after 2 h with Aqua Libra and after 3 h with Quosh. Serum osmolality fell ($P<0.01$) after 2 h with Aqua Libra and after 3 h with Quosh.

Acid-base status of arterialized venous blood before and after ingestion of 500 ml Aqua Libra

Period after drink (h) . . .	0		1		2		3	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma pH	7·41	0·02	7·42**	0·01	7·43***	0·02	7·43**	0·02
Plasma HCO_3^- (mmol/l)	24·7	2·5	25·7**	2·8	26·3**	2·9	25·7	3·1
Blood base excess (mmol/l)	0·2	2·2	1·2**	2·6	1·9***	2·5	1·4**	2·5

**$P<0.01$, ***$P<0.001$.

It appears from these results that ingestion of Aqua Libra does result in an acute alkaline shift, without large changes in the major circulating electrolytes, although the magnitude of this effect is small. The component of Aqua Libra which causes this alkalosis is not clear. The citrate content of Quosh (7·2 mmol/l) is similar to that of Aqua Libra (8·5 mmol/l), so differences in citrate content between the two drinks can be excluded.

Forster, H. V., Dempsey, J. A., Thomson, J., Vidruk, E. & DoPico, G. A. (1972). *Journal of Applied Physiology* **32**, 134–137.

Greenhaff, P. L., Gleeson, M. & Maughan, R. J. (1988). *European Journal of Applied Physiology* **57**, 583–590.

Siggaard-Andersen, O. (1963). *Scandinavian Journal of Clinical and Laboratory Investigation* **15**, 211–217.