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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Seventy-second Meeting of the Nutrition Society was held in the Barnes Lecture Theatre of the Royal Society of Medicine, London, on Tuesday, 18 *May* 1982, *when the following papers were read:*

https://doi.org/10.1079/PNS19820060 Published online by Cambridge University Press

Energy balance in rats maintained on 'cafeteria' diets of varying protein content. By K. R. BRYANT, N. J. ROTHWELL, M. J. STOCK and R. S. TYZBIR, *Department of Physiology, St. George's Hospital Medical School, Tooting, London SW17*

Feeding rats a varied and palatable cafeteria diet causes marked increases in energy intake and expenditure. (Rothwell & Stock, 1979). It has been assumed that hyperphagia is the primary stimulus to diet-induced thermogenesis (DIT) in these rats, but the nutrient composition of the diet may also influence energy expenditure. We have therefore studied energy balance in rats maintained on cafeteria diets of varying protein content.

Male, Sprague-Dawley rats (Charles River, UK) aged $5\frac{1}{2}$ weeks, were divided into four groups of equal body-weight, and maintained on pelleted stock diet (PRD, Christopher Hill) (control), or a choice of cafeteria foods of mixed composition (mixed), low protein (LP) or high protein (HP) content, for 15 d. The final protein content of the diets eaten (% metabolizable energy (ME) intake) were control 27, Mixed 23, LP 7, and HP 33%. The fat content of all cafeteria diets was considerably higher (42–51% ME) than the stock diet (9%). ME intake was elevated by 52 and 40% in the mixed and HP cafeteria groups, respectively, compared to controls, but LP rats overate by only 7%. However, intake corrected for body size (kJ/kg$^{0.75}$ per d) was similar for all cafeteria groups (mixed 1335±30; LP 1160±35; HP 1250±40) and was significantly greater than that of controls (945±30). Body-weight gain was similar for control and HP rats but was elevated in the mixed group and was markedly depressed in LP animals. Body fat content (%) was increased by all cafeteria diets, but energy gain (kJ) varied considerably between groups (control 1065±60; mixed 1745±95; LP 835±65; HP 1385±145).

Energy expenditure, calculated from ME intake and body energy gain was increased by 48 and 44% in mixed and HP rats respectively compared to controls, and was slightly, but not significantly elevated (18%) in LP animals. When corrected for body size (kJ/kg$^{0.75}$ per d), expenditure was elevated by 36% in all cafeteria groups compared to controls. Gross energetic efficiency was significantly reduced in LP rats, but was similar for other groups, whereas net efficiency was depressed in all cafeteria fed animals. Resting oxygen consumption ($\dot{V}O_2$) was increased in all cafeteria groups and propranolol caused significant reductions in $\dot{V}O_2$ in all groups apart from controls.

These results demonstrate that large increases in DIT can be achieved in cafeteria fed rats independently of protein intake, but is accompanied by growth retardation on very low protein diets.

Rothwell, N. J. & Stock, M. J. (1979). *Nature, Lond.* **281**, 31.

Biochemical effects of varying protein intake in cafeteria fed rats. By N. J. ROTHWELL, M. J. STOCK, R. S. TYZBIR and P. D. WINTER, *Department of Physiology, St. George's Hospital Medical School, Tooting, London SW17*

Previous work (Bryant *et al.* 1982) has demonstrated that the thermogenic response to cafeteria feeding in rats occurred with diets varying in protein energy content between 7% (LP), 23% (mixed) and 33% (HP). Compared to stock-fed controls, all cafeteria fed rats gained more body fat (LP 5 g, mixed 16 g, HP 8 g), but the energy cost of depositing this (assuming 14 kJ/g for de novo lipogenesis) accounted for less than 17% of the diet-induced thermogenesis (DIT).

Measurements of brown adipose tissue (BAT) oxygen consumption in vivo (Rothwell & Stock, 1981) have shown that BAT thermogenesis can account for all of the diet-induced changes in thermogenic capacity of rats fed a mixed cafeteria diet (18–22% protein), and this has been linked to changes in mitochondrial proton conductance by measuring purine nucleotide (GDP) binding. In the present study, hypertrophy of the interscapular BAT depot was seen in all cafeteria groups, irrespective of dietary composition (control 236±9 mg, LP 499±24 mg, mixed 572±39 mg, HP 414±36 mg). Mitochondrial GDP binding capacity (pmol/mg protein) was significantly elevated in all cafeteria-fed rats, with the greatest increase occurring in the LP group (control 316±30, LP 971±186, mixed 638±92, HP 510±45). Binding affinity (Scatchard analysis) was unaffected by cafeteria feeding or diet composition.

Studies on protein restricted animals have previously implicated changes in hepatic α-glycerophosphate (GP) shuttle activity in the thermogenic response (Tyzbir *et al.* 1981). In LP cafeteria rats, liver weight (g) was significantly decreased (8·5±0·9) compared to the mixed and HP groups (13·5±0·7 and 12·2±0·8, respectively), but not compared to controls (11·1±0·7). The specific activity (nmol NADH oxidized/mg protein per min) of the hepatic GP shuttle was significantly increased only in the LP group (control 7·3±1·1, LP 18·3±2·2, mixed 9·4±1·6, HP 7·5±0·9), but total mitochondrial activity was increased in all cafeteria groups. The specific activity of the GP shuttle in BAT was exceptionally high, but unaffected by cafeteria feeding, although total activity was significantly increased in the LP group.

The results suggest that DIT originating in BAT is not necessarily a consequence of a dietary nutrient imbalance. Increased BAT thermogenic capacity, either alone or in concert with altered hepatic mitochondrial metabolism, may explain the increased DIT associated with poor growth in protein restricted rats.

Bryant, K. R., Rothwell, N. J., Stock, M. J. & Tyzbir, R. S. (1982). *Proc. Nutr. Soc.* **41**, 127A.

Rothwell, N. J. & Stock, M. J. (1981). *Pflugers Archiv.* **389**, 237.

Tyzbir, R. S., Kunin, A. S., Sims, N. M. & Danforth, E. (1981). *J. Nutr.* **111**, 252.

The effect of dietary n-3 and n-6 polyunsaturated fatty acids on platelet aggregation and aortic prostacyclin production in lean and obese rats. By CAROLINE BOLTON-SMITH, W. F. VAS DIAS, M. J. GIBNEY and K. HILLIER, *Departments of Nutrition and Clinical Pharmacology, Faculty of Medicine, University of Southampton*

Dietary eicosapentaenoic acid (C20:5, n-3) which is found in high concentrations in marine oil is known to inhibit platelet aggregation in several species. It achieves this by partial displacement of arachidonic acid (C20:4, n-6) from platelet phospholipids and by inhibiting the conversion of C20:4, n-6 to thromboxane A_2 (TxA_2) by platelet cyclo-oxygenase. The pro-aggregatory properties of TxA_2 are counterbalanced by the anti-aggregatory properties of prostacyclin (PgI_2) which is produced by the arterial endothelium, also from C20:4, n-6. The present experiment examined the relative effects of dietary fats rich in n-3 or n-6 polyunsaturated fatty acids (PUFA) on platelet aggregation and aortic prostacyclin production in lean and obese Zucker rats. Coconut oil was used as a low-PUFA control. The oils were admixed with stock diet (70 g/kg). Platelet aggregation (% light transmission) was measured in an aggregometer and prostacyclin production by radio-immunoassay of 6-keto-$PGF_{1\alpha}$. The results are summarized in the Table.

	Corn oil (*n* 6)	Linseed oil (*n* 3)	Coconut oil (—)	Fish oil* (*n* 3)	Pooled SEM
Platelet aggregation (% maximum light transmission)					
			0·3 μM ADP		
Lean	36	39	46	32	6·1
Obese	50	43	43	45	
			2·5 μg collagen		
Lean	38^{ab}	26^{a}	51^{b}	15^{a}	8·6
Obese	56^{b}	32^{a}	34^{ab}	19^{a}	
Aortic prostacyclin production (ng 6-keto-$PGF_{1\alpha}$/g tissue)					
Lean	820^{ab}	210^{b}	371^{bc}	510^{c}	26
Obese	817^{a}	457^{b}	389^{b}	475^{b}	

*Maxepa, British Cod Liver Oils Ltd.

a,b,c Mean values that do not share a common superscript letter differ significantly ($P<0·05$).

Platelet aggregation by ADP (0·07–2·32 μM) was significantly greater ($P<0·001$) in obese than lean rats. Collagen induced aggregation was significantly greater in PUFA fed obese rats and significantly less in coconut oil fed obese rats. In general, n-3 PUFA reduced platelet aggregation but this was significant ($P<0·05$) only at lower doses of collagen 2·5–5·0 μg. Prostacyclin production was strikingly higher in n-6 PUFA fed rats while little quantitative difference was observed in prostacyclin production in rats fed n-3 PUFA or a saturated fat.

The results highlight the complex role of dietary fat in haemostatic function.

A transplantable rat insulinoma induces hyperphagia and abolishes diurnal changes of food consumption, plasma glucose and plasma insulin concentrations. By S. K. SWANSTON-FLATT, P. R. FLATT, K. TAN and V. MARKS, *Divisions of Nutrition and Food Science, and Clinical Biochemistry, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH*

Subcutaneous implantation of small fragments (approximately 0·2 g) of a radiation-induced transplantable islet cell tumour into the subscapular region of pre-adult male NEDH rats results in rapid tumour growth, marked hyperinsulinaemia, severe hypoglycaemia with the resulting death of the recipient by 28 d (Tan *et al.* 1981). To obtain further information on the metabolic effects and functional properties of the tumour (predominantly composed of β-cells with small numbers of D-cells) diurnal changes of food consumption, plasma glucose and plasma insulin concentrations were examined at 3-hourly intervals for 24 h in tumour-bearing NEDH rats. 20 d after transplantation, the rats characteristically exhibited (mean ± SEM) hyperphagia (25·1±1·8 g/24 h), hypoglycaemia (1·6±0·1 mmol/l) and hyperinsulinaemia (10·1±0·5 ng/ml). Food consumption and mean plasma glucose and insulin concentrations of control rats similarly monitored over the full 24 h period were 11·0±0·9 g/24 h, 5·6±0·2 mmol/l and 2·9±0·3 ng/ml respectively. The control animals exhibited distinct diurnal changes in the parameters studied. Food consumption was greatest between 17.00 hours and 23.00 hours; plasma insulin was greatest between 20.00 hours and 23.00 hours; and plasma glucose was raised at 20.00 hours, 02.00 hours and 05.00 hours compared with the other times. In contrast, insulinoma-bearing rats displayed no diurnal changes, other than a small reduction of food consumption between 05.00 hours and 11.00 hours. Plasma glucose and insulin concentrations were significantly different from control rats at all times, as was food consumption between 23.00 hours and 17.00 hours. These observations demonstrate that the transplantable islet cell tumour not only causes hypoglycaemia and hyperinsulinaemia but also results in hyperphagia and defective diurnal changes of food consumption, plasma glucose and plasma insulin concentrations. Interruption of continual nutrient intake by the withdrawal of food, exacerbated the hypoglycaemia of the tumour-bearing rats resulting in death by 4 h.

Supported by a Cancer Research Campaign Grant (SP1630) to P.R.F.

Tan, K., Flatt, P. R., Webster, J. D. & Marks, V. (1981). *Diabetologia* **21**, 514.

The interaction of diet, diethylmaleate and methionine on the content and resynthesis of glutathione in rat liver and kidney. By D. J. WITTS and A. E. M. MCLEAN, *Toxicology Laboratory, University College Hospital Medical School, London WC1E 6JJ*

The glutathione (GSH) content of liver and kidney are important in detoxication and are known to be dependent on food intake (Leaf & Neuberger, 1947).

Male Wistar rats were fed *ad lib.* on stock 41B diet, 30 g casein/kg or 250 g yeast/kg diets (to provide standard, low protein (LP) and low S-amino acid (Y) dietary conditions; McLean & Day, 1975) for at least a month. They were then given an intraperitoneal (ip) dose of diethylmaleate (DEM; 0·05 ml/100 g body-weight). 90 min later some were also given methionine (MET; 40 mg/100 g ip). The rats were then killed at intervals to follow the time course of GSH depletion and subsequent resynthesis.

The low protein and yeast diets both caused a sharp decrease in hepatic GSH within a few days but had no consistent effect on kidney GSH levels. 90 min after DEM the GSH in both the liver and the kidney was markedly reduced from the control values in all groups. The rate of GSH resynthesis in both tissues was also reduced by the LP and Y diets. This effect was due primarily to a reduced availability of S-amino acids since the injection of methionine brought GSH levels to stock diet control values in all groups.

The effect of diethylmaleate and methionine on liver and kidney glutathione (μmol/g tissue, estimated by Ellman reagent)

(+Met, –Met, Indicates with and without methionine injection at 90 min; no. of animals/group in parentheses)

	Period of expt (h)	Standard diet Mean	SD	LP diet Mean	SD	Y diet Mean	SD
Liver:	0	11·5	1·5 (30)	4·5	1·1 (11)	4·7	0·6 (15)
	1·5	1·6	0·6 (4)	1·8	0·1 (4)	2·3	0·4 (4)
		+Met	–Met	+Met	–Met	+Met	–Met
	2·0	3·5 (2)	2·1 0·2 (4)	3·1 (2)	2·2 0·9 (4)	4·2 (2)	1·7 0·4 (4)
	3·0	7·6 (2)	3·2 0·5 (4)	7·3 (2)	2·2 0·5 (4)	6·7 (2)	1·9 0·3 (4)
	4·0	9·5 (2)	4·3 0·7 (4)	10·6 (2)	2·1 0·8 (4)	9·6 (2)	2·1 0·3 (4)
Kidney:	0	5·9	1·2 (13)	6·6	0·5 (4)	5·1	0·8 (4)
	1·5	2·7	0·8 (4)	1·7	0·3 (4)	2·5	0·7 (4)
		+Met	–Met	+Met	–Met	+Met	–Met
	2·0	2·3 (2)	2·8 0·6 (4)	2·0 (2)	2·0 0·7 (4)	2·6 (2)	2·2 0·4 (4)
	3·0	3·5 (2)	3·4 0·4 (4)	3·6 (2)	1·8 0·7 (4)	3·4 (2)	2·1 0·4 (4)
	4·0	4·9 (2)	3·9 0·3 (4)	5·0 (2)	2·0 1·1 (4)	6·0 (2)	1·7 0·4 (4)

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McLean, A. E. M. & Day, P. A. (1975). *Biochem. Pharmac.* **24**, 37.

A comparison between the effect of added sodium phytate and endogenous phytate in bran and oats on zinc absorption. By SUSAN J. FAIRWEATHER-TAIT and ANDREA CAPREZ, *ARC Food Research Institute, Colney Lane, Norwich NR4 7UA*

The inhibitory effect of phytate on zinc absorption is well-documented. Many investigations have involved adding sodium phytate to diets. Although phytic acid is present mainly in a soluble form in soya bean (Cheryan, 1980) and behaves similarly to added sodium phytate (Davies & Olpin, 1979), cereals contain calcium-magnesium phytate complexes which are less soluble. If Zn binding in the intestine is related to phytate solubility added sodium phytate will have a greater Zn binding effect than endogenous phytate in cereals. This hypothesis was tested by partially or totally dephytinizing cooked bran and adding an appropriate amount of sodium phytate to restore the total phytate to the original endogenous level. The bran was then incorporated into a semi-synthetic diet (100 g/kg) and given to rats.

Since Zn balance is believed to be partly controlled by Zn excretion into the intestine via pancreatic secretions (Matseshe *et al.* 1980) Zn absorption was measured using an isotope dilution technique (Evans *et al.* 1979). This differentiates between unabsorbed dietary Zn and faecal Zn of endogenous origin.

Young male Wistar rats were injected intra-muscularly with 5 μCi ^{65}Zn, to label body Zn before being given the test diets. Faeces were collected and food intakes measured. Apparent Zn absorption was calculated from Zn balance data. True Zn absorption was calculated taking into account the difference between specific activity of the faeces and specific activity of Zn of endogenous origin. There were no differences in Zn absorption from the diets: 32·9% on untreated bran diet (containing only endogenous phytate), 33·5% on partially dephytinized bran diet (containing some added sodium phytate), and 34·4% on totally dephytinized bran diet (containing only added sodium phytate).

The experimental procedure was then repeated using cooked rolled oats. Zn absorption was 30·8% from partially dephytinized oat diet (containing some added sodium phytate) and 32·4% from totally dephytinized oat diet (containing only added Na phytate).

It, therefore, appears that Zn absorption in the rat is not influenced by the form of phytate in cereals at phytate:Zn molar values of less than 5:1.

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Evans, G. W., Johnson, E. C. & Johnson, P. E. (1979). *J. Nutr.* **109**, 1258.
Matseshe, J. W., Phillips, S. F., Malagelada, J.-R. & McCall, J. T. (1980). *Am. J. clin. Nutr.* **33**, 1946.

The effect of apple fibre on calcium, magnesium, iron, copper and zinc balance in the rat. By ANDREA CAPREZ and SUSAN J. FAIRWEATHER-TAIT, *ARC Food Research Institute, Colney Lane, Norwich NR4 7UA*

Foods rich in dietary fibre exhibit different mineral-binding characteristics depending on the physical properties and chemical composition of the fibre (Thompson & Weber, 1979) and the phytate content. Fruits such as apples contain high amounts of pectic substances but no phytic acid and are, therefore, useful substances for testing the fibre-mineral-binding hypothesis without the added complication of binding to phytate. Kelsay *et al.* (1981) showed that fruit and vegetable fibre interfered with absorption of some minerals, e.g. zinc, but not others, e.g. iron. Calcium, magnesium and copper results were inconsistent and it is not clear whether the minerals are bound to pectic substances (as suggested by Anderson & Chen, 1979) or other components of dietary fibre.

This experiment used two forms of fibre derived from apples: pomace and residue. The pomace was the dried material left after juice extraction from apples (630 g/kg dietary fibre, 135 g/kg uronic acids). The residue was pomace from which a proportion of the high methoxyl pectin had been removed (812 g/kg dietary fibre, 91 g/kg uronic acids).

After acclimatization to individual housing in stainless steel metabolic cages (with plastic funnels for separate urine and faecal collections) twenty-four young male Wistar rats were given the following diets for 10 d: group 1 was given a control semi-synthetic diet, group 2 was given the control diet containing 100 g/kg apple pomace and group 3 was given the control diet containing 77 g/kg apple residue. Faeces and urine were quantitatively collected for 10 d and food intakes measured. Diets, faeces and urine were analysed for Ca, Mg, Fe, Cu and Zn by atomic absorption spectrometry.

The mineral content of the diets was similar except for Fe which was higher in the diets containing pomace and residue. When the mineral balances were calculated over the 10 d (intake minus output) it was found that both pomace and residue significantly reduced apparent retention of Cu ($P<0.001$), Zn ($P<0.05$) and Fe ($P<0.02$) but had no effect on Ca or Mg. Partial removal of the pectin improved Cu but not Zn retention which suggests that binding of Cu to apple fibre is related to pectin content. Alternatively, the higher Fe content of the diet may have reduced Cu availability. Further work using residue containing no pectin is required to confirm this finding.

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Kelsay, J. L., Clark, W. M., Herbst, B. J. & Pratter, E. S. (1981). *J. Agric. Fd Chem.* **29**, 461.
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Effect of zinc deficiency on intestinal uptake of galactose in the rat. By S. SOUTHON, I. T. JOHNSON, J. M. GEE and M. G. GEE, *ARC Food Research Institute, Colney Lane, Norwich NR4 7UA*

It has been reported that when rats are provided with less than 6 mg zinc/kg diet there is a decline in the efficiency of food conversion (Williams & Mills, 1970). We have also observed decreased food utilization by immature rats fed a diet containing only 1 mg Zn/kg, compared with pair-fed supplemented controls (60 mg Zn/kg diet). It is possible that some defect of intestinal absorption contributes to this effect. This study was undertaken to test this possibility using galactose as an index of hexose absorption. The effect of Zn deficiency on hexose uptake is also of interest since Zn deficiency has been reported to impair glucose homoeostasis (Hendricks & Mahoney, 1972).

Sixty-eight immature Sprague-Dawley rats were randomly divided into four groups. The first group was given a semi-synthetic diet containing 1 mg Zn/kg. The second and third groups were pair-fed and *ad lib.*-fed respectively with a similar diet supplemented with 60 mg Zn/kg. The fourth group received laboratory chow *ad lib.* After 35 d, eight rats from the first and second groups and six rats from the remaining two groups were killed. Kinetics of tritiated galactose uptake (0–40 mM) was measured by a tissue accumulation technique using everted rings of jejunum. Remaining rats in each group were killed by exsanguination from the heart and blood was analysed for Zn by atomic absorption spectroscopy.

During the Zn depletion period rats exhibited recognized signs of Zn deficiency—poor growth, erratic food intakes, reduced food utilization and skin lesions. Rats fed the low-Zn diet had a significantly ($P<0.05$) reduced mean plasma Zn level (1·11 μg/ml) when compared to control groups (pair-fed 1·70 μg/ml; *ad lib.*-fed 1·71 μg/ml; stock-fed 1·73 μg/ml). Dietary Zn deficiency resulted in an increase in the rate of galactose uptake by intestinal rings when compared to all three control groups. Kinetic analysis showed that this was primarily the result of an increase in the V_{max}.

	K_m (mmolar)		V_{max} (μM/g per min)	
Group	mean	SEM	Mean	SEM
Zn-deficient	9·0	1·0	7·5	0·3
Pair-fed control	4·1	1·4	3·7	0·3
Ad lib.-fed control	4·1	2·0	2·4	0·3
Stock-fed control	10·8	2·7	3·0	0·3

The findings of this in vitro study suggest that during Zn depletion the rate of hexose uptake is not impaired and may be significantly increased.

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Williams, R. G. & Mills, C. F. (1970). *Br. J. Nutr.* **24**, 989.

Some effects, on the domestic laying hen, of zinc oxide addition to the diet. By S. GIBSON, N. JACKSON and MARY H. STEVENSON, *Department of Agricultural and Food Chemistry, The Queen's University of Belfast and Department of Agriculture, Northern Ireland*

Although zinc compounds have been used for the induction of a resting phase for hens, there is little information on the effects of supplementing the diet of laying hens with high levels of Zn compounds. Using broiler fowl it has been shown that high levels of Zn compounds in the diet cause an elevation of liver Zn concentration (Johnson *et al.* 1962; Kincaid *et al.* 1976). Hermayer *et al.* (1977) and Palafox & Ho-A (1980) have shown an adverse effect of Zn compounds on egg production.

Seventy-two laying hens, 40 weeks of age (Hisex, thirty-six white, thirty-six brown) were randomly allocated to one of six treatment groups each containing six birds of each hybrid strain. The birds were fed on a control diet (treatment 1) and this diet supplemented with oxide to provide 4, 8, 12, 16 and 20 g added Zn/kg diet (treatments 2–6). The control diet as fed contained 56 mg Zn/kg.

Food consumption and body-weight were recorded weekly and egg number on a daily basis. After 3 weeks the birds of treatments 1 to 5 were slaughtered. The gizzard, oviduct and ovary were weighed and the liver, kidneys, pancreas, spleen and adrenals weighed, oven-dried and the Zn and Fe contents determined. The birds on treatment 6 were removed from the experiment at 10 d because food intake was severely depressed.

ZnO addition depressed food intake, body-weight and egg number (all $P<0.001$). Food intake was more severely depressed for the brown than the white birds. By the second week 4000 mg added Zn/kg diet induced an almost complete pause in lay for both breeds. The effects on the reproductive system are seen by the significant depressions ($P<0.001$) of both ovary and oviduct weight. Gizzard fresh weights were increased by dietary ZnO ($P<0.001$) and the spleen and adrenal weights were also significantly affected overall (both $P<0.05$). Liver, kidney and pancreas were decreased (both $P<0.001$).

Dietary supplementation with high levels of Zn substantially increased the concentration of Zn in the liver (treatment 1, 98 μg/g DM; treatment 5, 1365 μg/g DM), kidneys, spleen and pancreas (all $P<0.001$). The most extensive increase in Zn accumulation seems to have occurred in the pancreas of the birds fed diets containing at least 4000 mg Zn/kg diet (treatment 1, 95 μg/g DM; treatment 5, 4560 μg/g DM).

There was a marked increase in liver Fe concentration with increasing dietary Zn concentration. A similar trend also seems to exist for the Fe concentration of kidneys, pancreas and spleen as well as for the Cu concentration of the kidneys and pancreas.

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Palafox, A. L. & Elodie Ho-A. (1980). *Poult. Sci.* **59**, 2024.

Different forms of fat in the diet of dairy cows. By J. L. CLAPPERTON and W. STEELE, *The Hannah Research Institute, Ayr KA6 5HL*

'Dairy fat prills' are a mixture of free fatty acids and are a by-product in the manufacture of glycerol. They contain approximately (g/kg) 450 palmitic acid, 450 stearic acid and 100 oleic acid. A free fat with a very similar composition was obtained and was used either as the free fat or after protecting it by encapsulation in formaldehyde-treated casein (fat–casein, 2:1).

Eight Friesian dairy cows were given a basal diet of approximately 5 kg hay, 2·5 kg sugar-beet pulp and 6·9–8·0 kg/d barley-based concentrate. Different forms of fat (500 g) were mixed with the concentrates and the total quantity offered was adjusted so that the amounts of concentrate given were isoenergetic and isonitrogenous. The experiment was carried out as two 4×4 Latin Squares with each period being 28 d long.

	No added fat	'Fat prills'	Free fat	Protected fat	SED
Milk yield (l/d)	15·4	16·9	17·2	16·7	0·24***
Total solids (g/kg)	127·6	128·1	122·6	121·0	1·06***
Fat (g/kg)	40·4	41·4	37·7	36·4	0·82***
Crude protein (N×6·25; g/kg)	32·7	31·8	30·3	30·3	0·28***
Lactose (g/kg)	46·6	47·0	46·8	46·2	0·64

***At least one of the differences was significant at $P<0·001$.

The results are shown in the Table. All the treatments increased the milk yield when compared to the no-fat control diet. This must indicate an increase in the over-all efficiency of transfer of energy from the food to the milk. The fat prills did not cause any reduction in the fat content of the milk, whereas both the free fat and the protected fat did so. The latter result is surprising since previous experiments have shown that protected fats can be used to increase the milkfat content. All the fat additions reduced the protein content of the milk. It is suggested that free fatty acids may have less effect on the rumen fermentation than the same amount of fat given as the triglyceride.

Quantitative effects of ICI 111075 (an inhibitor of methanogenesis) on rumen fermentation and digestion in sheep. By A. DAVIES, J. B. ROWE and G. STANIER, *ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire*

Treatment of populations of mixed ruminal organisms in vitro with inhibitors of methanogenesis in general besides depressing methane and acetic acid production, also stimulate production of hydrogen, propionic and butyric acids (Chalupa, 1980). The highly effective methane inhibitor ICI 111075 (2 trichloromethyl-4-dichloromethenyl-benzo (1,3) dioxin-6-carboxylic acid) also shows this spectrum of activity (Stanier & Davies, 1981). In contrast to the amount of in vitro data, in vivo work is more limited. In the study reported here, ten mature sheep prepared with permanent cannulas in the rumen and duodenum were acclimatized to a ration of 700 g pelleted concentrate/d (g/kg; bran 320, soya-bean meal 320, straw 320, molasses 20, minerals and vitamins 20) and 300 g/d medium quality chopped hay, fed continuously. The animals were divided into two equal groups, one of which was dosed intra-ruminally with 100 mg ICI 111075/d each morning. After 2 weeks dosing, during which samples of rumen fluid were taken, the proportions of volatile fatty acids stabilized in response to treatment. The rates of irreversible loss of acetate, propionate, butyrate, HCO_3^- and blood HCO_3^- were then determined by isotope dilution techniques using continuous infusions of [^{14}C]-labelled tracers. After this the apparent digestibility of the diet and the amount of nitrogen and organic matter entering the duodenum were measured. The proportion of non-ammonia N of microbial origin was determined by infusion of $Na_2{}^{35}SO_4$. Samples of rumen gas were also analysed.

	Control	Treatment	*P*
Net VFA production (mol/d):			
Acetate	4·7	4·5	NS
Propionate	1·3	1·6	NS
Butyrate	0·9	1·2	NS
Energy in VFA: Energy in OM fermented (%)	74	81	NS
Duodenal N g/d	25·3	28·6	NS
% Microbial N in duodenal N	57·3	64·8	0·036
Faecal N g/d	6·3	5·9	NS
Nitrogen digestibility	76·3	78·7	0·10
Dry matter digestibility	57·7	59·0	NS
Volumetric composition of ruminal gas (after removal of O_2 and N_2):			
CO_2	73·9	75·6	NS
H_2	0·1	19·0	<0·001
CH_4	26·0	5·4	0·001

Thus ICI 111075 prevents transfer of electrons to CO_2 blocking methanogenesis; some appear as H_2 while the remainder tend to improve the daily ruminal output of butyric and propionic acids and the flow of N through the duodenum by increasing the quantity of microbial N. This is in agreement with the in vitro effects of this compound.

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https://doi.org/10.1079/PNS19820060 Published online by Cambridge University Press

Suppression of adrenocortical function in rats and sheep treated with the anabolic steroid trenbolone acetate. By K. M. THOMAS and R. G. RODWAY, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Trenbolone acetate (TBA) is an anabolic agent used in agriculture. Its effects on adrenal function are of interest since suppression of glucocorticoid secretion could lead to reduced rates of protein degradation. Hepatic tyrosine aminotransferase (TAT) is increased by glucocorticoids and high TAT is associated with a negative nitrogen balance.

Female rats (4–5 weeks old) were fed *ad lib.* and kept on a 12 h light–12 h dark cycle with lights on at 07.00 hours. Groups of four to six rats were injected once daily for 3–4 d with either 80 μg TBA in arachis oil or with vehicle alone. They were killed at various times of day and blood collected for corticosterone assay and liver samples taken for TAT measurement. The peak of the diurnal rhythm of corticosterone at 16.00 hours was significantly ($P<0.05$) depressed by TBA: 120 ± 55 *v.* 520 ± 110 nmol/l (mean ± SEM). Peak TAT activity at 22.00 hours was also significantly lower ($P<0.05$) in the TBA-treated rats: 15.5 ± 0.8 *v.* 23 ± 2.7 μmol/min per g liver. In a separate experiment, control and TBA-treated rats were injected with either saline or 4 i.u. ACTH (ACTHAR) and killed by decapitation 1 h later. The adrenals' response to ACTH was significantly ($P<0.01$) lower in the TBA-treated rats compared with controls: 970 ± 45 *v.* 1295 ± 85 nmol/l.

The diurnal pattern of cortisol secretion was studied in seven pairs of twin female lambs. One of each pair was implanted with 40 mg TBA while her twin acted as the control. Four weeks after implantation hourly blood samples were taken for 25 h via indwelling jugular catheters, starting at 13.00 hours. Four weeks later the lambs were reimplanted with 40 mg TBA and 1 week after this the 25 h sampling was repeated. On both occasions TBA-treated lambs exhibited lower cortisol levels. This was more noticeable after the second implant. Following the second period of sampling six pairs of twins were given 0.1 mg 1–24 ACTH (Synacthen) intravenously and their cortisol response measured over the next 2 h. TBA-treated lambs showed a significantly lower response: 135 ± 23 *v.* 221 ± 23 nmol/l ($P<0.05$).

These results suggest that TBA exerts some of its anabolic influence through its suppression of adrenal activity.

Effects of high molybdenum intake on the distribution of copper between fractions of sheep digesta. By J. K. CHESTERS, A. M. WILL, J. PRICE and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The availability of dietary Cu to ruminants is known to be reduced when the contents of Mo and sulphur in the diet are increased. It has been suggested that the synthesis of tetrathiomolybdate (MoS_4^{2-}) by micro-organisms within the rumen and its subsequent reaction with Cu might be involved in this process. Limitations to the concept that the freely soluble MoS_4^{2-} ion might be directly involved have been discussed elsewhere (Simpson & Mills, 1982) and prompted the following investigations of the influence of dietary Mo on the distribution of Cu within the digestive tract contents of sheep.

Sheep with cannulas in the alimentary tract were allowed to graze fresh grass (2 mg Mo/kg DM); the plots were then treated with ammonium molybdate and after 4 weeks the sheep were returned to graze the regrowth (50 or 100 mg Mo/kg DM). Samples of digesta obtained during each period were fractionated by differential sedimentation.

The Cu content of the grass varied little with Mo treatment yet the Cu content of the microbial fractions increased significantly with elevated Mo concentration in the grass. However, the Cu content of the soluble fraction was unaltered.

Dietary content		Cu concentrations in fractions of rumen contents (mg/kg DM)			
Mo (mg/kg DM)	Cu (mg/kg DM)	Protozoa	Large bacteria	Small bacteria	Soluble fraction
2	8·8	2·0±0·2	6·3±0·3	15·7±0·9	4·9±0·7
50	9·1	4·8±0·4	14·1±3·0	21·7±1·7	4·8±0·1
100	10·2	3·7±0·4	11·5±0·6	18·9±0·7	4·8±0·6

During passage of digesta from the rumen to the duodenum the proportion of soluble Cu remained constant with the low Mo grass but decreased by about half on both high Mo rations. Mo derived from high Mo ingesta was widely distributed among particulate and soluble fractions of rumen and ileal contents but concentrations of Mo were particularly low in the soluble fraction of duodenal digesta.

The above results indicate that Mo influenced the Cu metabolism of the micro-organisms and reduced the proportion of soluble Cu reaching the duodenum. The lack of an effect on the soluble Cu in the rumen suggests that any involvement of thiomolybdates in these changes occurs within the microbial cells and not in the soluble phase.

Simpson, W. B. & Mills, C. F. (1982). *J. Inorg. Biochem.* (In the Press).

Superoxide dismutase activity in erythrocytes of sheep fed low and high copper diets. By W. H. PARRY and GHASSAN AL-JEBOURI, *Department of Science, Bristol Polytechnic, Coldharbour Lane, Bristol BS16 1QY*

The role of singlet molecular oxygen ($^1O_2$) and of the superoxide anion radical (O_2^-) in enzyme mediated peroxidation reactions in vivo has been an area of considerable interest. Superoxide dismutase, a metalloprotein containing 2 g-atoms of Cu and Zn each, catalyzes the dismutation reaction of O_2^- to give ground state molecular oxygen. It has been proposed that the in vivo role of this enzyme is to scavenge the highly reactive O_2^- radical. These radicals are produced by a number of reaction mechanisms, including several enzyme systems as a part of normal cellular function. Little is known of the effect of dietary copper and zinc concentrations on superoxide dismutase.

Our experiments were conducted with 8–10-month-old wether lambs fed on diets containing different concentrations of Cu. Each diet was given to a different group of lambs for periods of 8 weeks. The Cu concentration in each of four diets was 1, 6, 20 and 250 mg kg respectively. The corresponding dietary Zn concentrations in these diets were 50, 50, 213 and 200 mg/kg respectively; the amount of Zn was based on previous work by Parry & Al-Mukhtar (1980).

The results of erythrocyte superoxide dismutase (SOD) activity from lambs fed on the 250 mg Cu/kg diet showed no difference from SOD activity in the groups fed on 6 mg and 20 mg Cu/kg diet; the mean SOD activity was 3764 ± 338 units/g Hb. The mean plasma Cu concentration of each group fed on the 20 mg Cu and the 250 mg Cu diet was significantly higher ($P<0.01$) than the plasma Cu concentration of the group fed on 6 mg Cu/kg diet. The Cu concentration of the erythrocytes showed no difference between groups. The results of feeding the low-Cu diet, 1.0 mg Cu/kg diet with 50 mg Zn/kg diet, showed that the erythrocyte SOD activity decreased by almost 50% of the SOD activity in erythrocytes from lambs fed on 6 mg Cu and 50 mg Zn/kg diet. However, the lowered activity of SOD in the lambs fed on the low-Cu diet was not maintained for longer than 3 to 4 weeks. After this period the erythrocyte SOD activity increased until it returned to activity values during the sixth week which were comparable with activity values in lambs fed on the 6 mg Cu/kg diet.

Parry, W. H. & Al-Mukhtar, F. (1980). *Proc. Nutr. Soc.* **39**, 54A.

The intestinal flow of vitamin B_{12} in sheep given diets of grass or barley. By JOY WOOTTEN, R. N. B. KAY, J. B. BRUCE and E. D. GOODALL, *The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Vitamin B_{12}, synthesized in the rumen, has an important role in propionate metabolism (Elliot, 1980). Preliminary experiments (Elliot *et al.* 1971) indicated that the flow of vitamin B_{12} in the abomasum of sheep is substantially greater with a diet of grass than with barley. The effect has been investigated in more detail as part of a study of absorption of the vitamin (Wootten, 1972).

Four 3-year-old Scottish Blackface ewes, weighing 42 to 50 kg, were fitted under anaesthesia with cannulas in the rumen, abomasum (pyloric antrum) and terminal ileum. Two pelleted diets were given continuously from a feeder: a grass diet, 900 g daily, dry matter (DM) digestibility 70%, and a barley diet (85% rolled barley, 15% supplement), 800 g daily, DM digestibility 80%. Polyethylene glycol (PEG) was added to each diet as a reference substance. The diets were alternated according to a 2×2 Latin Square. After a 3-week introductory period on each diet, rumen samples were drawn at 09.00 hours while abomasum and ileum samples were taken at 3 h intervals, and pooled at −20°, during two consecutive 24 h periods. There were two such sampling sessions, separated by a week, on each sheep for each diet. Digesta flow was estimated from the concentrations of PEG and DM in the samples. Vitamin B_{12} was estimated microbiologically using *Ochromonas malhamensis*.

Digesta characteristics (n 4) are shown in the Table. Digesta flow was much greater with the grass diet than with the barley. The concentrations of vitamin B_{12} in the abomasum tended to be greater with the grass diet, though this was less evident in the ileum. Consequently, the flows of the vitamin into and out of the small intestine, and its net disappearance between abomasum and ileum, were more than twice as great with the grass diet than with the barley.

	Grass diet				Barley diet			
	Abomasum		Ileum		Abomasum		Ileum	
Digesta	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Dry matter (%)	7·6	0·50	9·0	0·25	6·4	0·84	10·5	1·25
Flow (g/d)	8720	428	4135	229	5092	379	1514	408
Vitamin B_{12} (ng/g)	254	15	310	12	156	31	263	25
Vitamin B_{12} (μg/d)	2207	176	1278	158	816	190	378	35

Vitamin B_{12} concentration and flow were positively correlated with rumen pH when both diets were considered, but there was no relationship within diets. These parameters did not correlate with the molar percentage of propionic acid in the rumen. To this extent there was no indication of an association between vitamin B_{12} synthesis and fermentation pattern in the rumen.

Elliot, J. M. (1980). In *Digestive Physiology and Metabolism in Ruminants*, p. 485 [Y. Ruckebusch and P. Thivend, editors]. Lancaster: MTP Press Ltd.
Elliot, J. M., Kay, R. N. B. & Goodall, E. D. (1971). *Life Sci.* **10**, 647.
Wootten, J. (1972). M.Sc. thesis. University of Aberdeen.

The stability of ß carotene in preserved, moist leaf protein. By N. W. PIRIE, *Rothamsted Experimental Station, Harpenden AL5 2JQ*

In feeding trials in India, Jamaica, Nigeria and Pakistan dried leaf protein (LP) was used. Had fresh material been used, the results would probably have been even better because drying, especially with foods containing as much unsaturated fatty acid as LP, is usually deleterious. The moist press-cake of LP, which is usually at about pH 4, keeps for a few days at room temperature even in the tropics, but it is not always convenient to make LP so frequently as to allow unpreserved, moist material to be used. Press-cake containing 50 to 60% dry matter (DM) keeps for several weeks at 27° if the fluid pressed from it at the end of the isolation contains 170 g sodium chloride or 6 g acetic acid/l (Pirie, 1980).

Vitamin A deficiency is a serious problem in several countries where LP would be a useful dietary supplement. It is therefore important to know how much of the β carotene usually present (0·1 to 0·2%) in fresh LP is lost when preserved material is stored. There is little or no loss of β carotene when LP from elder, nettle, potato or wheat is stored for 2 weeks in the dark in the presence of air at 34° when it contains NaCl and 50% DM. In the same conditions, LP from lucerne loses 20 to 30% of its β carotene, but nearly all of it is lost with LP from Brussels sprouts tops or rape. With these two species, destruction is slower if the LP is preserved with acetic acid, is pressed so thoroughly that it contains 60–70% DM, if air is excluded, or if cyanide is present. Exclusion of air would be troublesome, and hostility to the addition of even a minute amount of cyanide is to be expected. Other inhibitors are therefore being sought. Destruction is not, in a strict sense, enzymic: the LP used had been heated to 100°. The nature of the difference between NaCl and acetic acid, and between the species of leaf, is not yet clear.

Some food tables record similar differences between the amounts of β carotene in fresh and salted vegetables. For example, the *Food composition table for use in East Asia* (Food and Agriculture Organisation, 1972) shows large differences with cabbage, Chinese cabbage, turnip greens and olives.

Pirie, N. W. (1980). *Ind. J. Nutr. Diet.* **17**, 349.

Vitamin E status of children recovering from severe malnutrition. By P. M. MATHIAS*, *Tropical Metabolism Research Unit, University of the West Indies, Jamaica*

The children (*n* 22) and treatment used in this study were as described previously (Mathias, 1982). Mean vitamin E intakes were 3 mg/d during maintenance (10±1 d, mean ± SEM), 13 mg/d during rapid growth (33±4 d), and normal intakes in the period on mixed diet (9±1 d). Tocopherol, carotene and total lipids in plasma, and red cell peroxide haemolysis (RCPH) were measured.

		Tocopherol (mg/l)		Carotene (μg/l)		Total lipids (g/l)		Tocopherol: total lipids (mg:g)		RCPH (%)	
Period	No. of samples	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Admission	22	3·9	$0·6^{a}$	180	40^{a}	3·65	$0·27^{a}$	1·06	$0·13^{a}$	31	7^{a}
Maintenance	22	6·7	$0·7^{bd}$	130	20^{a}	4·43	$0·30^{b}$	1·56	$0·16^{b}$	13	4^{b}
Rapid growth	42	8·2	$0·6^{c}$	120	20^{a}	4·44	$0·26^{b}$	2·06	$0·25^{c}$	4	3^{c}
Mixed diet	22	7·0	$0·6^{d}$	290	60^{a}	4·27	$0·28^{ab}$	1·76	$0·13^{bc}$	8	4^{bc}

a,b,c,d, Values in the same column that do not share a common superscript letter are significantly different ($P<0·05$).

On admission there was marked hypocarotenaemia and a significant correlation (r 0·50, $P<0·05$) between carotene and tocopherol. This suggested that the low (<5·0 mg/l) mean tocopherol value was partly due to fat malabsorption. In addition, children with low tocopherols (*n* 14) had significantly lower ($P<0·05$) total lipids (3·24±0·33 g/l) than those with normal tocopherols (*n* 8, 4·36±0·37 g/l). Most of the increase in plasma tocopherol during maintenance was probably due to the rise in lipids, while the further increase seen in rapid growth was mainly the result of an increased vitamin E intake, although there was a positive correlation between plasma tocopherol and total lipids in several individuals throughout recovery. The highly significant ($P<0·005$) increase in plasma tocopherol over admission values, and an increase in the tocopherol:total lipids value (Horwitt *et al.* 1972) as well as a decrease in RCPH (Muller *et al.* 1974), signified an improved vitamin E status on discharge. Most raised RCPHs (>2%) were associated with low plasma tocopherols, although some children had abnormal RCPHs with normal tocopherols either on admission or during recovery. Additional studies suggested this may have been due to selenium deficiency (Mathias & Jackson, 1982).

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Mathias, P. M. (1982). *Proc. Nutr. Soc.* **41**, 52A.
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Preliminary results of a longitudinal assessment of the dietary habits of 405 11–13-year-old children. By A. F. HACKETT, A. J. RUGG-GUNN, D. R. APPLETON and J. E. EASTOE, *Departments of Oral Biology and Medical Statistics, University of Newcastle upon Tyne*

In a previous communication Hackett *et al.* (1982) described the use of a 3 d diary–interview method to record the dietary habits of more than 400, 11–13-year-old children over a 2 year period. The principle aim of this study was to discover which dietary habits best explain the observed 2 year dental caries increment.

This paper describes the nutrient intake of 405 children recorded between September 1979 and July 1981. Each of these children completed five diary–interviews and recorded their intake in household measures. These values were then converted at the interview to weight (g) by A.F.H., using a variety of calibrated visual aids. Computerized food tables (Paul & Southgate, 1978) were then used to calculate the mean nutrient intake recorded.

The following results were found for 193 boys (M) and 212 girls (F).

Survey . . .	1 September 1979–		2 March 1980–		3 September 1980–		4 December 1980–		5 April 1981–	
Nutrient	M	F	M	F	M	F	M	F	M	F
Protein (g)	64	54	60	53	63	55	68	57	68	59
SEM	1·2	0·9	1·0	1·0	1·1	1·0	1·2	1·0	1·1	1·2
Fat (g)	94	87	98	94	101	91	111	97	108	96
SEM	1·9	1·5	1·9	1·6	2·2	1·6	2·1	1·7	1·9	2·1
Carbohydrate	278	251	272	256	286	252	301	259	317	270
SEM	4·4	4·3	4·8	4·4	4·9	3·8	5·2	5·0	5·2	5·1
Energy (kJ)	9024	8151	9010	8473	9392	8338	10074	8717	10254	8885
SEM	95	92	103	94	105	87	112	136	109	115
Sugars (g)	121	115	119	116	123	109	125	113	133	117
SEM	2·1	2·1	2·2	2·2	2·5	1·8	2·3	2·4	2·2	2·1

It can be seen that the boys consistently ate more than the girls and their intake steadily increased as did the difference between the boys' and girls' mean intake. The girls' intake increased very slowly and they ate less sugars (g) than the boys. The percentage contribution of each nutrient to the energy intake can be calculated and is in fact very similar between the two groups at all times. Sugars consistently provided approximately 21% of the total energy intake.

This study was supported by an MRC project grant.

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Studies on the energy requirements of human pregnancy and lactation. By J. V. G. A. DURNIN, *Institute of Physiology, University of Glasgow,* J. HAUTVAST, *Department of Human Nutrition, Agricultural University, Wageningen,* A. VALYASEVI, *Institute of Nutrition, Mahidol University, Bangkok,* and R. G. WHITEHEAD, *M.R.C. Dunn Nutrition Unit, Cambridge*

There is still considerable controversy over the extra requirements for energy necessitated by pregnancy and lactation in healthy women. It is not difficult to understand why this is so, because the measurements required to elucidate this problem are complex and tedious and would need to be done on a longitudinal basis on a sufficiently large sample of women. Nevertheless, the situation at present is that virtually all the studies of the food intake of pregnant and lactating women, in both developing and developed countries, show intakes of energy which are highly unlikely to provide the theoretical extra requirements.

Because this is a problem of very wide nutritional importance, the present investigation has been organized to provide data, in groups of women, fifty to sixty in number, in each of four countries; Glasgow Scotland, Wageningen Holland, Ubol Thailand and in the Gambia. The data will be collected on young women between 20 and 30 years of age, in their second or third pregnancy, and should allow fairly exact calculation of their energy balance.

The women will thus be, hopefully, representative of societies in both developing and developed countries and, in Thailand and in Scotland, will comprose both the poor and the economically-favoured groups.

From the early stages of pregnancy (the sixth to the twelfth week), every 6 weeks each woman will have the following measurements taken: (1) food intake during 5–7 consecutive days by the individual weighed inventory method, (2) energy expenditure during the same period by a combination of the 24 h diary record and measurements by indirect calorimetry, with special care being given to careful assessment of the duration of any physical activity, (3) measurements in the laboratory of basal metabolic rate and metabolic rate in standardized exercise (usually walking on a treadmill), (4) anthropometric measurements of height, weight, skinfold thicknesses, skeletal diameters and limb circumferences and (5) body density by underwater weighing to allow calculation of the fat mass of each woman. Each woman will also record her body-weight at weekly intervals.

When the baby is born, all of the above measurements will be continued every 4 weeks, as well as monitoring the baby's weight, and measuring breast-milk production by test-weighing during 48 h.

So far, the study has been continuing in Glasgow for more than 1 year, in Wageningen for about 6 months, has just started in Thailand, and will soon begin in the Gambia. Results from these localities will be described in later communications to the Society.

This research is financed by a grant from the Nestlé Foundation.

Studies on the energy requirements of human pregnancy and lactation—Glasgow. By J. V. G. A. DURNIN, F. M. MCKILLOP, S. GRANT and G. FITZGERALD, *Institute of Physiology, University of Glasgow*

This paper describes some preliminary findings of the Glasgow section of the multi-centre investigation into the energy requirements of pregnancy and lactation, the details of which have been described in the previous paper.

Data have been collected on twenty-two women who have continued to participate throughout pregnancy and into lactation. Seven other women provided data during some months of pregnancy but had to discontinue for various reasons (abortion, etc.). None of the subjects apparently altered their food intakes or way of life in any way because they were taking part in the study, except for one subject who tried to decrease her food intake for about 2 weeks.

Of the seventeen women who have so far given birth, the weight gain has been 17% of their initial recorded weight, and has an average value of $10 \cdot 1 \pm 2 \cdot 4$ kg. The mean amount of fat laid down during pregnancy—presumably as a physiological store of energy for breast-feeding—was $4 \cdot 6 \pm 2 \cdot 3$ kg.

Complex changes in basal metabolic rate occurred which will be more fully discussed. Even as a gross value, the BMR of several women actually remained fairly constant or even fell during pregnancy. Expressed as BMR/kg body-weight, almost all the women showed a decrease, which showed some surprising fluctuations, notably a marked fall during the first 30 weeks of gestation.

Energy expenditure at different stages of gestation have been calculated, and alterations in patterns of physical activity will be discussed.

This research is financed by a grant from the Nestlé Foundation.

Dietary intakes of zinc, copper, calcium and iron in human pregnancy. By JULIE ARMSTRONG, *Clinical Research Unit, Aberdeen Maternity Hospital, Aberdeen*

Animal studies by Hurley (1981) showed that adequate zinc and copper nutrition was essential for normal foetal growth and development. There is an increased demand during pregnancy for mineral nutrition. Investigation of the trace element intake in human pregnancy is, therefore, indicated in relation to the outcome of pregnancy.

Eighteen primigravidae who subsequently delivered infants of birth weight <10th centile (Thomson *et al.* 1968), i.e. group 1, were compared to a similar number of primigravidae whose babies weighed >10th centile, i.e. group 2. The latter control group were matched with group 1 for height, weight at 20 weeks, weight for height at 20 weeks and weight gain between 20 and 28 weeks. None of the subjects were receiving any nutritional supplementation. Nutritional intakes were assessed by 7 d weighed dietary survey at 30 weeks gestation, the validity of which has been confirmed by Leitch (1950). Daily dietary intakes were calculated from the published composition of food tables (Paul & Southgate, 1978). As limited data are available for the Zn and Cu content of all foods a value for Zn and Cu in a related food was used in the calculation. This was to avoid grossly underestimating Zn and Cu intakes. The results are shown in the Table. The two groups were compared using a paired *t* test.

	Group 1	Group 2	*t*
Zinc (μmol)	151 ± 36·6	155 ± 47·4	−0·3
Copper (mmol)	28·3 ± 21·7	24·4 ± 12·3	0·7
Iron (μmol)	165 ± 43·7	190 ± 71·1	−1·5
Calcium (mmol)	21·9 ± 6·25	23·8 ± 11·20	−1·0
Energy (MJ)	8·0 ± 1·43	8·6 ± 1·68	−1·0
Protein (g)	70·4 ± 13·07	77·2 ± 19·27	−1·5

There was no significant difference found for any of the nutrients between the women who had growth retarded babies and matched controls. Some of the levels of dietary intake are less than recommended and this will be discussed.

Dr D. M. Campbell, Dr M. Campbell-Brown, Dr L. Jandial and Professor I. MacGillivray were responsible for selection and clinical care of the patients in the study.

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Citrate ingestion and exercise metabolism in man. By ADRIANNE E. HARDMAN and C. WILLIAMS, *Department of Physical Education and Sports Science, Loughborough University of Technology, Loughborough, Leicestershire LE11 3TU*

The progressive shift away from carbohydrate metabolism during prolonged exercise has been attributed to reduced glycolysis as a result of the accelerated oxidation of free fatty acids (FFA). The mechanism proposed (Newsholme & Start, 1973) is the inhibition of the glycolytic enzyme phosphofructokinase by the accumulation of citrate. We have observed in previous experiments increases in plasma citrate concentration from 0·10±0·01 mM (mean ± SEM) at rest to 0·16±0·01 mM during prolonged heavy exercise (100 min; n 13).

We therefore sought to investigate the influence of increased plasma citrate concentration on carbohydrate metabolism during exercise, by artificially elevating plasma citrate concentrations. Nine subjects ingested sodium citrate (70 mg/kg) in water and, on a separate occasion, water alone, after 30 min of a 75 min exercise test on a cycle ergometer. This procedure has previously been shown to produce significant increases in plasma citrate concentrations (Hardman & Williams, 1982).

		Plasma citrate (mM)				Blood lactic acid (mM)					
		Pre	28′	50′	75′	Pre	10′	25′	45′	60′	70′
Water trial	Mean	0·10	0·12	0·12	0·13	0·49	2·34	1·89	1·40	1·21	1·21
	SEM	0·01	0·01	0·01	0·01	0·07	0·23	0·17	0·13	0·11	0·12
Citrate trial	Mean	0·11	0·12	0·21***	0·20***	0·57	2·47	2·04	1·91**	1·75*	1·43
	SEM	0·01	0·01	0·01	0·01	0·09	0·20	0·18	0·22	0·21	0·16

Significantly different between trials *$P<0·005$, **$P<0·01$, ***$P<0·001$.

No effect was seen on plasma FFA or plasma glycerol concentrations but blood lactic acid concentrations were higher at 45 and 60 min of exercise on the citrate trial. Oxygen uptake and the respiratory exchange ratio did not differ between trials. Thus these artificially elevated plasma citrate concentrations appeared to increase rather than decrease glycolysis, as measured by blood lactic acid concentration.

C.W. and this work are supported by the Sports Council.

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