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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A meeting of the Nutrition Society was held at Lady Spencer-Churchill College, Oxford Polytechnic, Wheatley, Oxford from Wednesday to Friday, 26–28 July 1989, when the following papers were presented:

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The nutrition and environment of the early human embryo. By A. L. GOTT[1], K. HARDY[2], R. M. L. WINSTON[2] and H. J. LEESE[1], [1]*Department of Biology, University of York, York YO1 5DD* and [2]*Institute of Obstetrics and Gynaecology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN*

The fallopian tube, or oviduct, provides the environment for fertilization and early embryonic development. In view of this, it is not surprising that tubal disease is a major cause of female infertility. In many cases, the tube becomes blocked, leading to the accumulation of excessive amounts of fluid within the lumen. We have analysed the nutrient composition of thirty-five samples of such hydrosalpinx fluid, since the values may reflect those found in normal oviduct fluid. The concentrations of the components studied are shown in the Table and are compared with the values in human plasma and in T6, a common human embryo culture media. Such nutritional information is important in optimizing the composition of the media used for in vitro fertilization and embryo culture in man and domestic species.

	Source of fluid		
Component	Hydrosalpinx fluid	Human plasma	T6
Glucose (mM)	1·11	5·00	5·50
Pyruvate (mM)	0·24	0·10	0·47
Lactate (mM)	1·98	0·60	25·0
Lactate dehydrogenase (μmol/mg per min)	0·043	0·012	nd
Glutamine (mM)	0·30	0·60	nd
Glutamate (mM)	0·38	0·10	nd
Protein (mg/ml)	26·2	80·00	10·0

nd, not determined.

Pyruvate is consumed preferentially by early mammalian embryos with glucose becoming the preferred substrate at the blastocyst stage of development. We have used a non-invasive technique to investigate the consumption of pyruvate and glucose by thirty-nine, individual, spare human embryos incubated in successive 4-μl drops of culture medium from day 2 to day 6 post-insemination. The medium contained pyruvate and glucose at concentrations similar to those found in the hydrosalpinx fluid. Lactate production was also measured.

The pyruvate and glucose uptakes of the embryos which developed to the blastocyst stage (49%) were significantly higher than those which arrested. Furthermore, in such degenerate embryos, glucose uptake did not increase at the blastocyst stage as it did in the healthy embryos.

Lactate was produced at all stages of development in amounts greater than could be accounted for by the consumption of glucose. Healthy embryos produced significantly more lactate than arrested ones.

The capacity of non-invasive nutritional assays to distinguish between healthy and arrested embryos could have potential in selecting those embryos most likely to implant following transfer into the mother. This may aid in increasing the success rates in IVF clinics.

This study was supported by The Wellcome Trust. Full ethical permission for the work has been obtained from the relevant authorities.

Differential effects of dietary fatty acid saturation and chain length on lipogenesis in liver and mammary gland of lactating rats. By PAULO F. A. SOUZA and DERMOT H. WILLIAMSON, *Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE*

An oral load of triolein or medium-chain triacylglycerols can rapidly inhibit lipogenesis in the mammary gland (Agius & Williamson, 1980). It is also known that increased dietary fat intake can inhibit lipogenesis in liver and mammary gland of lactating rats, when compared with a semi-purified, high-carbohydrate, fat-free diet (Grigor & Warren, 1980). In the present experiments the effects of incorporation (150 g/kg) of fatty acids of different saturation (stearic, oleic and linoleic acids) or chain-length (a mixture of medium-chain acids, C_8 and C_{10}) into a standard pelleted chow diet (approximate composition (g/kg) 520 carbohydrate, 210 protein and 40 fat; residue non-digestible material) have been studied. The diets were given as pellets to lactating Wistar rats for 10–11 d post partum and rates of lipogenesis were measured with ^3H_2O at the end of this period. Arteriovenous differences for glucose and lactate across the mammary gland were also measured.

The dietary intake of the various fat diets was similar to that of the chow-only diet, except in the case of the medium-chain diet where the intake was decreased by 33% ($P<0.001$). This was accompanied by a decrease in litter-weight gain (45%; $P<0.001$); weight gain was normal in the other groups.

Lipogenesis (μmol ^3H_2O/g tissue per h)

	Liver			Mammary gland		
Diet	*n*	Mean	SEM	*n*	Mean	SEM
Chow only	4	13·5	2·3	4	138·7	13·9
Stearic acid	5	11·6	1·1	5	34·3***	9·9
Oleic acid	6	7·9*	1·3	6	23·9***	5·0
Linoleic acid	4	5·4**	0·5	4	12·1***	4·4
Medium-chain fatty acids	5	38·1**	4·2	5	60·1**	9·3

Significantly different from chow diet: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

All the fatty-acid diets decreased lipogenesis in the mammary gland and the arteriovenous differences for glucose and lactate across the gland; the polyunsaturated fatty acid diet being the most effective. In contrast, only the unsaturated fatty acid diets inhibited hepatic lipogenesis. Despite the decreased dietary intake, the medium-chain fatty acid diet increased lipogenesis in the liver threefold. It is possible that medium-chain fatty acids can act as a carbon source for hepatic lipogenesis.

Agius, L. & Williamson, D. H. (1980). *Biochemical Journal* **192**, 361–364.
Grigor, M. R. & Warren, S. M. (1980). *Biochemical Journal* **188**, 61–65.

Breast-milk calcium and phosphorus concentrations of British and Gambian mothers during prolonged lactation. By ANN PRENTICE, M. A. LASKEY, B. DIBBA and J. SHAW, *MRC Dunn Nutrition Unit, Cambridge CB4 1XJ* and *Keneba, The Gambia*

Breast-milk is a major source of calcium and phosphorus during infancy and early childhood in areas of the world where extended breast-feeding is customary and dairy produce scarce. The contribution of breast-milk to Ca and P nutrition, however, is difficult to evaluate as little information is available on breast-milk composition after the first weeks of lactation.

Breast-milk Ca and P concentrations have been measured in two communities practising prolonged lactation: Cambridge, UK, and rural West Africa. Breast-milk samples were collected cross-sectionally from 144 rural Gambian mothers (0·5–25 months post partum, 16–49 years old, parity 1–12) and from seventy-two Cambridge mothers (0·5–26 months post partum, 20–42 years old, parity 1–5). Ca was measured by atomic absorption spectrometry, and P by a centrifugal analyser assay (Roche) after dry ashing and mild acid digestion.

Breast-milk Ca and P concentrations

Stage of lactation (months)		Ca concentration (mg/l)			P concentration (mg/l)			Ca:P ratio		
		Gambia	Cambridge	Country effect†	Gambia	Cambridge	Country effect†	Gambia	Cambridge	Country effect†
0·5–2·99	Mean (SE)	247(7)	301(10)	$P<0·001$	165(5)	159(7)	NS	1·54(0·08)	1·95(0·07)	$P<0·001$
	n	33	29		29	18		29	18	
3·00–5·99	Mean (SE)	234(6)	266(9)	$P<0·01$	161(4)	136(3)	$P<0·001$	1·44(0·04)	1·99(0·11)	$P<0·001$
	n	42	19		32	17		32	17	
6·00–8·99	Mean (SE)	200(11)	250(9)	$P<0·01$	157(8)	134(9)	NS	1·14(0·09)	1·99(0·17)	$P<0·01$
	n	17	12		8	11		8	11	
9·00+	Mean (SE)	181(5)	217(11)	$P<0·01$	158(4)	124(11)	$P<0·01$	1·13(0·05)	1·94(0·23)	$P<0·001$
	n	52	12		30	10		30	10	
Stage of lactation effect‡		$P<0·001$	$P<0·001$		NS	$P<0·01$		$P<0·001$	NS	

NS, not significant.
†Significance of *t* test comparing Cambridge and Gambia at the same stage of lactation.
‡Significance of *t* test comparing 0·50–2·99 months with 9·00+ within country.

The results are summarized in the Table. Ca concentration in both countries decreased as lactation progressed, reaching a low plateau after 9 months. The difference between early and late lactation averaged 27%. A parallel decrease in P concentration occurred in Cambridge with the result that Ca:P ratios remained unchanged. No similar decrease in P concentration was apparent in The Gambia, resulting in declining Ca:P ratios with increasing stage of lactation. Between-individual differences in each country were considerable. Coefficient of variation (CV) values for both Ca and P concentrations averaged 17% after stage of lactation adjustment. No significant correlations were noted between an individual's Ca and P concentrations and the CV for Ca:P ratios was 23%. Ca and P concentrations were largely independent of maternal age and parity, except that in Cambridge primiparous mothers aged 35+ had significantly elevated P levels.

Significant differences were observed in Ca and P concentrations and Ca:P ratios between Gambian and Cambridge milks. These differences were substantial, e.g. after 6 months lactation Gambian milks contained 20% less Ca, 22% more P and 43% lower Ca:P ratios than Cambridge milks. Future studies will examine the extent to which diet is responsible for these differences in breast-milk composition between Gambian and Cambridge mothers.

M.A.L. and J.S. were successive holders of the Rank–Widdowson Fellowship.

Background variations in breath carbon-13 enrichment in free-living infants and toddlers.

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Breath-test techniques using the stable isotope carbon-13 (^{13}C) have been developed to study nutrient digestion and metabolic utilization. Previous studies in adults and children have been conducted under highly controlled conditions, e.g. periods of fasting, ^{13}C-poor diet, rest. For investigations in healthy infants and toddlers, such restrictions are impracticable and may not be necessary if the variations in background breath ^{13}C under less-controlled conditions are small. The aim of the present study was to determine the background fluctuations of breath ^{13}C enrichment in free-living infants and toddlers and to evaluate two simple devices for the collection of serial breath samples.

A 'bubble-box' for collecting breath from toddlers was designed and made in the Dunn Nutrition Unit. The child blows through a plastic tube fitted with a one-way valve into an internal compartment (75 ml) of a perspex cylinder (250 ml) half-filled with coloured water. This displaces the water, making bubbles which amuse the child. Trapped breath is immediately sampled. For children younger than about 2 years old a face mask fitted with one-way valves is used (Shulman *et al.* 1983). Breath samples were collected every 30 min for 6 h in five exclusively breast-fed infants 7–29 weeks old; seven older infants 6–11·5 months old; and ten toddlers aged 15–35·5 months. The older infants were given rice porridge (^{13}C-poor) at the beginning of the study. Mothers of all subjects were asked to avoid giving their children ^{13}C-rich foods the evening before the test, and during the test, otherwise all subjects were free to eat, drink and play as usual. The acceptability of both devices by children and mothers was high. ^{13}C enrichment was measured by the ratio of ^{13}C:^{12}C in breath carbon dioxide after correction for the abundance of oxygen isotopes, and expressed as delta ^{13}C relative to PDB ($\partial_{PDB}{}^{13}C$). PDB (Pee Dee Belemnite limestone) is the international reference for ^{13}C. The contribution of CO_2 from ambient air to ^{13}C:^{12}C ratios in breath samples was regarded as negligible if the sample CO_2 concentration exceeded 0·5%. All the bubble-box samples contained adequate CO_2 concentrations (1·9–4·6%) but 11% of samples obtained with the mask had CO_2 levels <0·5% due to shallow breathing and were rejected.

Within-individual variability of ^{13}C enrichment in breath was very low. The coefficient of variation ($\partial 13$) was smallest in exclusively breast-fed infants (1%) but was only 2–3% in older children regardless of whether they had eaten their normal breakfast or rice porridge.

These results allow further developments of ^{13}C breath-tests for studying digestive function in free-living infants and children.

The study was supported by the EEC, research project contract ST2*0447. The protocol was approved by the Ethical Committee of the Dunn Nutrition Unit.

Shulman, R. J., Wong, W. W., Irving, C. S., Nichols, B. L. & Klein, P. D. (1983). *Journal of Pediatrics* **103**, 23–28.

The urinary excretion of 5-oxoproline in healthy term infants. By C. PERSAUD[1], N. EVANS[3], N. RUTTER[3], M. HALL[2], S. SMITH[2] and A. A. JACKSON[1], *Departments of [1]Human Nutrition and [2]Child Health, University of Southampton, Southampton SO9 3TU* and *[3]Department of Child Health, Queen's Medical Centre, Nottingham*

The protein requirements to satisfy normal growth during early infancy are poorly defined. The provision of nitrogen in breast-milk appears to be barely adequate to satisfy the demands for growth. The evidence suggests that the provisions of glycine from breast-milk is no more than one-fifth that required for tissue accumulation, implying the need for substantial endogenous production of the amino acid. Glycine might be acting as a rate-limiting nutrient for lean tissue deposition during the early months of life. One non-invasive approach that we have adopted to assess glycine status is to measure the excretion of 5-oxoproline (5-OP) in urine (Jackson *et al.* 1987).

Random samples of urine were collected from a group of nineteen healthy, term infants at birth and 1, 3, 6 and 9 weeks of age. Fifteen infants were receiving exclusively breast-milk and four exclusively a milk formula (SMA; glycine content 360 mg/l). A series of random urine samples were collected from twenty-six normal adults for comparison. In a number of infants random samples of urine were collected and the 5-OP:creatinine ratio compared with that in a 72 h collection from a similar group of eight infants. 5-OP was isolated by column chromatography, and measured enzymically after acid hydrolysis to glutamic acid. Creatinine in urine was measured using the Jaffe reaction. There was no significant difference in the ratio of 5-OP:creatinine in a random sample from the 72 h collections (206 (SD 88) μmol/mmol). The adults had an excretion of 21 (SD 14) μmol/mmol.

Age (weeks) . . .	48 h	1	3	6	9
n	16	17	18	17	17
5-OP:creatinine (μmol/mmol)					
Mean	65	155	191	174	192
SD	46	98	176	155	145
Range	11–177	22–413	33–777	22–506	31–502

There was a wide variation in the excretion of 5-OP:creatinine at each age. At all ages the excretion of 5-OP was significantly greater than that seen in adults, and most infants for most of the first 9 weeks of life had an excretion beyond the normal adult range. There was no difference in 5-OP excretion in relation to birth weight or feeding pattern.

If the urinary excretion of 5-OP can be used as an index of glycine status, these results would provide evidence to support the suggestion that the demands for glycine in the newborn period may be greater than that available from the diet and endogenous production.

This work was supported by Nestlé Nutrition Research Foundation.

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

The effects of gonadectomy on weight gain and corticosterone metabolism in rats.

By C. J. H. WOODWARD[1], G. R. HERVEY[1], R. E. OAKEY[2] and E. M. WHITAKER[1], [1]*Department of Physiology* and [2]*Division of Steroid Endocrinology, Department of Chemical Pathology, University of Leeds, Leeds LS2 9NQ*

Female rats have a higher turnover of plasma corticosterone than do males, a difference which is reduced by gonadectomy (Colby & Kitay, 1972). Gonadectomy also influences body-weight, especially in females (Hervey & Hervey, 1981). The purpose of the present experiment was to investigate whether there is a causal relation between these two effects.

Groups of five male or female rats were gonadectomized or sham-operated at 3 months of age. The animals were killed 4 and 12 weeks later. Gonadectomy significantly increased body-weight in females but had no effect in males (see Table). The activity of the rate-limiting enzyme for hepatic corticosterone degradation (corticosterone 4,5-reductase; *EC* 1.3.1.3; Colby & Kitay, 1972), and the binding capacity of plasma corticosterone-binding globulin (CBG; Martin *et al.* 1977) were higher in females than in males. Gonadectomy significantly increased both these variables in males but had no effect in females. Plasma corticosterone concentration was reduced in ovariectomized females at 4 but not at 12 weeks.

	Male								Female							
	4 weeks				12 weeks				4 weeks				12 weeks			
	Intact		Castrate		Intact		Castrate		Intact		Castrate		Intact		Castrate	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Final wt (g)	329	4·4	326	6·8	370	12·6	346	11·5	186	3·5	227***	6·0	204	2·6	250***	5·0
Corticosterone 4,5-reductase (mU/g liver)	213	7	353***	23	204	19	395***	13	647	18	684	50	731	11	683	20
CBG capacity (pmol/ml plasma)	773	41	1050*	81	797	36	1060**	62	1875	50	1964	56	1808	79	1882	46
Plasma corticosterone concentration (ng/ml)	211	22	216	23	297	34	337	13	625	98	337*	44	718	141	546	29

*$P<0·05$, **$P<0·01$, ***$P<0·001$ compared with intact group of same sex.

In a separate experiment, the turnover rate of plasma corticosterone (Woodward *et al.* 1990) was measured under halothane anaesthesia 7 weeks after castration. Expressed as %/min, mean turnover in ovariectomized females (8·0 (SE 0·6)) did not differ from that in intact controls (7·8 (SE 0·4)). In castrated males, however, fractional turnover was significantly higher (7·0 (SE 0·4)) than in controls (6·0 (SE 0·2); $P<0·05$).

Since gonadectomy affects body-weight only in females, while corticosterone metabolism is altered predominantly in males, it is unlikely that the two effects are causally related. The results suggest that the male sex hormone is mainly responsible for the sex difference in corticosteroid metabolism in the rat.

This study was carried out under a contract with MAFF.

Colby, H. D. & Kitay, J. I. (1972). *Endocrinology* **90**, 473–478.

Hervey, E. & Hervey, G. R. (1981). In *The Body Weight Regulatory System: Normal and Disturbed Mechanisms*, pp. 345–352 [L. A. Cioffi, W. P. T. James and T. B. van Itallie, editors]. New York: Raven Press.

Martin, C. E., Cake, M. H., Hartmann, P. E. & Cook, I. F. (1977). *Acta Endocrinologica* **84**, 167–176.

Woodward, C. J. H., Hervey, G. R., Oakey, R. E. & Whitaker, E. M. (1990). *Proceedings of the Nutrition Society* **49**, 79A.

Plasma testosterone, oestradiol and sex hormone binding globulin in Indian vegetarian women compared with Caucasian vegetarians and omnivores. By SHEELA REDDY[1], T. J. A. KEY[2], J. W. MOORE[3], G. M. G. CLARK[3] and T. A. B. SANDERS[1], [1]*Department of Food and Nutritional Sciences, King's College, London W8 7AH,* [2]*Imperial Cancer Research Fund, Radcliffe Infirmary, Oxford* and [3]*Lincoln's Inn Fields, London*

Mortality rates from breast cancer in England and Wales are lower in Indian women than in the general female population (Marmot *et al.* 1984). This may be related to differences in sex hormone levels or to dietary influences since a high proportion of Indian women are vegetarians. Although sex hormone levels and diet are believed to be associated with breast cancer risk (Wynder, 1980; Moore *et al.* 1986), the links between them are unclear. We report plasma sex hormone levels in pre-menopausal Indian vegetarian women compared with Caucasian vegetarians and omnivores living in London. Compared with the omnivores, total energy, protein and alcohol intakes were lower in Indians but those of fat and fibre were similar; in Caucasian vegetarians fibre intakes were greater, fat and protein intakes were slightly lower. Blood samples were obtained 12–17 d after the onset of the last menstrual period for determination of oestradiol (E_2), testosterone (T) and sex hormone binding globulin (SHBG). Free T and free E_2 (non-protein bound and thought to be the biologically active fraction) were calculated by the formulas of Miller *et al.* (1985) and Moore *et al.* (1982) respectively. Statistical analysis was carried out making adjustments for body mass index, age and oral contraceptive use.

	Indian vegetarians (*n*21)		Caucasian vegetarians (*n*18)		Caucasian omnivores (*n*22)	
	Mean	SE	Mean	SE	Mean	SE
T (nmol/1)	1·10	0·116	1·89*	0·161	1·32	0·115
Free T (pmol/1)	24·5	3·12	38·7*	4·18	27·5	3·15
E_2 (nmol/1)	0·61	0·096	0·59	0·119	0·71	0·120
Free E_2 (pmol/1)	11·5	1·90	10·20	2·24	11·9	1·96
SHBG (nmol/1)	42**	13·2	56	9·1	58	5·4

Significantly different from Caucasian omnivores and Indian vegetarians: *$P<0·01$.
Significantly different from Caucasians: **$P<0·005$.

Plasma SHBG levels were significantly lower in the Indian women than in the Caucasians, similar to the observation made by Dunkley *et al.* (1989) in postmenopausal women. Total and free testosterone levels were greater in Caucasian vegetarians but total and free oestradiol levels were similar in all groups. This suggests that the difference in incidence of breast cancer in Indian and Caucasian women cannot be explained by differences in bioavailability of oestradiol.

Dunkley, S. A., Reed, M. J., Thomas, B. S., Cruickshank, J. K. & James, V. H. T. (1989). *Journal of Endocrinology* **121,** Suppl., Abstract 304.
Marmot, M. G., Adelsteins, A. N. & Bulusu, L. (1984). *Immigrant Mortality in England and Wales (1970).* London: H.M. Stationery Office.
Miller, G. J., Wheeler, M. J., Price, S. G. K., Beckles, G. L. A., Kirkwood, B. R. & Carson, D. C. (1985). *Atherosclerosis* **55,** 251–258.
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Moore, J. W., Thomas, B. S. & Wang, D. Y. (1986). *Cancer Surveys* **5,** 537–559.
Wynder, E. L. (1980). *Cancer* **46,** 889–904.

Testosterone, sex hormone binding globulin, and calculated free testosterone in male vegans and omnivores. By T. J. A. KEY, *Imperial Cancer Research Fund, Cancer Epidemiology Unit, Radcliffe Infirmary, Oxford OX2 6HE*, L. S. ROE, M. THOROGOOD and J. I. MANN, *Department of Community Medicine and General Practice, Radcliffe Infirmary, Oxford OX2 6HE* and J. W. MOORE, G. M. G. CLARK and D. WANG, *Imperial Cancer Research Fund, Clinical Endocrinology Laboratory, Lincoln's Inn Fields, London WC2A 3PX*

Testosterone (T) may influence the risk of developing prostate cancer (Henderson *et al.* 1982), and there is some evidence that vegetarian diets influence plasma T (Rose, 1986). To investigate this further we measured the concentrations of T and of sex hormone binding globulin (SHBG) in plasma samples from fifty-one male vegans and fifty-seven male omnivores who were non-smokers, were not using long-term medication and had no history of cardiovascular disease. An estimate of the percentage of free T was calculated (% free T = 6·11 −2·38 × $\log_{10}$ SHBG; Nanjee & Wheeler, 1985) and used to estimate the concentration of free T. Hormonal variables were logarithmically transformed for calculations.

		Vegans		Omnivores		
Variable	Adjusted*	Mean	SE Range	Mean	SE Range	P†
Age (years)	No	41·4	39·8–43·0	40·3	38·7–41·9	0·629
Body mass index (kg/m^2)	No	22·2	21·9–22·5	23·2	22·9–23·5	0·035
Alcohol (g/d)	No	14·1	10·9–17·3	19·6	16·6–22·6	0·216
T (nmol/l)	No	15·2	14·6–15·9	14·3	13·8–14·9	0·297
T (nmol/l)	Yes	15·3	14·7–15·9	14·3	13·8–14·8	0·250
SHBG (nmol/l)	No	41·3	39·2–43·5	30·7	29·2–32·3	<0·001
SHBG (nmol/l)	Yes	39·5	37·8–41·4	32·0	30·6–33·3	0·001
Free T (nmol/l)	No	0·34	0·33–0·35	0·36	0·35–0·38	0·253
Free T (nmol/l)	Yes	0·35	0·33–0·36	0·36	0·35–0·37	0·580

*Adjusted for age, body mass index, alcohol, all as untransformed continuous variables.
†2-sided test for difference between groups.

There were no significant differences between vegans and omnivores in total T or calculated free T (Table). SHBG was 23% higher (adjusted value) in vegans than omnivores.

Nutrient intakes calculated from 4 d diet records were available for a subset of eighteen vegans and twenty-two omnivores. The vegans had significantly higher intakes of polyunsaturated fatty acids (PUFA), carbohydrate and dietary fibre, and significantly lower intakes of saturated fatty acids, protein and alcohol. After adjusting for age and body mass index, there were significant correlations between T and PUFA (r 0·38), SHBG and PUFA (r 0·39), SHBG and alcohol (r −0·46) and free T and alcohol (r 0·32).

We conclude that a vegan diet causes a substantial increase in SHBG, but that feedback mechanisms achieve effective homeostasis of free T.

Henderson, B. E., Ross, R. K., Pike, M. C. & Casagrande, J. T. (1982). *Cancer Research* **42**, 3232–3239.
Nanjee, M. N. & Wheeler, M. J. (1985). *Annals of Clinical Biochemistry* **22**, 387–390.
Rose, D. P. (1986). *Progress in Clinical and Biological Research* **222**, 43–68.

Methane production by ruminants and its influence on the doubly-labelled water technique. By A. J. MIDWOOD, P. HAGGARTY, B. A. MCGAW and J. J. ROBINSON, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB*

The doubly-labelled water (DLW) method permits an estimate of carbon dioxide production (r_{CO_2}) in a free-living animal. This technique involves labelling the body water pool with deuterium (^2H) and oxygen-18 (^{18}O) and measuring the difference in the elimination rates of these two isotopes. The difference occurs because the ^{18}O is lost as CO_2 and water, whereas the ^2H is lost as water alone. Any loss of isotope as products other than CO_2 and water will introduce an error into the calculation of r_{CO_2}. Methane production (r_{CH_4}) represents an additional means by which ^2H may leave the body. In order to quantify losses in methane, four sheep were dosed with deuterated water (^2H_2O). A relationship was then established between the enrichment of the methane and urine of these animals which, when expressed as a ratio, had a mean value of 0·654 (see Table). This ratio was unaffected by the level of r_{CH_4} but showed some dependence on the absolute ^2H_2O enrichment of the urine. Hence, analysis of background isotope level in four sheep given no ^2H_2O resulted in a methane:urine enrichment ratio of 0·689. This ratio was unaffected by the composition of the diet.

Animal no.	Initial urine enrichment (ppm ^2H)	Ratio	SD	No. of observations
1	280·99	0·631	0·027	16
2	269·70	0·639	0·010	16
3	241·95	0·674	0·011	14
4	233·38	0·674	0·008	16
Mean		0·654	0·025	62

The proportional error on the isotopically estimated value for r_{CO_2} is dependent on the relative magnitude of r_{CH_4} and r_{CO_2}. Limits to this error were calculated by correcting the observed ^2H_2O flux thus:

$$^2H_2O \text{ flux}_{CH_4} \text{ (mol/d)} = \text{methane:urine enrichment ratio} \times (2 \times r_{CH_4} \text{ (mol/d)})$$

$$\text{Corrected } ^2H_2O \text{ flux} = \text{measured } ^2H_2O \text{ flux} - ^2H_2O \text{ flux}_{CH_4}$$

This corrected ^2H_2O flux can then be used in standard DLW equations (see, for example, Haggarty *et al.* 1988) to calculate r_{CO_2} for a ruminant. By considering the measured ratio of r_{CO_2}:r_{CH_4} from ninety-two studies at this Institute, we can estimate the error on r_{CO_2} that would have been incurred had this correction not been used. The ratio ranged from 20 to 10, and gave rise to error limits on the estimated r_{CO_2} when r_{CH_4} was not taken into account, of between –3·17 and –6·14% respectively using an enrichment ratio of 0·654.

Haggarty, P., McGaw, B. A. & Franklin, M. F. (1988). *Journal of Theoretical Biology* **134**, 291–308.

Repeated periods of dieting by women using a very low energy diet. 1. Effect on metabolic rate. By GAIL R. GOLDBERG, SUSAN A. PARKINSON, JUDITH M. SAVAGE, P. R. MURGATROYD and A. M. PRENTICE, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

Animal studies have shown an increased energetic efficiency and more rapid weight gain in rats subjected to periods of fasting and *ad lib.* refeeding (Brownell, 1986). This situation of weight loss and regain is paralleled in humans by those who frequently diet for short periods of time. However, no studies have investigated changes in metabolic rate, composition of weight lost and regained, or the effects of short-term repeated use of a very low energy diet (VLED) in these 'yoyo dieters' (Department of Health and Social Security, 1987).

We have studied seven healthy women (mean age 44·57 (SD 5·50) years, height 1·61 (SD 0·06) m, weight 84·13 (SD 16·60) kg, body mass index 32·34 (SD 5·65) kg/m^2) for 18 weeks throughout three consecutive cycles of 2 weeks on a VLED (Modifast, Kent Pharmaceuticals Ltd) followed by 4 weeks free-living (FL). The VLED was used as the only source of nutrition during the dieting periods and provided 1891 kJ (445 kcal) and 50 g protein/d. After initial and final measurements (weeks 0 and 18) and at the end of each VLED (weeks 2, 8 and 14) and intervening FL periods (weeks 6 and 12) the following measurements were made: basal metabolic rate (BMR) by whole-body indirect calorimetry; body-weight (BW); total body water (TBW) by deuterium dilution and whole-body density by underwater weighing. Fat free mass (FFM) was calculated from TBW and density using the equation of Siri (1961). Initial values at week 0 and changes are presented in the Table.

Week . . .		0	0–2	2–6	6–8	8–12	12–14	14–18	0–18
BW (kg)	Mean	84·13	–4·35***	1·03	–3·45***	2·61***	–2·62**	1·66	–5·11**
	SD	16·60	1·25	1·31	1·29	0·92	1·80	2·05	2·37
FFM (kg)	Mean	48·60	–0·78	0·65	–1·37	1·12	–0·68	0·23	–0·83
	SD	7·68	1·42	2·08	1·84	1·86	1·36	2·94	2·46
BMR (kJ/d)	Mean	6247	–458**	517**	–234	220*	–320*	261	–15
	SD	1096	259	286	420	220	325	313	238
BMR	Mean	74·59	–1·40	5·56*	–0·03	0·21	–1·15	1·92	–5·17*
(kJ/BW per d)	SD	3·82	2·52	4·17	4·36	2·72	3·76	3·26	4·60
BMR	Mean	128·53	–7·23**	8·72**	–1·31	1·37	–4·34	4·73	–1·94
(kJ/FFM	SD	10·62	4·41	5·11	6·83	8·91	6·38	5·65	8·05
per d)									

*P<0·05, **P<0·01, ***P<0·001 by paired t test v. previous measurement.

On each occasion absolute BMR decreased in response to dieting. These differences were not significant when corrected for BW and the change in BMR/kg FFM was only significant after the first period of dieting. Overall, between weeks 0 and 18 absolute BMR and BMR/kg FFM did not change. BMR/kg BW increased, indicative of the loss of less metabolically active adipose tissue.

Our results in this group of obese women do not suggest that repeated short periods of dieting using a VLED lead to a cumulative decrease in metabolic rate.

Brownell, K. D. (1986). *Physiology and Behaviour* **38**, 459–469.

Department of Health and Social Security (1987). *The Use of Very Low Calorie Diets in Obesity*, COMA Report no. 31. London: H.M. Stationery Office.

Siri, W. E. (1961). In *Techniques for Measuring Body Composition*, pp. 223–243 [J. Brozek and A. Henschel, editors]. Washington DC: National Academy of Sciences/National Research Council.

Repeated periods of dieting by women using a very low energy diet. 2. Effect on body composition. By SUSAN A. PARKINSON, GAIL R. GOLDBERG, JUDITH M. SAVAGE, P. R. MURGATROYD, W. A. COWARD and A. M. PRENTICE, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

One of the principal concerns regarding the use of very low energy diets centres on the relative loss of fat and lean tissue. Further, it has been suggested that the 'yoyo syndrome' of repeated loss and regain of weight experienced by many dieters (using a very low energy diet or conventional low energy diets) leads to a progressive increase in the proportion of body fat, popularly interpreted as 'dieting makes you fat'.

This study attempted to address both these questions. Body composition measurements were carried out on the seven women described previously by Goldberg *et al.* (1990), following their overnight stay in the calorimeter, and after voiding and before food or water. Measurements were made using hydrodensitometry, deuterium dilution, bioelectrical impedance analysis and skinfold thicknesses. The data were analysed using the method described by Murgatroyd & Coward (1989). This employs simultaneous measurements of body density and body water to make an accurate estimate of fat and protein changes, independently of changes in body water. The Table shows baseline measurements and changes in weight, fat, protein and water from one period to the next, assuming no changes in bone mineral.

Weeks		0†	0–2‡	2–6‡	6–8‡	8–12‡	12–14‡	14–18‡
Wt (kg)	Mean	84·13	−4·35***	1·03	−3·45***	2·61***	−2·62**	1·66**
	SD	16·60	1·25	1·31	1·29	0·92	1·80	2·05
Fat (kg)	Mean	35·53	−3·52***	−0·22	−1·63*	1·44	−1·85**	1·56
	SD	9·64	1·01	1·82	1·53	1·99	1·23	1·69
Protein	Mean	12·94	−0·28	0·95	−0·90	0·79	−0·40	−0·39
(kg)	SD	1·67	0·70	1·16	1·60	1·51	1·66	1·74
Water (kg)	Mean	35·67	−0·55	0·32	−0·92*	0·37	−0·36	0·49
	SD	6·23	1·53	1·72	1·08	1·76	1·21	1·71

*$P<0·05$, **$P<0·01$, ***$P<0·001$ by paired t test v. previous measurement.
†Baseline values calculated using the method of Siri (1961).
‡Changes calculated using the method of Murgatroyd & Coward (1989).

The loss of both weight and fat was significantly greater in the first dieting period than in successive dieting periods ($P<0·01$, $P<0·05$ respectively). Free-living periods were not significantly different from each other in terms of weight regain or change in fat or fat-free mass. Overall the weight loss of 5·11 (SD 2·37) kg ($P<0·01$) consisted of a highly significant loss of body fat 4·22 (SD 2·42) kg ($P<0·01$) representing 82·6% of the total weight loss. The loss of protein (0·25 kg, SD 1·68) and water (0·63 kg, SD 1·74) was not significant.

In this group of obese women we have not demonstrated an excessive loss of lean tissue whilst dieting and have no evidence of increasing adiposity despite significant regain in weight between dieting periods.

Goldberg, G. R., Parkinson, S. A., Savage, J. M., Murgatroyd, P. R. & Prentice, A. M. (1990). *Proceedings of the Nutrition Society* **49,** 11A.
Murgatroyd, P. R. & Coward, W. A. (1989). *British Journal of Nutrition* **62,** 311–314.
Siri, W. E. (1961). In *Techniques for Measuring Body Composition*, pp. 223–243 [J. Brozek and A. Henschel, editors]. Washington DC: National Academy of Sciences/National Research Council.

Weight changes during 7 d weighed energy intake studies in normal men. By A. C. BRUCE, A. ZAKARY, J. FELL and G. MCNEILL, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Measurements of free-living energy expenditure obtained using the doubly-labelled water technique suggest that in certain groups of individuals such as obese women there is a tendency for recorded energy intake to underestimate energy requirements during the weighed intake period. This underestimate can be due to either under-recording food eaten or to eating less than requirements while recording intake. The latter possibility can be explored by careful records of weight change over the weighed intake period.

In the present study thirty-nine healthy males (mean age 38 years (range 22–57), mean weight 77·7 kg (range 53–112), and mean body mass index (BMI) 24·4 (range 19·2–35·5)) kept two 7 d records of food intake and simultaneous records of physical activity over two separate weeks. None of the subjects was trying to lose weight and all were asked to eat normally. Energy intake was determined from food composition tables and physical activity level (PAL) was determined as described by McNeill *et al.* (1989). The subjects were weighed on electronic scales (Digi, CMS Weighing Equipment, London) to the nearest 50 g, in light clothing at the same time of day and time after voiding at the start and end of each 7 d period.

The recorded energy intake of the group was 11·30 (SD 2·19) MJ/d in week 1 and 10·66 (SD 2·17) MJ/d in week 2. There was a significant correlation between the energy intake of week 1 and week 2 (r +0·698; $P<0·01$). Analysis by paired t test revealed that the energy intake was significantly ($P<0·05$) lower in week 2. The mean weight loss was 0·28 (SD 0·78) kg/week in week 1 and 0·26 (SD 0·67) kg/week in week 2. This weight loss was significantly different from zero ($P<0·005$). If the energy content of the tissue lost was 29 kJ/g (Garrow, 1981), this weight loss could account for an average difference between actual intake and actual energy requirements of 1·10 MJ/d.

There was no significant correlation between weight change and BMI in week 1 (r −0·008; $P<0·05$) or in week 2 (r −0·027; $P<0·05$). In addition there was no significant correlation between energy intake and weight change in week 1 (r 0·182; $P>0·05$) or in week 2 (r 0·002; $P>0·05$). Energy intake was also correlated with some potential predictors of energy requirement (see Table). Only 20% of the variance in energy intake can be accounted for by estimated energy requirement (estimated basal metabolic rate (BMR) × PAL) even when corrected for weight change.

Correlations (r) with recorded energy intake

	Week 1	Week 2	Both weeks
Body-wt	0·22 (NS)	–0·26 (NS)	0·26 (NS)
Estimated BMR	0·25 (NS)	0·29 (NS)	0·30 (NS)
PAL	0·25 (NS)	0·50 ($P<0·001$)	0·39 ($P<0·02$)
Estimated BMR × PAL	0·33 ($P<0·05$)	0·52 ($P<0·001$)	0·46 ($P<0·001$)
Estimated BMR × PAL*	0·34 ($P<0·05$)	0·50 ($P<0·001$)	0·47 ($P<0·001$)

*Corrected for weight change (29 kJ/g).
NS, not significant.

We suggest that measuring weight change in groups of individuals may be a useful technique for assessing whether a general alteration in normal food intake occurs in response to keeping a 7 d weighed food record. However, correcting estimated energy requirements of individuals for weight change does not seem to help to explain the wide inter-individual variation in recorded energy intake.

Garrow, J. S. (1981). *Treat Obesity Seriously*. London: Churchill Livingstone.
McNeill, G., McBride, A., Smith, J. S. & James, W. P. T. (1989). *Nutrition Research* **9**, 363–370.

Energy intake and energy expenditure in post-obese women and weight-matched controls.

By G. McNeill, S. G. F. Bukkens, D. C. Morrison and J. S. Smith, *Rowett Research Institute, Aberdeen AB2 9SB*

Women who have lost weight often consider that their energy requirements are less than those of women of similar weight who have never been overweight. A recent study of 24 h energy expenditure (Geissler *et al.* 1987) lends support to this view, although the intake provided during the expenditure measurements was based on self-recorded intake, and activity during the measurements was not tightly controlled. We have carried out a similar study which attempts to avoid some of the difficulties in interpretation of the study cited.

Six women who had lost 15 kg or more and six women who had never been much above their current weight completed the 14 d residential protocol. Mean ages of the post-obese and lean groups were 42·3 (SD 13·2) and 38·8 (SD 13·1) years respectively. Weights and heights were 61·9 (SD 8·2) kg and 1·601 (SD 0·114) m in the post-obese group and 63·3 (SD 7·1) kg and 1·596 (SD 0·077) m in the control group. Fat-free masses (estimated from four skin-fold thickness sites) were 43·6 (SD 6·6) kg in the post-obese group and 42·6 (SD 5·6) kg in the lean group. None of these differences was statistically significant. Energy intakes for days 4–14 were based on observed weight changes from days 1 to 4, and were 7·79 (SD 0·55) MJ/d in the post-obese women and 8·87 (SD 1·04) MJ/d in the control women ($P<0·05$). Weight changes between days 4 and 14 were −0·70 (SD 0·48) kg in the post-obese women and −0·28 (SD 0·40) kg in the control women ($P>0·05$), suggesting that there was a small energy deficit, notably in the post-obese group.

The values for basal metabolic rate (BMR) (from duplicate measurements by ventilated hood indirect calorimetry) and those for 24 h energy expenditure (EE) and sleeping metabolic rate (SMR) from duplicate measurements by whole-body indirect calorimetry with a controlled activity programme, are shown in the Table. Means for all measurements were a little lower in the post-obese group, but none of the differences achieved statistical significance, due to the heterogeneity of both groups.

		BMR	BMR/kg FFM	EE	EE/kg	SMR	SMR/kg FFM
Post-obese	Mean	5·421	0·125	7·942	0·129	5·172	0·120
(*n* 6)	SD	0·692	0·007	0·759	0·014	0·564	0·010
Controls	Mean	5·509	0·129	8·497	0·135	5·549	0·131
(*n* 6)	SD	0·753	0·008	0·553	0·009	0·540	0·008
Post-obese as % control	Mean	98·4	96·9	93·5	95·6	93·2	91·6

All values in MJ/d.
$P>0·05$ for all measurements.

Free-living energy intakes, recorded by the weighed inventory method for 7 d after the residential study, were 6·24 (SD 1·43) MJ/d in the post-obese and 8·22 (SD 1·43) MJ/d in the controls ($P<0·02$). Isotopically labelled water studies are now in progress to assess whether this difference is due to lower free-living energy expenditure in the post-obese women, or to a greater tendency to change eating habits or under-report during weighed intake studies in women who have succeeded in losing weight.

The authors thank Scottish Slimmers (Aberdeen) for help with recruitment of the post-obese subjects.

Geissler, C. A., Miller, D. S. & Shah, M. (1987). *American Journal of Clinical Nutrition* **45**, 914–920.

Resting energy expenditure in cystic fibrosis. By S. A. WOOTTON, J. ELLIS, S. BOND and A. A. JACKSON, *Department of Human Nutrition, Southampton University, Southampton SO9 3TU*

Malnutrition and growth retardation have long been associated with cystic fibrosis (CF). Reduced gross energy intake from the diet and impaired maldigestion/malabsorption would limit metabolizable energy, whilst raised energy requirements through some basic cellular defect, laboured ventilation and undercurrent infection would also contribute to an energy deficit sufficient to limit growth or cause weight loss. Several workers have reported increases in energy expenditure in patients with CF (Vaisman *et al.* 1987; Buchdahl *et al.* 1988). However, one of the greatest difficulties in interpretation of the existing data has been the contentious issue of the selection of an appropriate control or reference group.

The aim of the present study was to compare the resting energy expenditure (REE) of twenty-five patients with CF (fourteen male, eleven female) with that of sex-matched healthy control (C) subjects of comparable body-weight (CF: 33·5 (SE 2·4) *v.* C: 32·8 (SE 2·0) kg), lean body mass (CF: 29·0 (SE 2·3) *v.* C: 28·0 (SE 1·7) kg) and predicted REE (CF: 5023 (SE 328) *v.* C: 5009 (SE 136) kJ/d). This was achieved by comparing the REE of CF patients against that of younger controls (CF: 13·5 (SE 0·9) *v.* C: 9·8 (SE 0·5) years; $P<0·01$).

REE was determined by indirect calorimetry using a ventilated hood in the CF group at a time when they were comparatively well and free from acute lung infection. The REE of the CF patients was approximately 22% greater than that of the control subjects whether expressed in absolute units (CF: 5444 (SE 328) *v.* C: 4414 (SE 290) kJ/d; $P<0·05$), per unit body-weight (CF: 167 (SE 5) *v.* C: 138 (SE 7) kJ/kg per d; $P<0·01$), per unit body-weight$^{0·75}$ (CF: 395 (SE 10) *v.* C: 325 (SE 16) kJ/kg$^{0·75}$ per d; $P<0·01$) or per unit lean body mass (LBM) (CF: 195 (SE 6) *v.* C: 161 (SE 8) kJ/kg LBM per d; $P<0·01$). The World Health Organization predictive formula (Food and Agriculture Organization/World Health Organization/United Nations University, 1985) over-estimated the REE of the control subjects by approximately 13%, whereas the REE measured in the CF patients was approximately 7% greater than predicted from age and body-weight.

These results add further weight to the view that energy requirements are raised in CF even when the patient is relatively well. The raised REE cannot be attributed to differences in relative adiposity between CF patients and controls in this study. It remains to be seen, however, the extent to which energy requirements may alter further during periods of infection and the subsequent period of convalescence.

The support of the Cystic Fibrosis Research Trust (UK) is gratefully acknowledged.

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Energy intakes and weight loss in institutionalized psychogeriatric patients. By RHODA SUTHERLAND, J. RUCKER and S. A. WOOTTON, *Department of Human Nutrition, Southampton University, Southampton SO9 3TU*

Many psychogeriatric patients (particularly Alzheimers and dementia) are underweight on admission to long-stay institutions and lose weight during hospitalization (Asplund *et al.* 1981). Demented patients may refuse food and fail to either recognize or communicate hunger. Energy intake could be further impaired as a result of inadequate meal provision and insufficient assistance with feeding. The aim of the present study was to specifically assess the food intake of a group of weight-losing institutionalized psychogeriatric patients.

Weighed food intake was recorded over 7 d in ten weight-losing female patients with advanced dementia (three Alzheimer and seven multiinfarct dementia) in a long-stay psychogeriatric unit (age 77 (SE 2) years, 43·1 (SE 1·9) kg, body mass index 18·5 (SE 0·6) kg/m^2, lean body mass 35·7 (SE 1·4) kg, weight loss since admission 15 (SE 2)%). Energy intake was estimated from a computerized food composition database. Resting energy expenditure (REE) was determined by indirect calorimetry using a ventilated hood.

Energy intake was generally low (5·7 (SE 0·5, range 2·1–7·9) MJ/d) and each of the ten patients consumed less than that generally recommended for elderly healthy populations (Department of Health and Social Security, 1979). REE ranged from 4·0 to 6·2 (mean 5·0 (SE 0·3) MJ/d). The ratio of energy intake:REE ranged from 0·51 to 1·53 (mean 1·11 (SE 0·10)) with nine of the ten patients consuming less than 25% more energy than that used at rest. The difference between energy served and energy consumed (plate waste) ranged from 0 to 54% (mean 16 (SE 6)%). Nine of the ten patients would still have energy intakes less than that generally recommended even if all of the food served was consumed. Energy served:REE ranged from 1·06 to 1·61 (mean 1·30 (SE 0·06)) with eight patients being served energy intakes less than 1·4 times their REE. These results suggest that inadequate food provision, assistance with feeding and/or appetite resulted in low energy intakes sufficient to cause weight loss in this group of patients.

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Arm circumference and energy balance in critical illness. By CERI J. GREEN[1], T. R. HELLIWELL[2], P. MCCLELLAND[3], A. A. GILBERTSON[1], R. G. WILKES[1], J. M. BONE[3] and I. T. CAMPBELL[1], [1]*Intensive Therapy Unit, University Department of Anaesthesia,* [2]*Department of Pathology* and [3]*Department of Renal Medicine, Royal Liverpool Hospital, Liverpool L69 3BX*

An imbalance of energy intake (EI) and expenditure (EE) is normally manifest as a change in body mass. In the critically ill, anthropometric or isotope dilution techniques of measuring energy balance are confounded by acute changes in fluid balance. We have investigated the relation between energy balance (EB), calculated from EI and EE, and changes in mid-arm circumference (MAC) and total body water (TBW), both measured every 1–3 d. In five patients, changes in muscle fibre size obtained by biopsy (tibialis anterior) were also quantified.

Twenty-eight ventilated patients were studied. EI was intravenous. EE was measured continuously using an Engstrom metabolic computer (Engstrom, Bromma, Sweden). Patients were studied for 4–30 (median 12) d. Eight were in respiratory failure secondary to cardiovascular or respiratory disease and twenty to surgical sepsis or trauma. Twenty-one were in acute renal failure. Sixteen died and twelve returned to the ward.

Total body impedance was measured using a simple impedance meter (Green *et al.* 1989) and TBW was calculated using the regression equation of Lukaski (1985). Changes in MAC with time were quantified using linear regression. In twelve patients there was a significant negative correlation. Energy and urinary nitrogen balances of the two groups are shown in the Table.

	Energy balance (kJ/d)		Energy balance (kcal/d)		N balance† (g/d)		% EE derived from protein	
	Median	Range	Median	Range	Median	Range	Median	Range
Negative correlation	−795	−2510 to +2092	−190	−600 to +500	−10·5	−19·7 to +2·2	22·8	14 to 35·6
No correlation	−460	−11882 to +2656	−110	−2840 to +635	−10·2	−21·8 to +0·3	22·0	13 to 41·2

†Abnormal losses ignored.

In the patients whose MAC decreased with time, five were in positive EB and seven were in negative EB. Of the patients with no change in MAC, six were in positive balance and ten were in negative energy balance. TBW decreased significantly ($P<0·005$) in the group in which MAC decreased. In the other group it did not alter overall but showed wide variations from day to day.

Muscle biopsies were performed 4–30 (median 7) d apart in five patients. In two patients in the 'wasting' group, fibre size decreased by 0·9 and 1·5%/d, and in the non-wasting group by 7·5 (SD 2·0)%/d.

It appears that these patients were losing lean body mass (LBM) regardless of EB. MAC appears to be a useful index of LBM in some patients, particularly if some method is available of assessing TBW.

Green, C. J., Hammond, P. & Campbell, I. T. (1989). *Proceedings of the Nutrition Society* **48**, 107A.
Lukaski, H. C. (1985). In *Human Body Composition and Fat Distribution*, p. 153 [N. G. Norgan, editor]. Wageningen: Euro Nut.

Plasma noradrenaline concentrations and thermogenic responses to injected noradrenaline in the rat. By A. Y. SIYAMAK and I. A. MACDONALD, *Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH*

Diet-induced thermogenesis and cold-induced non-shivering thermogenesis are frequently assessed by determining the metabolic rate (MR) response to noradrenaline injected subcutaneously (s.c.) or intraperitoneally (i.p.). There have been many studies assessing the dose-response characteristics in the rat, but information is lacking on the plasma noradrenaline (NA) concentrations achieved. The purpose of this study was to determine the changes in MR and plasma NA concentrations occurring following the s.c. injection or intravenous (i.v.) infusion of NA in the conscious rat.

Twenty-one male Charles River CD rats (280–320 g) were housed in pairs and fed *ad lib.* on stock diet. Each rat was anaesthetized with sodium methohexitone (60 mg/kg i.p.; Brietal, Lilly) and catheters implanted in the abdominal aorta, via the caudal artery, and the right atrium via the jugular vein. Catheters were filled with heparinized saline and exteriorized at the back of the neck, being held in place with a collar and spring. All rats were allowed to recover for 16–18 h before experimentation. In eight rats the arterial or right atrial plasma NA and MR responses to s.c. noradrenaline (400 μg/kg) were determined over a 2 h period. In two other groups of rats, NA was infused i.v. for 20 min at rates of 0·4 μg/kg per min (five rats) or 1·5 then 3·0 μg/kg per min (eight rats) with arterial plasma NA and MR being determined before, during and after each infusion. Plasma NA (0·2 ml sample) was measured as described by Macdonald & Lake (1985), and MR was measured continuously with an open circuit system using an oxygen analyser (Servomex OA 272) and flowmeter (Hastings ENALL–5K) connected to a BBC–B computer.

The resting MR was similar in all three groups (mean (SEM) ranging from 334 (33) to 360 (14) J/kg per min). The s.c. injection of NA increased MR by 54·0 (10·2)% over a 2 h period (with a peak response of 68 (15)%). In five rats arterial plasma NA increased from 2·5 (1) (baseline) to a peak value of 54·4 (6·6) nmol/l at 90 min, whereas in three other rats right atrial plasma NA increased from 2·4 (1) to 147 (6·6) nmol/l at 90 min. Although the i.v. infusion of 0·4 μg NA/kg per min increased arterial plasma NA to 18 nmol/l, there was no significant increase in MR. By contrast the infusion of 1·5 μg/kg per min increased arterial plasma NA to 72·4 nmol/l and MR by 71·3 (6·9)%, whilst doubling the infusion rate caused plasma NA to double but only slightly affected MR (91·3 (7·4)% above baseline).

Thus, injection of NA s.c. produces a marked, sustained increase in plasma NA and an associated prolonged MR response. The plasma NA threshold for increasing MR is in excess of 18 nmol/l, i.e. more than six times higher than baseline plasma NA levels.

Macdonald, I. A. & Lake, D. M. (1985). *Journal of Neuroscience Methods* **13**, 239–248.

Thermic effect of artificial sweeteners in humans: aspartame. By C. A. GEISSLER and D. A. JAMES, *Department of Food and Nutritional Sciences, King's College, University of London, Campden Hill Road, London W8 7AH*

Artificial sweeteners are widely used in food products and beverages, often to reduce energy content. It was of interest to investigate whether sweeteners, in this case aspartame, had an effect on human energy expenditure as well as intake.

Thermic effect (TE) of aspartame was measured by indirect calorimetry in twelve female subjects (mean (SD) weight 60·6 (7·1) kg, body mass index 21·63 (1·89)). Aspartame (A) was administered as two tablets of 'Flix' (Nutrasweet, Searle, High Wycombe, Bucks), each weighing 72·09 mg and containing 12·2 mg aspartame, 0·05 g carbohydrate, 0·015 g protein and 0 g fat (1·1 kJ (0·25 kcal)), along with a 37·9 mg gelatin capsule (G) and 120 g water (W). The three reported treatments, administered on separate days in a Latin-square design after fasting resting metabolic rates were measured, were: (1) W + G only (control); (2) A in W + G (dissolved aspartame); (3) A in G + W (encapsulated aspartame). TE was then measured every 20 min for 160 min.

Over 160 min the mean (SD) % rise in metabolic rate above baseline (TE) was 1·82 (1·95) for control, 8·27 (2·65) for dissolved aspartame, and 5·83 (3·98) for encapsulated aspartame. Dissolved aspartame elicited a response with an initial peak at 20 min (10·68%) and a second at 140 min (8·94%) and had not returned to baseline levels at 160 min (5·02%). Encapsulated aspartame gave a single main peak at 100 min (8·51%) and the response at 160 min was also still above baseline (4·23%).

Aspartame tablets, both dissolved and encapsulated, therefore have a significant TE ($P<0·001$), dissolved aspartame significantly greater than encapsulated aspartame (critical differences ($P<0·05$) between treatments 1·61). Part of the response is therefore cephalic, although not mediated by insulin release (Okuno *et al.* 1986; Horwitz *et al.* 1988; Carlson & Shah, 1989), the rest due to the later metabolism of aspartame or carrier substances. The lactose carrier is unlikely to be responsible for the thermic effect in the quantities (0·1 g) involved (Macdonald, 1984). Aspartame is metabolized to aspartate, phenylalanine and methanol and may affect energy metabolism by mechanisms including catecholamine synthesis. At the levels ingested this could not be due to changes in plasma amino acids (Stegink *et al.* 1987). These effects may be mediated by the dipeptide aspartame acting on gastrointestinal neuroendocrine systems.

These results indicate that aspartame could affect energy balance to a greater extent than by reduction of energy intake alone, resulting in a total difference in the order of 200 kJ (50 kcal) for a drink sweetened with aspartame compared with a sucrose-sweetened drink.

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The effect of a 3 d high-fat or high-carbohydrate diet on the responses to glucose ingestion in man. By M. B. SIDERY, I. W. GALLEN and I. A. MACDONALD, *Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH*

We have reported that underfeeding at 60 kJ/kg for 7 d (Mansell & Macdonald, 1988) and a 48 h fast (Gallen *et al.* 1989) alter some of the physiological responses to the ingestion of a test meal, and produce a degree of glucose intolerance in healthy subjects. This study was designed to examine whether a similar effect could be produced by modifying carbohydrate intake, whilst maintaining energy intake.

Eight normal weight, healthy subjects (five male, aged 18–24 years), were studied on two occasions, either after 3 d on a high-carbohydrate diet (C) or a high-fat diet (F) (70 and 60% of total energy intake respectively) with normal total daily energy intake. Subjects were studied whilst resting supine, wearing a T-shirt and shorts only, in a temperature-controlled room (30°). Measurements were made of metabolic rate (MR), heart rate (HR), blood pressure (BP) and forearm blood flow (FBF) by venous occlusion plethesmography for 30 min before and 80 min after the ingestion of a 1·5 g glucose/kg load. Analysis of arterialized venous blood glucose and insulin, and forearm muscle effluent venous blood glucose concentrations were made over the same period.

The high-fat diet had no effect on baseline MR (mean (SE) C, 4·7 (0·3); F, 4·6 (0·3) kJ/min), plasma glucose (C, 4·2 (0·3); F, 4·2 (0·1) mmol/l), insulin (C, 5·4 (1·0); F, 5·1 (1·4) mU/l) or upon forearm glucose uptake (C, 1·1 (0·5); F, 1·0 (0·4) μmol/l per min). Baseline HR, systolic BP, diastolic BP and FBF were also similar. After glucose ingestion, the rises in MR (C, +0·9 (0·3); F, +0·4 (0·2) kJ/min), glucose (F, +6·3 (0·7); C, +4·9 (0·8) mmol/l), HR and systolic BP were similar in the two states. However, the rise in FBF was significantly ($P<0·05$) less (F, +8 (2); C, +23 (3) ml/l per min), the fall in diastolic BP significantly ($P<0·01$) greater (F, −8·5 (2); C, −1·0 (3) mmHg) and the rise in insulin significantly ($P<0·01$) higher (F, +87 (11); C, +53 (10) mU/l) in F than C subjects. Forearm glucose uptake increased to a similar extent in both states (C, +7·3 (3); F, +7·7 (3) μmol/kg per min). Hence 3 d on a high-fat diet had little effect on the thermic and cardiovascular responses to glucose, but induced a modest degree of insulin resistance. Thus the changes seen in the physiological response to food after 7 d of underfeeding or a 48 h fast are likely to be due to a reduction in energy intake, whereas the impaired glucose tolerance is simply due to a reduction in carbohydrate intake.

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Net substrate deposition in human adipose tissue in vivo after glucose ingestion and during insulin infusion. By KEITH N. FRAYN, SIMON W. COPPACK and SANDY M. HUMPHREYS, *Sheikh Rashid Diabetes Unit, Radcliffe Infirmary, Oxford OX2 6HE*

Despite current interest in the regulation of body-weight, we still understand little of the normal physiology of human adipose tissue. We have studied adipose tissue metabolism in vivo in healthy subjects. Blood samples were collected from a vein draining the subcutaneous adipose tissue of the abdominal wall, from a deep antecubital vein draining forearm muscle, and from a vein draining a heated hand (arterialized), after an overnight fast and then either for 120 min following ingestion of 75 g glucose monohydrate (eight studies), or during insulin infusion at 35 mU/m^2 per min for 120 min with the plasma glucose concentration 'clamped' at a euglycaemic level (eight studies). Fluxes of different substrates were compared in terms of gram-atoms of carbon (per litre of blood flow, which was not measured in the adipose tissue in these studies).

In the fasting state, adipose tissue was a net exporter of C, mainly in the form of non-esterified fatty acids (NEFA), with smaller contributions from glycerol, lactate and carbon dioxide. Ketone bodies and acetate were taken up, but their contributions were small. Glucose and triacylglycerol (TAG) were taken up with small percentage extractions (1·4 and 4·5% respectively). Forearm muscle was almost in 'C balance', with uptake of NEFA the main source of incoming C, and release of CO_2 the main route of loss. The mean values were: adipose tissue, −5530; muscle, −90 μg-atoms C/l blood, where the minus sign denotes net C efflux. (These measurements do not include amino acids, which would make both tissues more 'catabolic'.)

After glucose ingestion, both adipose tissue and forearm muscle became 'anabolic'; at 60 min mean C fluxes were: adipose tissue, +5800 μg-atoms C/l; muscle, +8150 μg-atoms C/l blood. The main cause for the switch from 'catabolic' to 'anabolic' in adipose tissue was suppression of NEFA release (mean change from basal 4870 μg-atoms C/l), although increased extraction of glucose and of TAG also contributed. In muscle the main change from fasting was increased glucose extraction (to 19%; mean change from basal 9800 μg-atoms C/l).

During insulin infusion, forearm muscle became 'anabolic' to an extent similar to that seen after glucose ingestion (at 60 min, mean C flux +5480 μg-atoms C/l). Adipose tissue, however, did not become 'anabolic' even after 60 min of infusion (0 μg-atoms C/l) and was only just so after 120 min (+90 μg-atoms C/l; cf muscle +6720 μg-atoms C/l).

We conclude that net substrate deposition in adipose tissue after carbohydrate loads involves sparing of fat mobilization to a greater extent than increased substrate uptake; and that the switch to 'anabolism' in adipose tissue after glucose ingestion may not be due simply to the rise in insulin concentration.

A modified TRAP assay to measure pro-oxidant activity in serum. By D. I. THURNHAM, *Dunn Nutrition Laboratories, Milton Road, Cambridge CB4 1XJ* and D. KWIATOWSKI, *MRC Laboratories, Fajara, The Gambia, West Africa*

Free radicals are trapped in plasma by a variety of antioxidants including vitamins E and C. The total radical-trapping potential (TRAP) of serum or plasma can be measured by exposure to a free radical initiator 2,2'-azo-bis-(2-amidinopropane hydrochloride) (ABAP; Polysciences, Warrington, PA, USA) in an oxygen electrode and the delay in the onset of ABAP-induced peroxidation of added linoleic acid (LA) can be internally calibrated in μmol/l by comparison with the delay produced by a known amount of added antioxidant (TROLOX; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Wayner *et al.* 1987). In theory any pro-oxidant (PO) activity in serum can be ignored since its effect will be similar on sample and standard but, in practice, large discrepancies between measured and theoretical TRAP activities have been observed (Thurnham *et al.* 1988). A modified method is described to measure PO activity by carrying out the following steps at 5 min intervals.

Buffer in the cell was aerated, O_2 uptake measured without (basal) then with the sample-LA mixture and finally with ABAP. PO activity was calculated as the increase in O_2 uptake above basal over 5 min as a percentage of the maximum possible. In most samples less than 4% of O_2 within the O_2 electrode cell is consumed by PO activity over 5 min. Haemoglobin, as a result of haemolysis, is the main cause of PO activity in serum but serial dilutions of erythrocyte lysate in non-haemolysed plasma suggest that, provided samples contain no visible pink coloration ($\geq$1:800), measurements of TRAP are unaffected and PO activity is very low. The only consistent exception to this of which we are aware is in serum from malaria patients where non-haemolysed samples can contain considerable PO activity and this is currently being investigated. Discrepancies between measured and theoretical TRAP values occur probably because PO activity is initiated by vigorous mixing necessary to suspend LA in serum before addition to the O_2 electrode and any delay in adding the mixture to the electrode will deplete the antioxidant capacity of the plasma before it can be measured by the TRAP assay.

In conclusion, haemoglobin has auto-oxidative properties and interferes with the TRAP assay; thus, any samples showing pink coloration due to haemolysis cannot be used. Where discrepancies between calculated and experimentally determined TRAP values still occur, the above modification will detect and assess the magnitude of PO activity in such samples.

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Alcohol consumption and measurements of iron status. By J. J. STRAIN[1], K. A. THOMPSON[2], M. E. BARKER[2] and P. G. MCKENNA[1], [1]*Biological and Biomedical Sciences Department* and [2]*Centre for Applied Health Studies, University of Ulster at Coleraine, Cromore Road, Coleraine BT52 1SA, Northern Ireland*

Alcohol can cause liver damage resulting in a rise in serum ferritin (SF) but there is also a high occurrence of hepatic iron overload in alcoholics (Chapman *et al.* 1982). Indeed in alcoholics with mild liver disease there is a significant correlation between Fe stores (FeS) and SF (Kristenson *et al.* 1981).

In a random sample of the Northern Ireland population (aged 18–64 years) alcohol consumption was elicited by questionnaire, and haemoglobin (Hb), SF, transferrin saturation (TS) and mean corpuscular haemoglobin concentration (MCHC) were measured in blood samples by standard methods (Barker *et al.* 1989). FeS were calculated using the equations of Ballot *et al.* (1989). Three distinct groups of subjects with respect to FeS were obtained on the basis of sex and menstrual status. Subjects were classified as regular drinkers (once a week or more often) or non-drinkers (including occasional drinkers). Reported mean (SD) alcohol consumption of regular drinkers in units/week (see Barker *et al.* 1989) for men, women, aged 18–44 years and women, aged 45–64 years were: 24 (21·5), 11 (9·5) and 9 (6·2) respectively. Distributions of SF were normalized by logarithmic transformation for analysis of variance with age as a covariate and all measurements are given in the Table. Approximately 5% of subjects had missing TS values.

		Hb (g/l)		SF (μg/l)		TS (%)		MCHC (%)		FeS (mM)	
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Men aged 18–64 years											
Drinkers	134	150	11·2	121·9***	100·09	26·1*	11·30	33·8	1·13	14·3***	5·80
Non-drinkers	84	147	11·4	82·7	59·79	22·2	7·01	33·8	2·02	11·8	5·95
Women aged 18–44 years											
Drinkers	75	132	11·7	34·0	29·72	21·5	8·75	33·5	1·62	5·4	5·76
Non-drinkers	115	130	9·7	35·0	25·51	21·5	9·59	33·3	1·53	5·2	6·26
Women aged 45–64 years											
Drinkers	30	139**	9·2	88·7***	88·47	21·3	6·67	33·7	1·05	11·8**	6·07
Non-drinkers	61	132	13·7	47·5	34·81	18·6	5·95	32·8	4·38	6·9	6·47

Significantly different with respect to non-drinkers: $*P<0·05$, $**P<0·01$, $***P<0·001$.

Results indicate that regular drinking increased some indicators of Fe status in men and older women. However, there were no significant correlations (Spearman) between quantity of alcohol consumed and measurements of Fe status apart from TS (r 0·29, $P<0·01$) in the younger women.

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Relation of plasma levels of antioxidants and cholesterol to the risk of colorectal cancer and polyps in Ireland. By KATHRYN R. O'SULLIVAN[1], P. M. MATHIAS[1], J. J. STRAIN[3], D. G. M. CARVILLE[3], A. TOBIN[2] and C. O'MORAIN[2], [1]*Dublin Institute of Technology, Kevin Street, Dublin,* [2]*Adelaide and Meath Hospitals, Dublin, Irish Republic* and [3]*Biomedical Sciences Research Centre, University of Ulster, Coleraine, Northern Ireland*

In a previous communication (O'Sullivan *et al.* 1989) preliminary results were presented on blood profiles of cholesterol and certain antioxidants in normal (N) patients and those with colonic polyps (P) and colorectal cancer (C). This communication summarizes results from the completed study, with an estimation of relative risks in relation to these nutritional profiles. The Table shows the values for cholesterol (CH), tocopherol (T), tocopherol:cholesterol ratios (T:C), retinol (R) and selenium in plasma, and activities of glutathione peroxidase (GSHPx; *EC* 1.11.1.9) and superoxide dismutase (SOD; *EC* 1.15.1.1) in erythrocyte lysates.

		CH (mmol/l)		R (μmol/l)		T (μmol/l)		T:C (μmol:mmol)		Se (μmol/l)		GSHPx (U/gHb)		SOD (U/gHb)	
Group	*n*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
N	71	5·7	1·3	1·52	0·85	16·4	5·5	3·1	1·2	0·9	0·3	147	148	26	15
P	57	6·2	1·4	1·33	0·58	12·5	7·5	2·1	1·4	0·7	0·36	162	124	28	11
C	53	5·0	1·6	1·08	0·61	11·9	7·2	2·6	1·7	0·69	0·3	112	80	29	17

Hb, haemoglobin.

Significant differences were found between the C and N groups for T ($P<0·001$), R ($P<0·005$) and Se ($P<0·005$), and between the N and P groups for T ($P<0·005$), Se ($P<0·025$) and T:C ($P<0·001$). CH was lower in the C group when compared with either P or N ($P<0·025$). Relative risks for polyps and cancer in relation to these nutrient levels were estimated by Mantel–Haenszel methodology. The Table below gives odds ratios for relative risk by tertiles (lowest *v.* highest) of cancer among normals (RR-CN), polyps among normals (RR-PN) and cancer among polyps (RR-CP), together with the 95% confidence interval (CI).

Lowest tertile	RR-CN	95% CI	RR-PN	95% CI	RR-CP	95% CI
R ≤ 0·98 μmol/l	3·0*	1·2– 7·8	1·3	0·5–3·0	2·4*	0·9–6·6
T ≤ 12·9 μmol/l	4·7*	1·6–13·2	3·9*	1·6–9·2	1·4	0·5–4·2
T:C ≤ 2·3	2·8*	1·0– 7·9	3·2*	1·1–9·1	1·1	0·3–3·9
Se ≤ 0·7 μmol/l	2·8*	1·1– 7·4	2·8*	1·2–6·2	1·0	0·4–2·9
GSHPx ≤ 76·9 U/Hb	1·8*	0·5– 6·2	0·5	0·2–1·6	3·6*	1·0–14
CH ≤ 5 mmol/l	2·1*	0·9– 5·2	0·5	0·2–1·2	4·2*	1·6–11

*Significant risks where odds ratio >1·8.

A significant risk for cancer in normals was present when Se, R, T, CH and T:C were below the lowest tertiles. There was a significant risk for polyps in normals when T, T:C and Se were below the lowest tertiles, and a significant risk of cancer among polyps when R, GSHPx and CH were below the lowest tertiles.

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Diet and cancer of the stomach, colon, rectum, breast and lung: a prospective cohort study. By P. A. VAN DEN BRANDT[1], R. A. GOLDBOHM[2], P. VAN'T VEER[2], R. J. J. HERMUS[2] and F. STURMANS[1], [1]*Department of Epidemiology, University of Limburg, PO Box 616, 620 MD Maastricht, The Netherlands* and [2]*Department of Nutrition, TNO-CIVO Toxicology and Nutrition Institute, Zeist, The Netherlands*

In 1986, a prospective cohort study on diet and cancer was started in The Netherlands. The purpose of this study is to investigate the promotional or preventive effects of various nutrients on the development of gastric, colorectal, breast and lung tumours. In particular, the effects of fat, fibre, alcohol, β-carotene, vitamins C and E, selenium, nitrate and sodium will be evaluated.

In this study, the cohort is selected from population registers of 204 municipalities with various degrees of urbanization. The cohort (*n* 120852) is composed of 48·2% men and 51·8% women from the age group 55–69 years. The contrast in dietary habits in the cohort is increased by over-representation of individuals with special dietary habits. At baseline (1986), the cohort members completed a self-administered questionnaire on diet and potential confounding variables (e.g. smoking, occupation, medical history). In addition, about 67% of them also provided toenail clippings, which are being used to assess selenium status.

Follow-up for cancer incidence consists of record linkage to PALGA, a nationally operating register of pathology reports and to cancer registers. During the first 5 years of follow-up, approximately 250 cases of stomach cancer, 450 of colon cancer, 300 of rectal cancer, 800 of breast cancer and 1200 of lung cancer are expected to arise from this cohort, taking mortality into account.

For reasons of efficiency a case-cohort approach (Miettinen, 1985; Prentice, 1986) is applied, in which a random subcohort (*n* 5000) has been selected from the large cohort after the baseline measurement. Detailed follow-up information is being collected on the subcohort to estimate the person-time experience of the cohort. Questionnaires and toenail clippings of this group (that can already be processed during the course of the study) will be compared with those of incidence of cancer cases, yielding exposure-specific incidence rate ratios. Furthermore, the intra-individual variation in determinants is estimated by annually repeated measurements (*n* 250) within the subcohort.

Toenail Se levels from a random sample of the subcohort are shown in the Table together with results from other studies. Substantial differences probably reflect intake differences due to different Se contents of the soil.

			Toenail Se (ppm)	
Study	Country	*n*	Mean	SD
This cohort	Netherlands	48	0·64	0·15
Morris *et al.* (1983)	USA: Boston	9	0·74	0·13
	Georgia	24	0·18	0·14
	South Dakota	15	1·17	0·35
	New Zealand	14	0·26	0·09
Van Noord *et al.* (1987)	Netherlands: breast cancer	27	0·83	0·21
	controls	120	0·80	0·21
Van't Veer *et al.* (1990)	Netherlands: breast cancer	124	0·63	0·12
	controls	236	0·65	0·18

This study is supported by grants from the Netherlands Cancer Foundation, the Dutch Ministry of Welfare, Public Health and Cultural Affairs, and the EC (Europe against Cancer).

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Alzheimer's senile dementia: potential pathogenic significance of free radical and antioxidant micronutrient interactions. By P. H. EVANS, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*, J. KLINOWSKI, *University Department of Physical Chemistry, Cambridge* and E. YANO and N. URANO, *Department of Public Health, Teikyo University, Tokyo, Japan*

The identification of aluminosilicate deposits within the characteristic neuritic plaques present in the brains of subjects with Alzheimer's senile dementia (Candy *et al.* 1986), has prompted hypothetical proposals as to the pathogenic significance of such potentially hazardous inorganic particulate material (Evans, 1988).

We have examined the capacity of model natural and synthetic aluminosilicate zeolite particles of differing crystal structure, size, shape and ionic composition, to stimulate the phagocytic respiratory burst of human blood polymorphonuclear leukocytes (PMN) in vitro. The associated production of oxygen-derived free radicals was monitored in real time by luminol-dependent chemiluminescence.

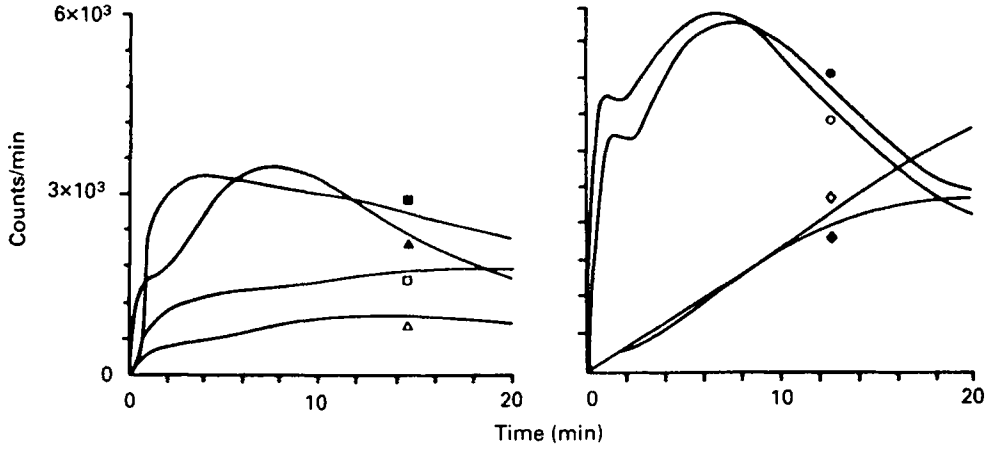

Fig. PMN chemiluminescent response to calcium and sodium cationic forms of natural and synthetic zeolite aluminosilicate particulates.

	Na^+ form	Ca^{2+} form
Erionite (Turkey)	○	●
Erionite (California)	△	▲
Offretite (synthetic)	□	■
Zeolite omega (synthetic)	◇	◆

The results indicate that the natural fibriform mineral and calcium and iron cationic particulate forms generally exhibited the greatest reactivity.

The findings suggest that analogous fibrillary and ionic forms of aluminosilicate material present in cerebral plaques may thus similarly contribute to the neurodegenerative process. Reported changes in serum vitamin E (Burns & Holland, 1986) and brain zinc (Ward & Mason, 1987) content in Alzheimer's disease, also indicate a possibly significant interaction with micronutrient antioxidants.

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Blood antioxidant status of runners in relation to training load. By J. D. ROBERTSON[1], G. G. DUTHIE[1], R. J. MAUGHAN[2] and P. C. MORRICE[1], [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and [2]*University Medical School, Foresterhill, Aberdeen AB9 2ZD*

Prolonged exercise such as distance running may result in increased oxidative stress. We wished to assess whether regular physical training causes adaptive changes in the protective capacity of the antioxidant defence mechanisms.

Blood antioxidants, antioxidant-related enzymes and indices of free radical-mediated damage were measured (Table) in venous blood from thirty-two healthy young (age 21–39 years) male subjects after an overnight fast; six were sedentary and twenty-six were recreational or competitive runners who had run an approximately constant weekly training distance for at least 10 weeks before the study.

Blood antioxidants and related erythrocyte antioxidant enzymes for three groups of subjects: sedentary (SED, n *6), low mileage (16–64 km/week,* n *11) runners (LOW) and high mileage (69–147 km/week,* n *15) runners (HIGH)*

	SED		LOW		HIGH	
Group	Mean	SE	Mean	SE	Mean	SE
Plasma						
Vitamin C (μM)	22·8	5·8	18·2	2·8	27·7	4·6
Vitamin E (μg/ml)	7·5	0·5	7·1	0·4	8·2	0·5
TBARS (μM)	1·35	0·10	1·25	0·06	1·31	0·04
CK (U/l)	107	14	218**	29	308*	70
Erythrocytes						
GSH (mg/g Hb)	0·79	0·12	1·25**	0·06	1·10*	0·07
GSHPx (U/g Hb)	79·5	6·9	87·4	3·3	95·6	3·8
Cat (U/g Hb)	892	252	1260	160	1391	170
SOD (U/g Hb)	1·67	0·20	1·47	0·12	1·51	0·10
Vitamin E (μg/g Hb)	7·6	3·5	17·1*	1·6	16·7*	1·9

Hb, haemoglobin; TBARS, thiobarituric acid reactive substances; CK, creatine kinase (*EC* 2.7.3.2); GSH, glutathione; GSHPx, glutathione peroxidase (*EC* 1.11.1.9); Cat, catalase (*EC* 1.11.1.6); SOD, superoxide dismutase (*EC* 1.15.1.1).

Student's *t* test for unpaired data was used for comparison of the running groups with the sedentary group: $*P<0·05$, $**P<0·01$.

The exercise groups had higher levels of plasma CK activity than the sedentary group (Table); plasma CK activity is accepted as an indicator of muscle damage (Ebbeling & Clarkson, 1989). However, they had similar plasma concentrations of the products of free radical lipid peroxidation measured as TBARS. Mean weekly mileage was significantly correlated with erythrocyte enzymes, GSHPx (r 0·454, $P<0·01$) and Cat (r 0·427, $P<0·05$) total erythrocyte GSH (r 0·340, $P<0·05$) and plasma vitamin E (r 0·350, $P<0·05$). Erythrocyte vitamin E was significantly higher in the runners than in the sedentary individuals (Table).

The results indicate that the protective antioxidant capacity of blood is enhanced in endurance runners but, despite these changes, habitual physical activity may still produce evidence of muscle damage. As energy intake was significantly correlated with training mileage (Maughan *et al.* 1989), the increased concentration of antioxidants may reflect increased dietary intakes of antioxidant vitamins and trace element cofactors of antioxidant enzymes.

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Effect of dietary α-tocopherol level on susceptibility of chicken tissues to lipid peroxidation. By P. J. A. SHEEHY, P. A. MORRISSEY and A. FLYNN, *Department of Nutrition, University College, Cork, Irish Republic*

Free radical-initiated lipid peroxidation occurs readily in subcellular membranes because of the presence of substantial amounts of polyunsaturated fatty acids (PUFA) (Slater *et al.* 1987). It is generally accepted that susceptibility to lipid peroxidation is influenced by tissue levels of α-tocopherol. In the present study, the effect of dietary α-tocopherol supplementation on the susceptibility of muscle and other tissues from chickens to in vitro iron-induced lipid peroxidation was investigated.

Twenty-four 1-d-old male ISA Brown chicks were randomized into four groups and fed on diets containing 5, 25, 65 or 180 μg α-tocopherol/g feed. The chicks were killed by cervical dislocation on day 24. Liver, heart, lung, brain and thigh muscle were removed and stored at –20° until required. The lability of the various tissues to lipid peroxidation was determined by a modification of the method of Kornbrust & Mavis (1980). Thiobarbituric acid-reacting substances (TBARS) were determined by the method of Beuge & Aust (1984) and reported as nmol malonaldehyde/mg protein.

Fe-induced lipid peroxidation (nmol malonaldehyde/mg protein) as affected by dietary α-tocopherol levels

		Dietary α-tocopherol (μg/g)							
	Incubation time	5		25		65		180	
Tissue	(min)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Muscle	0	0·85^{a}	0·05	0·69^{b}	0·06	0·59^{b}	0·05	0·57^{b}	0·07
	40	3·23^{a}	0·37	1·55^{b}	0·49	0·73^{b}	0·08	0·65^{b}	0·07
Liver	0	0·37^{a}	0·05	0·38^{a}	0·08	0·36^{a}	0·05	0·25^{a}	0·06
	40	1·36^{a}	0·25	0·48^{bc}	0·06	0·46^{b}	0·03	0·33^{c}	0·05
Heart	0	1·26^{b}	0·15	1·79^{a}	0·12	1·02^{bc}	0·08	0·79^{c}	0·10
	40	9·37^{ab}	1·19	10·2^{a}	0·63	6·88^{bc}	1·07	4·11^{c}	0·88
Lung	0	1·88^{a}	0·15	2·02^{a}	0·20	1·95^{a}	0·24	1·48^{a}	0·19
	120	2·74^{a}	0·17	2·52^{a}	0·07	2·22^{a}	0·17	2·10^{a}	0·15
Brain	0	4·44^{ab}	0·18	4·97^{a}	0·21	4·33^{ab}	0·22	4·11^{b}	0·28
	40	28·2^{ab}	2·27	31·1^{a}	2·19	27·0^{ab}	1·85	23·50^{b}	1·12

$^{a-c}$Values in horizontal rows with unlike superscript letters are significantly different ($P<0·05$)

The results show that lung tissue peroxidized at a much lower rate than liver, heart or thigh muscle. Brain showed significantly higher TBARS than did the other tissues after 40 min incubation. Significant reduction ($P<0·05$) in the rates of peroxidation were observed in the liver, heart, brain and muscle tissues from chicks fed on high levels of α-tocopherol. However, in the case of lung tissue, no significant differences between groups were observed. The protection of lung against lipid peroxidation could be due to either the presence of other biological protective agents in the membranes or to the presence of low concentrations of PUFA.

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Vitamin E alters T cell subsets in elderly patients. By L. PURKINS[1], N. D. PENN, J. KELLEHER[1] and R. V. HEATLEY[1], *Departments of [1]Medicine and [2]Medicine for the Elderly, St James's University Hospital, Leeds LS9 7TF*

Elderly, long-stay patients have been shown to have impaired immune functions when compared with young controls (Pahwa *et al.* 1981). Such impairment of the immune system may be due, in part, to vitamin deficiency. Vitamin E acts as an in vitro antioxidant, protecting cell membranes from free radical attack and, in this context, it may well play an important role in modulating the immune system (Sheffy & Shultz, 1979).

A double blind, randomized, controlled trial was designed to evaluate the effect of short-term supplementation of vitamin E on the ageing immune system. Nutritional status and cell-mediated immune function were assessed in twenty elderly, long-stay patients. Patients were then randomly allocated to two groups: ten patients were supplemented for 28 d with 100 mg vitamin E/d; the remaining ten patients received a placebo. After 28 d nutritional status and immune function were reassessed.

Weight, mid-arm circumference and plasma albumin remained constant in both groups over the 28 d period. The placebo group mean plasma vitamin A concentration significantly decreased from (mean (SD)) 594 (217) mg/l to 509 (216) mg/l ($P<0{\cdot}01$). Plasma vitamin E decreased slightly from 8·2 (2·5) mg/l to 7·7 (2·4) mg/l; however, this was not significant. Supplementation with vitamin E significantly increased mean plasma vitamin E from 8·2 (1·5) mg/l to 13·1 (2·1) mg/l ($P<0{\cdot}001$) while the vitamin A concentration decreased slightly from 440 (103) to 406 (135) mg/l (not significant). The increased plasma vitamin E concentrations were associated with a significant increase in T cell number and an alteration in T cell subsets, as assessed by immunofluorescence. The supplemented individuals had a significant increase in the percentage of $CD3^+$ cells (44·7 (15) to 70·9 (9·6)); this represents almost a 50% increase in T cell number. The expansion of the $CD3^+$ cell population was due to an increase in the $CD4^+$ (helper/inducer) subset; the $CD8^+$ (cytotoxic/suppressor) subset of T cells remaining unchanged. There was no improvement in the mitogenic stimulation of the cells using phytohaemaglutinin.

In conclusion, vitamin E supplementation appears to protect vitamin A concentrations in plasma and stimulate an increase of $CD4^+$ cells in this elderly group. Further work is required to assess whether such an alteration in T subsets would ultimately improve the patients' clinical state and well-being.

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Immune function in vitamin E-deficient rats. By L. PURKINS, J. KELLEHER and R. V. HEATLEY, *Department of Medicine, St James's University Hospital, Leeds LS9 7TF*

Vitamin E deficiency has been shown to affect some aspects of immune function in susceptible animal models. This study was designed to assess the effect of an 8-week vitamin E-deficient diet on the developing immune system of weanling Wistar rats. Sixteen weanling rats were allocated to either a vitamin E-deficient diet or a comparable diet with addition of 100 mg vitamin E/kg diet (both diets obtained from Dyets Inc., USA). The animals were housed four per cage and were weighed weekly; food intake per cage was carefully monitored. After 8 weeks on the appropriate diet, the animals were killed and a number of immune variables were measured.

Mean (SD) plasma vitamin E concentration in vitamin E-supplemented animals was 15·09 (2·68) mg/l compared with 0·50 (0·53) mg/l in the deficient animals ($P<0·001$). Spontaneous haemolysis in vitro of erythrocytes in the deficient group was 100% compared with <10% in the supplemented animals. Body-weight increase was comparable in both groups over the 8 week period of study. The vitamin E-deficient group did tend to gain less weight than the supplemented group, particularly at the end of the study period, but the difference did not reach significance. Spleen weights from the animals were again comparable; however, if expressed as a percentage of body-weight, the deficient animals had significantly larger spleens than the supplemented group ($P<0·05$). Despite the apparently larger spleens, the deficient animals had significantly lower numbers of white cells per gram of splenic tissue (793 (126·6) $\times 10^6$) compared with 1178 (257·9) $\times 10^6$ ($P<0·01$). Whole blood leucocyte counts indicated that there was no reduction in circulating white cells; there was a trend, however, for the deficient group to have more neutrophils and less lymphocytes than the supplemented animals. Thymus weights were greater in the supplemented group (0·78 (0·18) g compared with 0·65 (0·15) g), though the difference did not reach significance. Immunofluorescence of peripheral blood cells and splenocytes indicated no apparent alteration in T cell subsets, monocyte number or T cell activation. Mitogenic stimulation with optimal concentrations of Concanavalin A indicated that peripheral blood lymphocytes and splenocytes from deficient animals had a lower [^{3}H]thymidine incorporation compared with the supplemented group, but this difference was not significant.

In conclusion, it appears that despite achieving vitamin E deficiency in this strain of rat, as assessed by plasma vitamin E levels and the haemolysis test, no overt immune dysfunction was apparent. However, the trend seems to suggest that a longer period of vitamin E deficiency may be required to show a significant effect on immune function.

Gastric juice ascorbic acid: effects of disease, hypochlorhydria and stimulation of gastric secretion. By C. J. SCHORAH[1], J. N. PRIMROSE[2], G. M. SOBALA[3], M. J. SANDERSON[1] and M. ROGERS[2], *Departments of* [1]*Chemical Pathology,* [2]*Surgery* and [3]*Gastroenterology, University of Leeds, Leeds LS2 9JT*

In a previous study we found low concentrations of ascorbic acid in small samples of gastric juice taken at endoscopy in patients with gastritis and hypochlorhydria (Sobala *et al.* 1989). We have now investigated whether continuous collection of gastric juice and stimulation of secretion can enhance ascorbic acid levels in patients with low initial concentrations.

Control subjects (four male, five female), with no endoscopic or histological evidence of gastrointestinal disease, and twelve male and four female at high risk of gastritis because of refractory duodenal ulcer (DU), who were not taking antisecretory agents, had fasting resting gastric juice sampled. DU patients then had juice collected continuously for 1 h (basal output) and for 2 h following a sham-feed. All samples were acid (pH ≤5·0, mean 1·67). The procedure was repeated in seven patients (DU-H) in whom hypochlorhydria was produced in all samples (pH>5, mean 7·5) by 1 month's treatment with antisecretory agents Omeprazole (40 mg/d) or Cimetidine (800 mg/d). Gastric juice ascorbic acid was measured by high performance liquid chromatography (Sanderson & Schorah, 1987). Intake of vitamin C was estimated by food frequency questionnaire (Sobala *et al.* 1989) and plasma total vitamin C was measured by 2·4-nitrophenylhydrazine.

Group		Age (years)	Plasma total vitamin C (μmol/l)	Gastric juice ascorbic acid (μmol/l)		
				Resting	Basal	Sham-feed
Controls	Mean	32	49·4	182	—	—
	Range	19–58	18·7–95·5	23·8–490	—	—
DU (pretreatment)	Mean	45	45·5	23·3**	17·6	21·6
	Range	22–72	15·3–106	0·6–146	0·6–70·5	2·8–80·1
DU-H (post-treatment)	Mean	46	42·1	2·8***†	3·4†	6·3
	Range	22–72	11·9–88·1	0·6–6·3	0·6–9·7	2·2–10·2

Significantly different from controls (Wilcoxon's rank test): $^{**}P<0·01$, $^{***}P<0·001$.
Significantly different from DU (pretreatment) (Wilcoxon's paired rank test): $^{\dagger}P<0·05$.

The Table shows that resting juice ascorbic acid was considerably lower in DU patients than in control subjects, although both groups had similar plasma vitamin C levels and identical mean intakes (58 mg/d). Subsequent drug-induced hypochlorhydria in ulcer patients (DU-H) reduced resting juice ascorbate values effectively to zero. Neither collecting basal juice continuously nor stimulation of its secretion by sham-feeding led to significant changes in ascorbate in either ulcer group although it increased H^+ concentration 340% in the pretreatment group. In this group acid concentration was stimulated sixfold by pentagastrin, but this led to no change in ascorbic acid (mean 15 μmol/l). Ascorbic acid may protect against stomach cancer by reducing nitrite and preventing formation of carinogenic *N*-nitroso compounds (Mirvish, 1986). However, our work shows that in hypochlorhydric conditions, where nitrite concentrations and stomach-cancer risk both increase, the levels of ascorbic acid found in gastric juice, even after stimulation of gastric secretion, are too low to afford protection.

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Selenium depletion and repletion: effects on the thyroid gland in the rat. By J. R. ARTHUR and F. NICOL, *Biochemistry Division, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and P. W. H. RAE and G. J. BECKETT, *University Department of Clinical Chemistry, The Royal Infirmary, Edinburgh EH3 9YW*

Selenium deficiency causes imbalances in thyroid hormone metabolism in both rats and cattle (Beckett *et al.* 1987; Arthur *et al.* 1988). In the rat these changes are accompanied by impaired conversion of thyroxine (T_4) to the more metabolically active 3,5,3,'-triiodothyronine (T_3) by type 1 thyroid hormone deiodinase in liver and kidney and type 2 deiodinase in brain (Beckett *et al.* 1989). Type 2 deiodinase activity in the pituitary gland is necessary for the local production from T_4 of T_3 which can exert control on thyroid-stimulating hormone (TSH) release into the plasma and thus thyroid gland metabolism.

Groups of male weanling rats consumed Se-deficient (<0·005 mg Se/kg diet) or Se-sufficient (0·1 mg Se/kg diet as Na_2SeO_3) diets for 5 weeks with a further group of deficient rats receiving a single intraperitoneal injection of 200 μg Se as Na_2SeO_3 5 d before the end of the experiment. Results are shown in the Table. Selenium deficiency significantly decreased thyroid iodine, T_4 and T_3 concentrations without any effect on thyroid weight. Thyroidal iodine and hormone concentrations of Se-repleted rats were not significantly different from those of the Se-supplemented rats.

Thyroid T_4, T_3, and total iodine concentrations in Se-deficient (–Se), Se-sufficient (+Se) and Se-repleted (–Se(200)) rats (six animals/group)

		+Se	–Se	–Se(200)
T_4 (nmol/g protein)	Mean	2186	1763*	2481
	SD	292	309	160
T_3 (nmol/g protein)	Mean	352	232*	288
	SD	112	57	36
Iodine (μmol/g protein)	Mean	49·3	36·9*	47·4
	SD	17	5·5	4·5

Significantly different from +Se group: $P<0·05$.

Thus Se deficiency alters iodine and thyroid hormone metabolism in the thyroid gland. This may be due to inhibition of T_4 deiodination in the pituitary causing changes in TSH release or to a direct effect of loss of Se-containing glutathione peroxidase activity in the thyroid, allowing increased hydrogen peroxide concentrations in the gland to stimulate T_4 and T_3 synthesis and release.

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Nitrosation of amino acids and peptides in gastric contents. By DOUGLAS S. ANNAN[1], BRIAN C. CHALLIS[2], JOHN D. HARRISON[3], JIM ILEY[2], DAVID L. MORRIS[3] and GILLIAN E. SHEARS[2], [1]*Chemistry Department, Imperial College, London SW7 2AZ*, [2]*POCRG, Chemistry Department, Open University, Milton Keynes MK7 6AA* and [3]*Department of Surgery, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH*

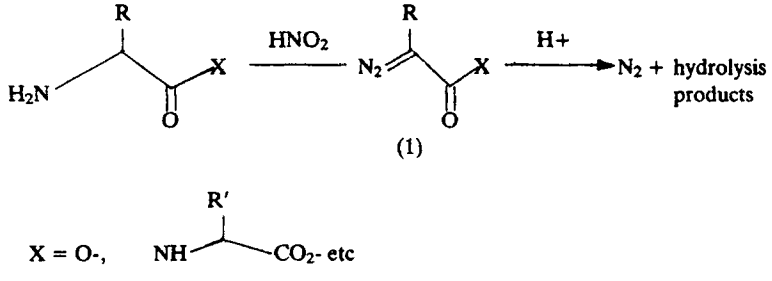
Nitrosation of dietary components resulting in the formation of carcinogenic *N*-nitroso compounds has been considered as a causal factor in cancer (Doll & Peto, 1981; Mirvish, 1983). We have found that the addition of nitrite to native human gastric aspirates in vitro at 37° produces substantial amounts (>80%) of N_2 and very much smaller amounts of *N*-nitroso compounds.

Extent of diazocompound formation in different clinical groups

Clinical Status	pH	$^{15}N_2$:^{15}NO	Amino end groups (mM)
Normal	1·6	6·0	6·2
Normal	4·1	19–62	6·2–9·9
Intestinal metaplasia	4·1	108–157	29–34
Dysplasia	4·1	305–408	42–57

No samples contained significant amounts of *N*-nitrosocompounds.

The principal nitrosation reaction must therefore be the deamination of amino acid and peptide constituents of the gastric aspirate (Scheme) via a diazo intermediate (1).



Scheme

Diazoderivatives (1) of amino acids and simple peptides are cytotoxic (Druckrey *et al.* 1965; Challis, 1989) and may also be implicated in human cancer.

We have shown that the amino acid and peptide content of human gastric aspirates is dependent on the clinical status of the stomach, being higher in the presence of gastric cancer. At this stage of the analysis, the higher concentration does not seem to relate to any specific amino acid or peptide constituents. It follows that the generation of cytotoxic diazocompounds by endogenous nitrosation is probably more extensive in patients with gastric cancer. Current analysis of gastric aspirates of pre-cancerous clinical status may reveal whether the elevated levels of amino acids and peptides are implicated in the initiation of cancer or only occur as a result of cancer.

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Human milk: a source of potentially toxic and carcinogenic halosubstituted biphenyls. By J. T. BORLAKOGLU, N. J. BORLAK and R. R. DILS, *Department of Physiology and Biochemistry, University of Reading, Whiteknights, PO Box 228, Reading RG6 2AJ*

Polychlorinated biphenyls (PCBs) contain up to eighty individual isomers and congeners and accumulate in animals including humans (Safe, 1984; Borlakoglu *et al.* 1988, 1989). Some PCBs are carcinogenic and may also be mutagenic in a number of laboratory animals, as judged by hepatocellular changes, e.g. trabecular carcinoma, adenocarcinoma, neoplastic nodules, as well as intestinal metaplasia, adenofibrosis, thymoma and bile duct hyperplasia (Safe, 1989).

Infants can be exposed to varying concentrations of PCBs during the ingestion of breast milk (Jensen, 1983; Safe *et al.* 1985). The toxicity of PCBs depends on specific substitution patterns, but little information is available on the precise molecular structures enriched in human milk. We examined the PCBs present in samples of breast milk obtained from mothers (*n* 10) living in the Reading area, and identified up to sixty individual PCBs. Using capillary gas chromatography (GC)–electron capture detection and capillary GC–mass spectrometry we estimated a mean concentration approximately 1200 μg total PCBs/kg milk, which is at least in 20-fold excess of a PCB concentration that has been shown to cause adverse effects in man (World Health Organization, 1976). Twelve of these, which accounted for up to 80% of the total PCBs present, were highly chlorinated and lacked vicinal protons in the *meta-para* position of the biphenyl moiety. Some of these, e.g. 3,3′,4,4′-tetrachloro-, 2,3′,4,4′,5-pentachloro-, 2,3,3′,4,4′,5-hexachloro- and 2,3,3′,4,4′,5′-hexachlorobiphenyls, are highly toxic to some laboratory species and bind with high affinity to an intracellular receptor that is involved in controlling both drug metabolism and the complex patterns of toxic responses observed.

A selection of PCB-congener specific analysis of human breast milk and a commercial mixture of PCBs (Aroclor 1260)

(Values are means; SD does not exceed 15% of the mean for (*n* 10) breast milk samples)

PCB structure	No. of pairs of vicinal H-atoms in: ortho/meta	meta/para	% PCB composition in Breast milk	Aroclor 1260	in vitro binding affinities to the intracellular Ah-receptor protein*
2,4,4′-Trichlorobiphenyl	3	0	1·26	0·04	nd
3,3′,4,4′-Tetrachlorobiphenyl	2	0	0·05	0·24	$7·1 \times 10^{-7}$M
2,3′,4,4′,5-Pentachlorobiphenyl	1	0	7·60	0·49	$9·1 \times 10^{-6}$M
2,3,3′,4,4′,5-Hexachlorobiphenyl	1	0	1·74	0·42	$7·1 \times 10^{-6}$M
2,3,3′,4,4′,5′-Hexachlorobiphenyl	1	0	0·71	0·72	$5·0 \times 10^{-6}$M
2,2′,3,4,4′,5-Hexachlorobiphenyl	1	0	17·62	6·50	nd
2,2′,4,4′,5,5′-Hexachlorobiphenyl	0	0	19·26	9·60	$7·9 \times 10^{-5}$M
2,2′,3,3′,4,4′,5-Heptachlorobiphenyl	1	0	5·20	3·78	nd
2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	0	0	9·74	8·60	nd
2,2′,3,4,4′,5′,6-Heptachlorobiphenyl	0	0	3·32	3·36	nd
2,2′,3,3′,5,5′,6,6′-Octachlorobiphenyl	0	0	0·59	0·03	nd

nd, not determined. *In vitro binding affinities are taken from Bandiera *et al.* (1982).
With the exception of one volunteer, all participants were first lactating, with an age range of 24–32 years.

In summary, highly toxic PCBs identified in human breast milk could be a source of potential carcinogens, and a detailed analysis of the effects of these xenobiotics is needed to assess the potential risk to the breast-fed infant.

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Arginine in benign and malignant disease of the breast and colon. By K. G. M. PARK[1,2], S. D. HEYS[1,2], C. I. HARRIS[2], M. A. MCNURLAN[2], R. J. STEELE[1], O. EREMIN[1] and P. J. GARLICK[2], [1]*Department of Surgery, Aberdeen Royal Infirmary, Foresterhill, Aberdeen AB9 2ZD* and [2]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The amino acid arginine has been demonstrated to decrease tumour growth and improve immune function when administered in pharmacological doses in a variety of experimental situations (Barbul, 1986). Plasma amino acid profiles are altered in malignancy and concentrations of arginine have been shown to be elevated (Glass *et al.* 1986). However, it is not known whether plasma concentrations reflect the situation in the tumour itself. Moreover, the activity of the enzyme arginase has been shown to be high in macrophages, which may comprise a substantial part of the cellular component of the tumour. We have therefore measured the free arginine concentration in benign and malignant tissues in relation to macrophage number and arginase activity.

Samples of benign breast tissue (*n* 14), malignant breast tissue (*n* 14), malignant colonic carcinomas (*n* 6) and colonic polyps (*n* 6) were obtained from patients undergoing surgery, and immediately frozen in liquid nitrogen. Free amino acid concentrations were measured by ion-exchange chromatography and arginase (*EC* 3.5.3.1) activity was assayed by urea production. Macrophages in frozen sections were stained with the monoclonal antibody Leu M3. The Table shows the results expressed as means (SEM).

	Arginine (μmol/g protein)		Arginase (unit/g protein)		Macrophages (per 4mm²)		Plasma arginine (mmol/l)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Breast								
Benign	2·8	0·8	53	16	29	11	111·3	10·4
Malignant	9·8	0·9	63	12	278	78	167·0	17·0
Colon								
Benign	7·0	4·0	70	20	3	42	115·0	11·2
Malignant	14·0	4·0	91	13	263	74	126·1	9·5

Arginine concentrations were significantly higher in malignant than in benign tissues ($P<0·05$), as were the macrophage counts ($P<0·001$), but arginase activity was no different (Mann-Whitney U test).

In this study arginine concentrations in the malignant tumours were high and arginase activity relatively low, despite the high macrophage count. This may represent either a reduced amount or an inhibition of arginase in tumour infiltrating macrophages.

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Glass, R. E., Goode, A. W., Houghton, B. J. & Rowell, L. W. (1986). *Gut* **27**, 844–849.

Do rates of protein synthesis differ in separate biopsies from the same tumour? By S. D. HEYS[1,2], K. G. M. PARK[1,2], M. A. MCNURLAN[1], K. BLESSING[2], O. EREMIN[2] and P. J. GARLICK[1], [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and [2]*Department of Surgery, Aberdeen Royal Infirmary, Foresterhill, Aberdeen AB9 2ZD*

Previous investigations of protein metabolism in patients with malignancy have centred on whole-body measurements and relatively few studies have measured protein synthesis in the tumour tissue itself. Mullen *et al.* (1980) determined fractional rates of protein synthesis in a range of tumours of gastrointestinal origin by constant infusion of [^{15}N]glycine and more recently we have measured protein synthesis in colorectal tumour tissue with a 'flooding dose' of L-[1^{13}C]leucine. The results from both these studies showed variability in colorectal tumour protein synthesis, ranging from 2 to 26%/d (Mullen *et al.* 1980) and 18 to 33%/d (Heys *et al.* 1989). The present study was therefore undertaken to show how much of this variability was due to variation within different parts of the same tumour or to variation between different tumours.

Patients with carcinoma of the breast undergoing surgery were studied. They were all fasted for 12 h before determination of protein synthesis rates by injection of 4 g/70 kg body-weight of L-[1^{13}C]leucine, 20 atoms %, in 200 ml saline (9 g sodium chloride/l) given intravenously over 10 min. Immediately after surgical removal of the tumour, one, two or three random biopsies (the number taken being limited by the requirement to leave adequate tumour tissue for histopathological examination), were taken from its periphery and the presence of tumour cells confirmed by light microscopy. The fractional rate of protein synthesis (%/d) was determined from the increase in enrichment of protein-bound leucine and the average free leucine enrichment in plasma as measured by isotope ratio- and gas chromatography-mass spectrometry.

The mean rate of protein synthesis (SD) in all breast tumour biopsies was 10·8 (3·0) %/d, ranging from 5·3 to 15·9% (*n* 27). By comparison, the mean difference in fractional rates of protein synthesis in multiple biopsies from the same tumour was 1·1 (0·9) %/d (*n* 9), and the difference between biopsies was small compared with the difference between individual tumours. Histologically, tumours were adenocarcinomas of no special type with nuclear grade III and there was no relation between the number of mitoses and rates of protein synthesis.

These results suggest that the differences in protein synthesis between tumours are real and do not arise from variability within the tumour itself. Furthermore, the rate of protein synthesis in breast tumours is only 48% of the mean value reported previously (Heys *et al.* 1989) for colorectal cancer.

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Effects of the intestinal microflora and dietary fibre on colonic epithelial cell proliferation.

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Two groups of fifteen conventional and two groups of fifteen germ-free Lister Hooded rats were fed on a fibre-free elemental diet, Flexical (Mead Johnson, Uxbridge) with or without a dietary fibre mixture (1 part Ispaghula gel: 9 parts Trifyba, w/w) for 2 weeks.

At the end of the investigation the animals were injected with vincristine (1 mg/kg) and killed at timed intervals. The wet weight of the sections of the gastrointestinal tract were recorded and intestinal crypt cell production rate (CCPR) measured by counting the rate of accumulation of arrested metaphases in microdissected crypts (Goodlad & Wright, 1982).

Animals . . .	Germ-free				Conventional			
Diet . . .	Flexical		Flexical + fibre		Flexical		Flexical + fibre	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	Tissue weight (g/kg body-weight)							
Stomach	3·66	0·04	4·73***	0·11	3·90	0·06	4·59***	0·11
Small intestine	20·56	0·37	21·40	0·33	19·44	0·34	21·20**	0·36
Caecum	5·37	0·23	6·30**	0·14	2·76	0·06	3·53***	0·13
Colon	3·60	0·14	5·61***	0·15	3·36	0·08	4·98***	0·15
	Cryptcell production rates (cells/crypt per h)							
50% of the small intestine	11·88	1·86	12·45	1·08	15·48	1·56	14·28	1·50
10% of the colon	2·82	0·54	3·48	0·60	2·16	0·54	7·68***	0·96
50% of the colon	4·20	1·26	2·94	0·96	3·12	0·48	9·24***	1·38
90% of the colon	2·88	0·78	4·38	1·32	4·74	0·72	9·36**	1·38

Significantly greater than respective Flexical diet: $^{**}P<0·01$, $^{***}P<0·001$.

Two-way analysis of variance showed that diet had a significant effect ($P<0·001$) in the stomach, small intestine, caecum and colon weights. The microflora had significant ($P<0·001$) effects on the caecum and colon weights only.

No effect of fibre on CCPR was seen in the germ-free groups, but fibre approximately doubled the CCPR in the proximal, mid- and distal colon in the conventional groups. Thus the above effects of fibre on the intestinal tissue weights of the germ-free rats would appear to be the result of alterations in muscle rather than epithelial mass.

It is concluded that it is the products of hind-gut fermentation (short-chain fatty acids?), not fibre *per se*, that stimulate colonic cell proliferation and that fibre has an independent trophic effect on the intestinal muscle.

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Stimulation of large bowel fermentation has no effects on duodenal epithelial proliferation in rats given white bread-based diets. By J. C. MATHERS, DEVINA MCCLEAN and FIONA B. KEY, *Department of Agricultural Biochemistry and Nutrition, The University, Newcastle upon Tyne NE1 7RU*

Excessive gastrointestinal epithelial proliferation rates (EPR) may result in polyp formation and could predispose to carcinoma. Infusion of volatile fatty acids (VFA), the major end-products of large bowel (LB) fermentation, into the ileum of rats fed on an elemental diet caused two- to fourfold increases in EPR in the proximal small intestine and in the LB (Sakata, 1987). If such effects followed dietary stimulation of LB fermentation, this could be a contraindication for diets rich in non-absorbable carbohydrates (dietary fibre).

LB fermentation in male Wistar rats (five animals per diet) was stimulated by including graded amounts (0, 150, 300 and 450 g/kg) of cooked, freeze-dried and ground haricot beans (*Phaseolus vulgaris*), at the expense of sucrose and casein, in diets containing 500 g freeze-dried white bread/kg. Rats were housed individually and offered 20 g diet/d for 16–17 d (final weight 269 g). Measurements were made of organic matter (OM) disappearance in and VFA absorption from the LB, duodenal mucosal EPR (Key & Mathers, 1989), sucrase (*EC* 3.2.1.48) activity (Dahlqvist, 1968) and villus height and crypt depth with the aid of an image analyser (IAS25 Joyce-Loebl, Gateshead).

Beans in diet (g/kg)	0	150	300	480	SE of mean
Diet composition (g/kg dry matter)					
Non-starch polysaccharides	15	44	78	112	
Resistant starch	5	11	14	18	
LB OM disappearance (g/d)	0·4	0·9	0·9	1·7	0·12***
LB VFA absorption (mmol/d)	4·7	10·2	9·7	19·1	1·38***
Villus height (mm)	0·51	0·48	0·50	0·51	0·021
Crypt depth (mm)	0·13	0·13	0·12	0·13	0·010
EPR†	91	103	123	107	12
Sucrase (IU/0·1 m mucosa)	2·2	2·8	2·3	2·2	0·20

†Arrested crypt cells/1000 crypt cells at 2 h following vincristine injection.
Statistically significant linear effects of dietary haricot beans inclusion rate: ***$P<0·001$.

Increasing intakes of beans were associated with large linear increases in OM disappearance in and estimated VFA absorption from the LB, but there was no significant ($P>0·05$) effects on duodenal EPR, morphology or functional capacity as indicated by sucrase activity. These results confirm our earlier observations with rats fed on wholemeal bread-based diets (Key & Mathers, 1989) and suggest that, when conventional foods are eaten, stimulation of LB fermentation is unlikely to affect duodenal EPR.

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Oats, gastrointestinal cell proliferation and serum cholesterol in the rat. By E. K. LUND and I. T. JOHNSON, *AFRC Institute of Food Research, Colney Lane, Norwich NR4 7UA*

Oats appear to have beneficial nutritional properties (Lockhart & Hurt, 1986). For example they lead to relatively low post-prandial glycaemia, and prolonged consumption has been shown to lower plasma cholesterol. The underlying physiological changes occurring in the intestine when oats are eaten are, however, relatively unknown.

Rats (twelve per group) fed on either a standard laboratory diet of (g/kg): sucrose 300, starch 280, casein 200, cellulose 100, maize oil 80, minerals and vitamins 40, or a diet of similar nutrient composition containing oats, i.e. rolled groats 500, sucrose 190, starch 20, casein 130, cellulose 60, maize oil 60, minerals and vitamins 40 (amino acid content was adjusted appropriately) were killed after 4 weeks. Differences in cell proliferation were measured in the jejunum and ileum, with the metaphase arrest technique (Johnson *et al.* 1988) and circulating levels of enteroglucagon, gastrin and cholesterol were determined.

Although neither diet contained cholesterol and both were low in saturated fat, rats fed on the oat diet had significantly lower plasma cholesterol concentrations than those fed on the control diet. (mean (SEM) 3·10 (0·09) and 3·83 (0·17) mmol/l respectively; $P<0·01$). Cell proliferation was markedly higher in rats fed on oats compared with controls, particularly in the ileum where values of 20·6 (1·2) compared with 9·4 (0·8) arrested metaphases/h per crypt were recorded ($P<0·001$). Similar changes have been shown to be associated with increased plasma enteroglucagon levels in rats fed on guar gum but no significant difference was found in this study (mean (SE) control 0·24 (0·03), oat diet 0·26 (0·03) pg/ml). Gastrin levels were not affected by eating oats (mean (SE) control 126·2 (8·1), oat diet 117·9 (7·9) pg/ml) and crypt depth also remained unchanged.

Since an effect of oats on dietary cholesterol uptake was excluded in this study the results suggest that the hypocholesterolaemic effect of oats is due to a reduction in endogenous synthesis. The small intestine is a major site of cholesterol metabolism in the rat, and increased mucosal cell turnover may have led to an increase in faecal cholesterol losses. The increase in cell proliferation does not appear to be associated with any change in level of the putative gastrointestinal growth hormones gastrin or enteroglucagon.

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Lipogenesis measured in vivo in tumour-bearing rats. By O. OBEID and P. W. EMERY, *Department of Food and Nutritional Sciences, King's College, London W8 7AH*

Cancer cachexia involves both a decrease in food intake and an inappropriately high rate of energy expenditure leading to a considerable loss of body fat. It is generally agreed that lipoprotein lipase (*EC* 3.1.1.34) activity is decreased and lipolysis is increased but there is some doubt as to whether lipogenesis is affected (Thompson *et al.* 1981; Evans & Williamson, 1988).

We measured rates of lipogenesis in vivo in tissues of male Fisher 344 rats bearing a transplantable Leydig cell tumour by measuring ^3H incorporation into saponifiable lipids 1 h after intraperitoneal injection of ^3H_2O. Experiments were carried out between 09.00 and 11.00 hours on days 1, 5 and 10 of palpable tumour growth, during which time the tumours reached 6% of body-weight. Food intake was recorded daily. *Ad lib.*-fed control rats were studied on days 1 and 10: results for these two groups did not differ significantly so they have been pooled.

			Tumour-bearing rats		
		Control	Day 1	Day 5	Day 10
n		12	6	5	6
Food intake (g/d)	Mean	15·5	15·5	12·5	8·8
	SD	0·4	0·6	0·7	0·3
Lipogenesis (μmol ^3H_2O incorporated/h per g tissue)					
Epididymal fat pad	Mean	2·71	3·27	2·28	1·86
	SD	0·92	1·36	0·56	0·60
Liver	Mean	17·88	13·25	8·79*	9·01*
	SD	7·15	4·71	2·13	3·59
Lipogenesis (μmol ^3H_2O incorporated/h per g tissue per g food intake)					
Epididymal fat pad	Mean	0·169	0·204	0·175	0·207
	SD	0·058	0·085	0·043	0·067
Liver	Mean	1·12	0·83	0·68	1·00
	SD	0·45	0·29	0·16	0·40

*$P<0·01$ *v.* control.

Absolute rates of lipogenesis in both liver and adipose tissue appeared to decrease with tumour growth, although the difference did not reach statistical significance in the fat pads. However, lipogenesis is known to be influenced by food intake (although the relationship may not be linear), and when rates of lipogenesis were corrected for food intake the differences between control and tumour-bearing rats disappeared. This suggests that the low rates of lipogenesis were entirely due to reduced food intake. Nevertheless tumour-bearing rats did lose considerably more fat than pair-fed controls: after 10 d of tumour growth body fat content was 30·5 g (SD 4·9) compared with 38·8 g (SD 4·4) in pair-fed controls (n 8, $P<0·01$). This suggests that reduced lipoprotein lipase activity and increased lipolysis are likely to have been the major causes of lipid depletion.

Financial assistance from the Hariri Foundation is gratefully acknowledged.

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Smoking and diet: is the diet of smokers different? By JANET CADE[1] and BARRIE MARGETTS[2], [1]*Community Medicine* and [2]*MRC Environmental Epidemiology Unit, Southampton General Hospital, Southampton SO9 4XY*

Smokers develop more cancers of the lung, mouth, throat, pancreas, kidney and urinary tract, and suffer more from coronary heart disease than do non-smokers. Apart from the smoking itself, other characteristics of smokers such as dietary habits may increase their risk of these diseases.

Smoking habits were assessed in our study of diet in 2340 men and women, aged 35–54 years, living in three English towns (Cade *et al.* 1988). Daily energy intakes were statistically significantly higher for current smokers than non-smokers or past smokers among the men but not among the women. Men who smoked tended to eat more fat and carbohydrate than non-smokers.

Daily nutrient intake by smoking status – men

(Mean values, ranges in parentheses)

	Non-smokers	Ex-smokers	Current smokers	Statistical significance
n . . .	294	305	512	*P*=
Energy (MJ)	10·2 (9·8–10·6)	10·5 (10·1–10·8)	11·0 (10·7–11·3)	
(kcal)	2440 (2349–2533)	2498 (2408–2590)	2626 (2546–2707)	0·008
Total fat (g)	102 (97–106)	103 (98–108)	105 (101–109)	0·5
P:S ratio	0·34 (0·31–0·36)	0·35 (0·33–0·37)	0·30 (0·29–0·32)	0·0004
Protein (g)	85 (82–89)	86 (83–89)	88 (85–91)	0·3
Carbohydrate (g)	281 (269–293)	285 (273–296)	297 (287–307)	0·09

P:S ratio, polyunsaturated:saturated fatty acid ratio.

For both men and women, total fibre, vitamin C, β-carotene and vitamin E intakes were lowest in the current smokers and highest in the non-smokers, with past smokers having intermediate values. In both men and women the polyunsaturated:saturated fatty acid ratio was lowest in the current smokers. After adjusting for social class there were still statistically significant differences between smokers and non-smokers (apart from vitamin C intake in men).

The differences seen are in line with suggestions that nutrients with antioxidant properties (such as vitamin C, β-carotene and vitamin E) are protective against cancer. A recent meta-analysis of cigarette smoking and altered serum lipid concentrations (Craig *et al.* 1989) showed that smokers had different serum cholesterol, triacylglycerols and lipoprotein concentrations compared with non-smokers. This could explain some of the excess risk of coronary heart disease among smokers and it was suggested that dietary differences could be involved. Some of the dietary differences found in our study would tend to increase cholesterol and triacylglycerols in the smokers.

It is not possible to assess from this cross-sectional study whether smoking is related to the dietary differences seen. The reasons why smokers have a different dietary pattern to non-smokers needs further investigation.

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Comparison of the diets of smokers and non-smokers. By MARGARET J. WHICHELOW and SHARON W. ERZINGLIOGLU, *Department of Community Medicine, University of Cambridge Clinical School, Fenner's, Gresham Road, Cambridge CB2 2ES*

Previous analysis using data from the Health and Lifestyle Survey, 1984–5 (Cox *et al.* 1987), revealed differences in the consumption of fresh fruit in summer, fried food, type of bread, type of spread used on bread, and breakfast between smokers and non-smokers (Whichelow *et al.* 1988; Whichelow, 1989). The dietary data from the 9003 randomly selected British adults in the Health and Lifestyle Survey was mostly in the form of the frequency of consumption of food items (frequently 'most days', 'daily' or 'more than once a day' for most items and 'once or twice a week' or more often for selected items). The present study reports comparisons of the frequent consumption of many items of the diet between non-smokers and current regular cigarette smokers. Each food item has been examined using logistic regression analysis, to allow for variations in the age and social class (non-manual and manual) structure of the smoking and non-smoking groups. Men and women were considered separately.

Non-smokers of both sexes were significantly more likely to consume frequently fresh fruit in winter, fruit juice and salad in summer and winter, breakfast cereals, biscuits, cakes, puddings, light desserts, jam, and skimmed/semi-skimmed milk. Smokers were more likely to eat chips and processed meats frequently, and to consume more alcohol and more cups of tea and coffee and more sugar in these beverages than non-smokers. The differences were less marked in those over 60 years of age.

Although portion sizes and therefore intakes could not be determined, the findings suggest that the diets of non-smokers are more in line with current recommended intakes than those of smokers. The overall pattern is for non-smokers to consume fruit, salads and cereal products more frequently, with implications for dietary fibre and vitamin C intake, and for smokers to consume many high-fat items, e.g. chips, processed meats and full-cream milk more often. These findings are of interest since both smoking and high-fat/low-fibre diets are risk factors for certain diseases.

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What YOU think causes obesity. By G. P. WEBB and C. A. GEISSLER, *Department of Food and Nutritional Sciences, King's College, London W8 7AH*

Negative attitudes towards the obese are still prevalent even amongst professionals (Mayer, 1968; Gilbert, 1986) probably due to a puritanical response to assumed over-indulgence. This is despite the boom in obesity research since the 1960s (Biological Abstracts obesity entries in 1960: 50, in 1985: 600) which has suggested causes that are not self-inflicted.

A study of the beliefs of nutritionists on the causes of obesity was carried out by questionnaire and related to personal research and the use of animal or human models. Responses (117) were obtained from 275 short questionnaires sent to all and randomly selected UK/Irish resident members of the Association for the Study of Obesity and the Nutrition Society respectively in 1986.

Only eighty-nine respondents ranked the six putative differences between obese and lean individuals in order of importance as causes of obesity: (A) diet composition, e.g. high-energy density, fat, sugar, alcohol; (B) physiological control of eating, e.g. defective hypothalamic satiety mechanism; (C) genetic and (D) psychological effects on eating; (E) metabolic response, e.g. defective thermogenesis; (F) inactivity. Mean rank scores according to research or clinical experience and per cent respondents ranking each cause first (1) or last (6) is shown in the Table.

		Cause					
Group	*n*	A	B	C	D	E	F
All respondents	89	2·5	4·7	3·3	3·4	3·6	3·6
No experience	20	2·9	4·8	3·2	3·7	3·2	3·3
Clinical experience	45	2·3	4·8	3·5	3·4	3·8	3·4
Research only	24	2·5	4·4	3·3	3·3	3·6	4·0
Only human research	32	2·3	5·0	3·6	3·3	3·7	3·5
Animal research	26	2·7	4·3	2·9	3·2	3·6	4·1
Genetic rodent models	13	2·5	3·9	2·4	4·0	3·7	4·4
Dietary rodent models	15	3·0	4·7	3·2	2·5	3·7	3·9
Dietitians	18	2·0	4·9	4·1	3·2	4·2	2·9
% Ranked first		45	2	25	16	12	5
% Ranked last		10	48	12	12	15	10

Diet composition was the favourite cause followed by genetics (45%, 25% respondents ranked first) and regulation of food intake the least favourite (48% ranked last), reflecting a decline in the once fashionable view of a hypothalamic defect (e.g. Davidson & Passmore, 1963) and in the use of hypothalamic models. The other causes had similar mean ranks but were polarized with over 10% of respondents ranking each first or last. Mean rank scores were similar between groups except for: researchers using genetic models who ranked genetics higher (more popular) and psychology lower than other respondents; those using dietary models who surprisingly ranked diet composition lower and psychology higher than others; and dietitians who ranked both diet and activity highest of all respondents, showing a more traditional overeating/inactivity view of obesity. A dietary view of causation therefore still prevails, despite the efforts of workers such as D. S. Miller to popularize the 'metabolic' view and the attention drawn to this by the media following the paper by Rothwell & Stock (1979).

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Effects of dietary supplementation on work performance in Gambian labourers. By E. DIAZ, G. R. GOLDBERG, M. TAYLOR, J. M. SAVAGE, D. SELLEN, W. A. COWARD and A. PRENTICE, *Dunn Nutrition Laboratory, Downham's Lane, Milton Road, Cambridge CB4 1XJ* and *Dunn Nutrition Field Station, Keneba, The Gambia*

The effect of food supplementation on work productivity was studied during a period of natural food shortage (wet season) in Keneba, The Gambia. Sixteen healthy male labourers (20–45 years of age), divided into two groups (G1, G2) matched for weight, height, haemoglobin and physical capacity, were each studied during 6-week supplementation (SUPPL) and control periods (CONT) using a cross-over design.

The supplement consisted of three meals each day, eaten *ad lib.* and contributing 15·7 (SE 1·96) MJ/d (3750 (SE 470) kcal/d) (protein 11%, fat 48%) to the food intake.

Men worked 8 h/d during the last 3 weeks of each period, having a lunch break (1·5 h) and 1 d off/week. Work paid on a piece-work basis, consisted of building a road by pushing wheelbarrow loads of gravel over a fixed distance (1·2 to 1·5 km). Work output was expressed as loads/d (LOADS) and loads/working hour (LWHOUR).

Basal metabolic rate (BMR), total energy expenditure (TEE) by the doubly-labelled water method ($^2H_2{}^{18}O$), weight, four skinfolds and daily heart rate were measured.

Both groups gained weight during SUPPL (G1, 1·8 kg; G2, 1·2 kg) and both groups lost weight during control periods but the loss was three times higher when CONT followed SUPPL than vice versa (G1, −3·4 kg; G2, −1·2 kg) with five subjects in G1 ending the study with 2–6 kg less weight than the beginning. Fat-free mass (FFM) and fat were also significantly reduced in G1 but not in G2.

Body-weight, body composition, work output and energy expenditure of Gambian labourers

(Mean values and standard deviations for eight subjects per group)

	1st Working period				2nd Working period			
	G1 SUPPL		G2 CONT		G1 CONT		G2 SUPPL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Wt (kg)	61·9*	6·6	60·3†	6·2	59·5*	6·5	62·9†	2·5
FFM (kg)	56*	7·2	53·0	3·7	53·3*	6·3	53·0	5·6
LOADS	163	18	158	30	178	28	175	4·0
LWHOUR	2·21	0·2	2·26	0·3	2·25	0·2	2·26	0·3
TEE (MJ)	19·8	4·1	16·6	2·7	19·8	4·0	17·8	1·7
BMR (MJ)	6·9	1·1	5·8	0·6	6·3	0·5	6·2	0·8
TEE/BMR	2·84	0·80	2·86	0·54	3·21	0·82	2·75	0·58
Skinfolds (mm)	23·9*	6·2	29·0†	12·5	21·1*	5·2	30·9†	14·5

*,†Significantly different individual values (paired *t* test (within a group) $P<0·05$).

It is concluded that 6 weeks of supplementation failed to demonstrate any significant effect on workers' productivity, TEE, BMR or TEE/BMR in spite of the fact that the subjects were in negative energy balance when unsupplemented. Careful interpretation of this finding is recommended since a high motivation factor could have been responsible for a constant productivity, even at the expense of important body-weight losses.

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Activity and energy expenditure of lactating women in rural Ghana. By MARGARET A. ARMAR-KLEMESU[1,2] and ERICA F. WHEELER[1], [1]*Centre for Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT* and [2]*Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana*

Most cultures forbid or restrict work and physical exertion after childbirth. However, a cross-cultural study of 202 traditional societies showed that women do not necessarily suspend work for prolonged periods of time; in about half, women were expected to resume all duties within 2 weeks (Jimenez & Newton, 1979).

We report a study of the activity patterns and energy expenditure of thirty lactating women from two subsistence agricultural communities in Ghana, at two stages of lactation. Subjects were recruited during two seasons. Activity diaries were used to record duration of activity from 07.00 to 19.00 hours and total energy expenditure (TEE) was calculated using published values of the energy cost of activities of female farmers in West Africa (Brun, 1984). Resting metabolic rate (RMR) was measured at both stages of lactation using the Oxylog (McNeill *et al.* 1987), and 3-d weighed food intake measurements were made.

Period post-partum (month) . . .	1st		3rd		
	Mean	SD	Mean	SD	*P*
Energy intake MJ (kcal)/d					
Dry season (*n* 12)	11·24 (2688)	2·44	10·78 (2579)	3·62	NS
Farming season (*n* 18)	10·41 (2488)	3·33	9·82 (2348)	3·55	NS
Energy expenditure MJ (kcal)/d					
Whole group (*n* 30)	8·41 (2012)	1·08	8·92 (2132)	1·46	***
Dry season (*n* 12)	8·16 (1951)	0·72	8·36 (1999)	0·80	†
Farming season (*n* 18)	8·59 (2054)	1·25	9·29 (2222)	1·69	***
Time spent on activities (min/12 h), whole-group (*n* 30)					
Farm work	2·2	9·7	49·2	74·4	***
Feeding baby	93·5	29·1	69·9	22·4	***
Resting	62·6	50·6	39·1	38·2	**

NS, not significant.
Significance of differences, paired *t* test, between months: $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.
Significance of differences, two sample *t* test, between seasons: $\dagger P<0{\cdot}05$.

The results show significant differences between months of lactation in time spent on certain activities. A between-season difference in energy expenditure was only found at the third month of lactation. There were no between-season or between-month differences in RMR or in dietary energy intake. The results confirm the traditional acceptance of a period of rest for at least 1 month after childbirth in Ghanaian communities, which overrides the requirements of farm work.

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Rationing and wartime food policies in Iran. By F. RABIEE and C. A. GEISSLER, *Department of Food and Nutritional Sciences, King's College, Campden Hill Road, London W8 7AH*

During the 1970s Iran imported and subsidized a large proportion of its food supply. Food prices have risen drastically since the Islamic revolution due to the removal of food subsidies, except on bread, as part of a government policy to become self-sufficient in food, trade sanctions following the American hostage crisis, and lack of foreign exchange during the Iran–Iraq war. For example between 1978 and 1982 the price of rice and meat rose by 170%. Food supply data were collected from Ministries, by food price survey and by survey of family food supply as part of a nutrition study in the Caspian province of Gilan. A survey of family food supply was made by questionnaire, administered at three seasons to 148 families covering a range of socio-economic groups. Information on the quantity of food bought and consumed over the previous 7 d, quantified from food weight, household measures and expenditure was obtained.

Rationing was one of the steps taken by the government to distribute scarce goods equitably. The quantities of rationed food were small but the price subsidized. Throughout most of the country the foods rationed were: chicken (one/family per week); cheese (1 kg/family per month); butter (100 g/person per month); vegetable fat and oil (450 g/person per month); sugar (800 g/person per month); powdered milk (450 g/child <2 years per week); baby cereal (100 g/child <2 years per week or 2 weeks). However, distribution was often less frequent in practice. In Teheran and some other cities additional rationing included: rice (1 kg/person per month); frozen red meat (400 g/person per week); pasteurized milk (0·5 litres/d per child <2 years or elderly or sick). All of these foods were also available on the open market at three to four times the rationing price.

Price control was also used to raise purchasing power but bread, the national staple, was the only food in this category. Vegetables, fruits, pulses and nuts were freely available and their prices subject to seasonal fluctuations. Meat, fish, eggs, milk and rice were in short supply and their very high prices severely restricted effective demand for them.

These policies have succeeded in reducing the gap in food consumption between economic groups (Table), however, little priority was given to vulnerable groups in contrast to similar rationing systems (Benjamin & Collins, 1985; Hollingsworth, 1985). For example the lack of appropriate low-cost weaning foods on the market and the very low fat content of the family diet (energy density 4·43 kJ (1·06 kcal) per g) were main reasons for the low energy intakes of young children (70% recommended daily intake). Rationing is still applied but since the end of the war in 1988 rations have doubled for chicken and meat and trebled for rice.

	Intake (g/person per d)							Energy Source			
	Cereals	Animal products	Legumes and nuts	Vegetables	Fruit	Oils and fats	Sugar	Protein (%)	Fat (%)	Carbo-hydrate (%)	MJ/person per d
High income (*n* 88)	540	115	57	308	263	18	44	11	16	74	10·53
Low income (*n* 56)	505	107	54	206	204	18	44	11	15	74	9·74

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The effects of yeast culture on yeast numbers and fermentation in the rumen of sheep. By C. J. NEWBOLD, P. E. V. WILLIAMS, N. MCKAIN, A. WALKER and R. J. WALLACE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The administration of live yeast culture (YC) to ruminants leads to improved nutritional efficiency (Harris & Lobo, 1988). The aim of this experiment was to determine the effects of YC on rumen fermentation and on yeast numbers in the digestive tract.

Five adult wether sheep were given 500 g/d each of rolled barley and dried grass using a continuous belt feeder. *Saccharomyces cerevisiae* yeast culture (Yeasacc, Alltech UK Ltd) was given twice daily (two times 2 g/d). Three sheep received YC in the first 28 d period, then YC administration was switched to the other two animals. Samples of rumen fluid were withdrawn on two separate days at least 20 d after the start of each period. Time of sampling had no influence on pH, volatile fatty acids (VFA), L-lactate or ammonia measurements, thus mean values are presented. YC caused a non-significant increase in rumen pH and had no effect on total VFA concentrations, but the acetate:propionate ratio decreased from 5·01 to 3·81 due mainly to an increase in propionate when YC was given. L-Lactate and ammonia concentrations were lowered by 22 and 35% respectively when YC was given.

Yeast cell numbers were determined aerobically as described by Lund (1974), with penicillin, tetracycline and streptomycin (60 mg/l) added to inhibit bacterial growth. Increased counts of yeasts were observed in rumen fluid 1 h after YC addition (Table). Numbers then fell by 61% after 6 h, but remained two orders of magnitude higher than that of control animals. Viable yeast persisted in the duodenum and ileum of treated animals, at values 6·5 and 6·8 times higher than controls.

	+YC	−YC	SED
pH	6·41	6·17	0·18
Total VFA (mmol/l)	113	105	9·1
Acetate (mmol/mol)	629	662	30·4
Propionate (mmol/mol)	165	132	13·3*
Butyrate (mmol/mol)	170	182	15·4
L-Lactate (mmol/l)	2·01	2·58	0·19*
NH_3-N (mg/l)	175	268	29·2*
Yeast numbers (colony forming units/ml)			
Rumen			
1 h after yeast addition	$3·34 \times 10^5$	$1·50 \times 10^3$	$8·09 \times 10^{4**}$
6 h after yeast addition	$1·31 \times 10^5$	$1·63 \times 10^3$	$3·44 \times 10^{4**}$
Duodenum			
2·5 h after yeast addition	$1·91 \times 10^5$	$2·93 \times 10^4$	$2·32 \times 10^{4**}$
Ileum			
5 h after yeast addition	$4·61 \times 10^5$	$6·90 \times 10^4$	$9·91 \times 10^{4**}$

*$P<0·05$, **$P<0·01$.

These results indicate that YC affected fermentation in the rumen, but that viable yeast numbers declined at a rate (0·17/h) similar to the likely rate of liquid outflow from the rumen. Post-ruminal effects on nutrition cannot be ruled out in view of the survival of live yeast in duodenal and ileal digesta.

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Protein digestibility measured by ^{15}N and homoarginine. By N. ROOS, H. HAGEMEISTER and J. SCHOLTISSEK, *Institut für Physiologie und Biochemie der Ernährung, Bundesanstalt für Milchforschung, Postfach 60 69, D-2300 Kiel 14, Federal Republic of Germany*

During absorption of ^{15}N-labelled proteins, parts of the tracer re-enter the intestine in the form of endogenous secretions and desquamations. Therefore resulting values for protein digestibility are falsely low. This is not the case with homoarginine (HA) labelling, because the intestinal disappearance of HA can be equated with the true digestibility (Hagemeister & Erbersdobler, 1985).

We tried to estimate the error of the ^{15}N-method by comparison with the HA method. Fourteen adult Göttingen minipigs received a diet with 150 g casein/kg. Of this casein, 27% was uniformly labelled with ^{15}N (0·086 atoms per cent excess) and additionally guanidinated (421 μmol HA/g casein). Cr_2O_3 was added as an indigestible marker. Six h postprandially, the animals were killed. The intestine was divided into three parts of equal length. In the chyme of the last two parts, Kjeldahl-N, ^{15}N and HA were determined.

Precaecal digestibility (%) of casein as calculated from Kjeldahl-N, ^{15}N and homoarginine (HA)

Segment of intestine . . .	Second third			Last third		
	Mean	SEM	Difference	Mean	SEM	Difference
^{15}N	89·3	1·5	5·4	91·2	0·5	4·6
HA	94·7	0·6	43·9	95·8	0·4	20·4
Kjeldahl-N	50·8	7·4		75·4	1·6	

As can be seen from the Table, the endogenous secreta, as calculated from the difference between HA and Kjeldahl-N digestibilities, amounted to 43·9 or 20·4% of ingested N in the second and last third of the small intestine, respectively. This demonstrates a considerable disappearance of endogenous secreta between these two parts of the small intestine. The part of endogenous secreta labelled with ^{15}N proved to be 12 and 22% respectively, in the second and last parts of the intestine. In this study the ^{15}N-method underestimated precaecal protein digestibility by 4·8% (HA-method = 100%).

We conclude that the ^{15}N-method can be used as a rather reliable method to measure precaecal protein digestibility.

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The movement of intact urea across the mucosa of the defunctioned human colon. By B. J. MORAN, S. J. KARRAN and A. A. JACKSON, *Departments of Surgery and Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU*

There is controversy and confusion in the literature as to the extent and ability of urea to cross the mucosa of the colon. In part this may be attributed to difficulties in carrying out appropriate investigations in the lower bowel, and in part to the extensive metabolic activity of the colonic microflora. We have shown that urea nitrogen placed in the colon at colonoscopy passes into the systemic circulation, but only 5% of the label administered was recovered as intact urea molecules in the urine. This implied that the majority of the dose was metabolized by the flora. In order to assess the extent to which urea might cross the colon wall without being metabolized, we have taken advantage of studying subjects in whom surgical intervention had produced a defunctioned loop of the left colon.

Five adults, aged 55–74 years, participated in the study. One subject had the study repeated on two separate occasions. A known dose of $^{15}N^{15}N$-urea (1·5 mg/kg body-weight) was placed into the lumen of the defunctioned loop of colon. Urine was collected into acid in four divided specimens over the following 72 h. Urea was isolated for mass spectrometry by column chromatography and the enrichment of ^{30}N- and ^{29}N-urea measured in a triple collector isotope ratio mass spectrometer.

Subject . . .	1	2	3	4	5A	5B	Mean
Age (years)	61	74	70	59	55	55	
Recovery isotope (% dose)							
^{30}N-urea	29	22	29	74	32	30	37
^{29}N-urea	6	14	10	6	13	13	10
Retained	65	64	61	20	55	57	54

These results confirm that the human colon is permeable to urea. The difficulties that have been experienced in demonstrating this in the past can be attributed to the efficient, rapid metabolism of urea by the colonic microflora. None of these subjects passed the contents of the defunctioned bowel, and so label not recovered in the urine had been retained; 54% on average. The observation is that substantial hydrolysis takes place in the defunctioned colon, despite the fact that the flora have been deprived of a normal provision of energy and substrate. These results emphasize the potential magnitude of floral metabolism under circumstances of normal physiology.

Hypocholesterolaemic and other responses to oat bran intake in humans. By R. W. WELCH, *Biomedical Sciences Research Centre, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB* and A. M. MCVEIGH and C. MURPHY, *St Mary's College, Falls Road, Belfast BT12 6FE*

Other workers have investigated the hypocholesterolaemic effects of dietary oat products in humans (e.g. Anderson *et al.* 1984; Turnbull & Leeds, 1987). However, in previous studies oats were usually included in the diet on an isonutrient basis or were combined with modifications in dietary fat intake, or both. The aims of the present work were to investigate the hypocholesterolaemic effects of oat bran intake in free-living subjects and to estimate concomitant voluntary diet modifications and changes in bowel function.

Cholesterol status was assessed throughout by measuring blood total cholesterol (CL) level on a pin-prick blood sample (Reflotron, BCL, Lewes, Sussex) taken after an overnight fast. Twelve subjects (six male; six female) with CL >5·0 mmol/l were selected after an initial screen (week 1). Ages (years) and body mass indices were (mean, range, standard deviation), 42·8, 13–61, 15·5; 27·6, 19·6–32·7, 4·32. One week later, CL concentrations were measured again (week 2) and the subjects incorporated 90 g oat bran/d (The Quaker Oats Company, Chicago, Illinois) into their normal diets for 3 weeks. CL levels were measured at the end of each week on the diet (weeks 3, 4, 5) and for the two subsequent weeks (weeks 6 and 7). Diet records were kept during the week preceding the oat bran diet and during the second week on this diet. Nutrient intakes were estimated using standard portion sizes and food tables. Body-weights were taken and bowel habits were assessed by questionnaire at the start and end of the oat bran period. CL levels were compared by analysis of variance and other data by paired *t* tests. There were significant ($P<0·001$) differences in CL levels during the experiment. Mean CL (mmol/l) at weeks 1–7 were 5·62, 5·37, 5·22, 4·82, 4·97, 5·17, 5·25 respectively (least significant difference between means, $P<0·05 = 0·244$). Thus after 2 and 3 weeks on the oat bran diet CL levels were significantly lower than the initial values and there was a gradual and significant increase in the two subsequent weeks. Comparison of the normal and oat bran periods (mean (SD)) showed no significant changes in body-weight (74·7 (14·8) *v.* 74·5 (14·2)kg), total energy intake (5·89 (0·97) *v.* 6·12 (0·73)MJ/d) or energy intake as fat (2·47 (0·52) *v.* 2·25 (0·31)MJ/d) but there were significant increases in energy intake from protein (0·81 (0·18) *v.* 0·96 (0·19)MJ/d; $P<0·001$), carbohydrate (2·50 (0·81) *v.* 2·81 (0·66)MJ/d; $P<0·01$) and total fibre intake (11·1 (5·9) *v.* 22·9 (5·6)g/d; $P<0·001$). Mean frequency of bowel movement was significantly increased ($P<0·001$) from 4·8 (2·0) on the normal diet to 6·9 (2·1)/week during the oat bran period.

The results indicate that the daily incorporation of 90 g oat bran into the diet of free-living individuals as the only intervention can yield significant hypocholesterolaemic effects and improve bowel function.

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Large bowel fermentation in rats given raw peas in the diet. By LAURENTINA M. R. PEDROSO, J. C. MATHERS and HEATHER J. FINLAYSON, *Department of Agricultural Biochemistry and Nutrition, The University, Newcastle upon Tyne NE1 7RU*

Peas are a rich source of complex carbohydrates, including starches which are slowly digested by pancreatic amylase, and non-starch polysaccharides which largely escape small intestinal digestion (Cummings & Englyst, 1987), and are therefore a useful dietary component for manipulating substrate supply to the large bowel (LB).

Groups of twelve male Wistar rats (initial weight 138 g) were housed individually in metabolism cages and offered 15 g/d of semi-purified diets containing 0 (control) or 500 g peas (*Pisum sativum*) (+ Peas)/kg for 22–26 d. Both diets contained 350 g maize starch/kg and raw milled peas were added at the expense of sucrose and fishmeal to ensure similar protein and digestible energy contents.

	Caecum							Colon	
					Molar proportions (mmol/mol)				
Diet	Tissue (g)	Wet contents (g)	pH	Total VFA†	Acetate	Propionate	Butyrate	Tissue (g)	Wet contents (g)
Control	0·57	1·87	6·7	95	607	226	85	0·81	1·23
+ Peas	0·90	4·28	6·1	136	606	163	197	0·94	1·80
SED*	0·036	0·325	0·07	8·1	1·9	9·6	14·5	0·032	0·154

*Dietary effects significant at $P<0·001$ except for acetate, not significant ($P>0·05$).
†mmol/kg caecal contents.

Pea-feeding was associated with larger caecums and colons with significant increases in both LB tissue and digesta contents, supporting the hypothesis that the LB enlarges to accommodate the tendency of residual material to accumulate within it (Wyatt *et al.* 1988). Caecal pH was reduced as volatile fatty acid (VFA) concentration increased. The molar proportions of individual VFA were markedly altered with a doubling of butyrate matched by reductions in propionate and longer chain VFA. The caecal butyrate pool was 7·6-fold greater in pea-fed animals (115 *v.* 15 μmol/caecum). Since butyrate is a major end-product of starch fermentation in vitro (Goodlad & Mathers, 1988), some pea-starch may have escaped small intestinal digestion. These results suggest that LB fermentation was stimulated by pea-feeding so that both the amount and pattern of VFA available for absorption were altered.

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

In a previous study, we observed that including wheat bran, at the expense of sucrose and casein, in the diet of rats was associated with significant reductions in the activities of the two principal NADPH-producing enzymes (glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; G6PDH) and malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$) (*EC* 1.1.1.40; ME)) and of ATP-citrate (*pro*-3S)-lyase (*EC* 4.1.3.8; CL) in liver (Pedroso *et al.* 1989). These effects may have been due to an alteration in the pattern of substrates supplied to the liver associated with stimulation of large bowel fermentation.

Livers were obtained from rats (twelve per diet) fed, once daily at 10.00 hours, on semi-purified diets formulated to be isoenergetic and containing 0 (control) or 500 g raw peas (*Pisum sativum*) (+ Peas)/kg (Pedroso *et al.* 1990) and killed approximately 4 h after feeding. Specific activities of G6PDH, ME, isocitrate dehydrogenase ($NADP^+$) (*EC* 1.1.1.42; IDH), CL and acetyl-CoA carboxylase (*EC* 6.4.1.2; ACC) were determined by standard methods. Results are expressed as nmol substrate utilized/min per mg protein except for ACC (nmol carbon dioxide incorporated/min per mg liver).

	Growth rate (g/7d)	Liver wt (g)	Liver glycogen (mg/g liver)	Liver enzyme activities				
				G6PDH	ME	IDH	CL	ACC
Control	25·1	11·4	38	26·4	26·2	151	17·6	0·86
+ Peas	24·5	9·6	25	15·9	15·0	166	11·5	0·68
SED	1·38^{NS}	0·41***	3·6***	2·16***	1·22***	12·0^{NS}	0·87***	0·059**

NS, not significant; **$P<0·01$; *** $P<0·001$.

Inclusion of peas in the diet was associated with substantial reductions (0·40 and 0·43) in the activities of the two principal NADPH-producing enzymes, G6PDH and ME respectively, and of CL (0·35). Coupled with the significant ($P<0·01$) reduction (0·21) in ACC (the rate-limiting enzyme for fatty acid synthesis from acetate), these observations suggest that the capacity for lipid synthesis in the liver was reduced by pea-feeding. Animals consuming peas would be expected to absorb less fructose and more volatile fatty acids than control rats but it is not clear how, or indeed whether, these changes in the pattern of substrates supplied to the liver are responsible for the altered hepatic enzyme activities.

We thank R. G. Vernon for advice on the ACC assay.

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Influence of guar gum flour of different molecular weights on viscosity of jejunal digesta in the pig. By F. G. ROBERTS, H. A. SMITH and A. G. LOW, *Institute for Grassland and Animal Production, Shinfield, Reading RG2 9AQ*, P. R. ELLIS, *King's College London, London W8 7AH*, E. R. MORRIS, *Cranfield Institute of Technology, Silsoe College, Bedford MK45 4DT* and I. E. SAMBROOK, *Institute for Food Research, Shinfield, Reading RG2 9AT*

Guar gum is known to reduce post-prandial blood glucose and plasma insulin but its physiological mode of action is not fully understood. Current evidence indicates that a key factor is the ability of guar gum to hydrate and increase the viscosity of the digesta, leading to delayed glucose absorption (Jenkins *et al.* 1978). Although the rheological properties of guar gum solutions in vitro are well established, there is a lack of reliable information about the levels of viscosity they induce in the small intestine. The aim of this study was to employ a novel technique to obtain information about the viscosity of guar gum in digesta in vivo. Such information could ultimately be useful for simple in vitro predictions of the physiological effectiveness of different types of guar gum.

Three Large White × Landrace boar pigs were fitted with re-entrant cannulae 2·0 m distal to the pylorus. A low-fat, semi-purified diet was given twice daily at a level of 40 g/kg body-weight per d. Guar gum flours of different relative molecular weights (Meyprogat 60, 90, 150; Meyhall Chemical (UK) Ltd, Wirral, Merseyside) were added as solutions, fully hydrated over 12 h, at levels of 20 or 40 g guar gum/kg diet 15 min before feeding in the proportions of 2·5 kg solution/kg diet.

Samples of jejunal digesta were collected immediately following the morning feed (time 0 min) and subsequently at 10- or 30-min intervals between 0 and 240 min for measurements of apparent viscosity at 39° (Brookfield DV-II rotaviscometer, Stoughton, USA), across a range of shear rate conditions. 'Zero shear' viscosity (Robinson *et al.* 1982) was then calculated.

Maximum 'zero shear' viscosity (mPa.s) of pure solutions of guar gum and jejunal digesta†

Guar gum grades . . .	Control	M60	M90	M150
20 g guar gum/kg diet				
Solution	0	360	2640	8200
Digesta				
Mean	57	82	536	465
SE	20	63	463	372
40 g guar gum/kg diet				
Solution	0	1600	8200	—
Digesta				
Mean	57	482	4220*	5160*
SE	20	352	2960	521

*Significantly different from control: $P<0·01$.
†Peak viscosities were all observed at 40 or 50 min after feeding.

The peak viscosities of the guar gum-containing digesta occurred within the first 60 min of the post-prandial period, corresponding to the time during which greatest changes have been seen in glycaemia and insulinaemia in previous studies. The tendency towards an increase in viscosity of digesta with increasing molecular weight of guar gum in the diet reached a plateau towards the highest molecular weights used at both concentrations. This is possibly a dilution effect resulting from increased endogenous secretions in the gut.

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Effect of two types of guar gum in solid and liquid foods on postprandial blood glucose, plasma insulin and C-peptide in healthy subjects. By R. M. FAIRCHILD and C. E. J. DANIELS, *School of Home Economics, University of Wales College Cardiff, Cardiff CF1 3AS* and P. R. ELLIS, *Department of Food and Nutritional Sciences, King's College London, London W8 7AH* and S. H. M. NAQVI, R. M. F. KWAN and M. A. MIR, *University Hospital of Wales, Cardiff CF4 4XW*

Guar gum is known to improve the carbohydrate and lipid metabolism of diabetic patients, but the most effective type and mode of administration of guar gum are still unknown (Peterson, 1985). We have investigated the effect of guar gum flour (RG30, Hercules Ltd) and granulate (Guarem, Rybar Laboratories Ltd), in solid and liquid foods, on blood glucose control.

After overnight fasts, eleven healthy subjects consumed breakfast meals (one control, three supplemented with guar gum) on separate days in random order. Each meal consisted of orange juice, toasted white bread (wheat) with butter and jam, cornflakes with whole milk and drinking water (available carbohydrate, 106 g; protein, 17 g; fat, 30 g; energy, 3095 kJ). Guarem (5·1 g) was either mixed into orange juice (immediately before consumption) or incorporated into bread; guar gum flour was incorporated into bread only. Venous blood samples were taken preprandially and 15, 30, 45, 60, 90, 120 and 150 min after commencement of each meal and analysed for blood glucose, plasma insulin and C-peptide.

Meals containing guar gum bread (both flour and Guarem), but not Guarem in orange juice, significantly reduced postprandial insulin at 60 (guar gum flour only), 90 and 120 min compared with the control (see Table). No significant differences in the glucose or C-peptide concentrations were observed between the control and guar gum meals.

Change in plasma insulin (mU/l) from fasting values

(Mean values with their standard errors, *n* 11)

	Postprandial time (min)													
	15		30		45		60		90		120		150	
Meal type	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	23	7	82	12	90	13	73	10	57	9	49	8	45	16
Guar gum flour in bread	27	2	76	8	83	15	51*	11	36**	8	26‡	4	26	5
Guarem in bread	21	7	69	9	74	9	53	12	34‡	5	29*	4	26	5
Guarem in juice	19	2	79	10	95	13	78	11	61	10	40	4	40	10

Significantly different from control: *$P<0·05$; **$P<0·01$; ‡$P<0·005$.

Guarem in orange juice taken at the start of a meal does not appear to be an effective method of reducing postprandial insulin. The C-peptide results suggest that the insulin-sparing effect of the guar gum breads may be due to an increase in hepatic extraction of insulin rather than a decrease in insulin secretion.

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Development and validation of a diet questionnaire for health education. By M. G. O'DONNELL[1,2], M. NELSON[2] and P. H. WISE[1], [1]*Department of Endocrinology, Charing Cross Hospital, London W6 8RF* and [2]*Department of Food and Nutritional Sciences, King's College London, London W8 7AH*

A self-administered diet questionnaire for use in public health was developed and validated using a representative sample of adults drawn from an inner London general practice. Questionnaire completion normally takes between 20 and 40 min. The subject's nutrient profile can be obtained within 5 min using a specially developed computer program. Two print-outs are provided. The first, for the health professional, gives a comprehensive listing of nutrient intakes. The second, for the subject, provides personalized dietary feedback on the following: energy and ideal body-weight, protein, total fat, saturated fat, polyunsaturated:saturated fat ratio, total sugars, dietary fibre, salt, alcohol, calcium, iron, zinc, and vitamins A, B_1, B_2, nicotinic acid, B_{12}, folate, C, D and E. This print-out, together with a nutrition information pack, gives practical suggestions for advised dietary changes.

The questionnaire, designed to assess habitual nutrient intake, was validated against 16 d weighed diet records (four 4 d records over 3 months) obtained from fifty-two male and female subjects aged 19–58 years. Four blood samples and four 24 h urine samples were obtained concurrent with the four diet records.

Pearson product-moment correlation coefficients between estimates of nutrient intake based on questionnaire and weighed records ranged from 0·73 for alcohol to 0·26 for vitamin D. The results revealed important differences between males and females in the strengths of the associations between questionnaires and weighed records, and emphasize the need to validate questionnaires for males and females separately. The Table shows the correlations between selected biochemical measurements and estimates of intake by weighed record and by questionnaire for all subjects.

	Weighed record	Questionnaire
Serum		
Zn	0·30*	0·26
γ-Glutamyltransferase (*EC* 2.3.2.2) *v.* alcohol intake	0·39*	0·26
Urine		
Sodium	0·40*	0·24
Potassium	0·50*	0·08
Nitrogen *v.* dietary protein	0·56*	0·23

*$P<0·05$.

Although the questionnaire showed weak correlations with biochemistry and with nutrients estimated by weighed records, careful exploration of the data using linear regression techniques allowed questionnaire responses to be used to classify subjects into intake groups with minimal misclassification for purposes of dietary feedback. A study is currently under way to evaluate the impact of the feedback on dietary modification.

Energy and nutrient intakes of disabled and of elderly subjects as assessed using a combination method of dietary survey. By N. L. BULL, *84 Grove Road, Tring, Herts* and S. J. GATENBY

As part of a larger study, nine disabled and twelve elderly people took part in a field trial of a 'combination' dietary survey method. The background to the study is given by Bull & Wheeler (1986) which describes dietary survey work carried out with thirty well-motivated and intelligent subjects, leading to the proposal of a new method of assessment for use in larger-scale surveys. The method operates as follows. First, the subject is interviewed, given a dietary diary and a set of weighing scales and asked to weigh all food and drink for a period of three consecutive days, including one weekend day. When this has been completed, the record is checked during a second interview and a food frequency questionnaire and 24 h recall of the previous day's consumption are carried out. Last, during the following 3 weeks a second 24 h recall is completed by telephone. This recall covers the other weekend day.

Results from the 5 d of record and recall, for the disabled and elderly groups, indicated that the disabled in particular had low intakes of energy, as assessed against current recommended daily amounts (RDAs) (Department of Health and Social Security, 1979). It appears that these subjects, six of whom had very limited mobility, had adapted their diets in such a way that nutrient intakes remained generally adequate compared with RDAs, while lower than average consumption of fat and carbohydrate resulted in low average energy intakes.

All elderly subjects were aged between 65 and 85 years, and all but two were assessed subjectively to be at or above ideal body-weight (IBW). The eight female disabled were aged between 18 and 54 years, and the male subject was between 18 and 34 years of age. All but one of the disabled subjects were assessed as at or above IBW.

		Average daily intakes							
		Energy (MJ)	Protein (g)	Fat (g)	Fat (%)	Carbo-hydrate (g)	Calcium (mg)	Iron (mg)	Vitamin C (mg)
Elderly (*n* 12)	Mean	7·53	71·9	80	40	206	1049	13·3	63
	% RDA	92	148				210	133	211
Disabled (*n* 9)	Mean	5·83	66·7	57	36	162	855	10·6	70
	% RDA	63	122				171	91	233
All subjects (*n* 51)	Mean	8·00	75·9	85	39	211	991	12·9	66
	% RDA	87	139				198	188	220

The authors thank Erica Wheeler for her help and gratefully acknowledge funding for this project from the Ministry of Agriculture, Fisheries and Food.

Bull, N. L. & Wheeler, E. F. (1986). *Human Nutrition: Applied Nutrition* **40A**, 60–66.
Department of Health and Social Security (1979). *Recommended amounts of food energy and nutrients for groups of people in the United Kingdom*. London: H.M. Stationery Office.

Diet and health in a random sample of British adults. By MARGARET J. WHICHELOW and F. P. TREASURE, *Department of Community Medicine, University of Cambridge Clinical School, Fenner's, Gresham Road, Cambridge CB2 2ES*

The relationship of diet to morbidity and mortality for various diseases, particularly cardiovascular disease, has been extensively investigated. This study reports the relationship of some indices of self-reported and measured health, with reported dietary habits in the 9003 randomly selected adults in the 1984–5, Health and Lifestyle Survey (Cox *et al.* 1987). The frequency of consumption of a large number of food items, the type of bread eaten and whether or not breakfast was eaten were amongst the dietary information recorded. Information was also obtained about other lifestyle habits. The measurements of health examined here are the respondents' assessment of their overall health status, the number of symptoms of physical ill health (illness) and of mental/psychological ill health (malaise) and lung function (FEV1).

The foods selected for analysis were those where, in the survey population as a whole, there appeared to be a difference in health status between the frequent and the infrequent consumers. Log linear analysis has been used to examine the relationship between the measures of health and the frequent ('most days' or 'daily' for most items) consumption of items of the diet, adjusting for age (three groups, ages 18–39, 40–59, 60+ years), social class (non-manual including students and manual) and smoking status (current regular cigarette smokers compared with non-smokers, ex-smokers and occasional smokers) (Whichelow *et al.* 1988). All the significant associations found are shown in the Table.

	Health, excellent/good		Low illness score		Low malaise score		FEV_1, excellent/good	
	♂	♀	♂	♀	♂	♀	♂	♀
Fruit	**	**		**	**	***		*
Juice	*	**		**				
Salad, summer	*	***			**	**	*	
Salad, winter	*	***		**	**	***	**	
Green vegetables					**	*		
Potatoes (excluding chips)				**				
Cheese		***						
'Brown' bread		*	*			*		
Cake	*				***	*		
Puddings	**	**		*	***	***		
Jam, preserves	**	*			**			
Breakfast	*				*	*		

*$P<0{\cdot}05$, **$P<0{\cdot}01$, ***$P<0{\cdot}001$.

The most striking findings are those of the close associations between the frequent consumption of fresh fruit, salads and puddings (including pies, tarts, steamed puddings, fruit 'crumbles' etc., but excluding ice cream, yoghurt, jellies, milk puddings etc.) with 'excellent/good' health and low malaise score. Few associations were found with symptoms of physical illness (low illness score), particularly amongst men, or with lung function. All the associations found were 'positive' – that is, of 'frequent' consumption with good health. No associations of 'frequent' consumption of any food item with poor health could be detected. In this context, in view of the role of fat in the aetiology of heart disease, the lack of a relationship between 'frequent' consumption of some of the fattier foods, e.g. chips, fried food and processed meats, with any measure of ill health is noteworthy.

This study was supported by The Health Promotion Research Trust.

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Intakes of individual sugars in Britain. By JANET LEWIS and D. H. BUSS, *Ministry of Agriculture, Fisheries and Food, 65 Romney Street, London SW1P 3RD*

Household purchases of packet sugar have declined steadily from an average of 70–75 g/d between 1955 and 1967 to just over 30 g/d in 1987. This compares with a wartime low of 34 g/d (Ministry of Agriculture, Fisheries and Food, 1951–1989). Although national supplies of sugar have also fallen, reductions in home baking and jam making may have led to an increase in sugars from pre-manufactured products. We have therefore used new analytical data (Holland *et al.* 1988, 1989; Ministry of Agriculture, Fisheries and Food, unpublished results) to determine the amounts of sucrose, other sugars and starch in every food recorded in the National Food Survey (NFS) in 1987. The Table shows that these other foods provide a further 25·2 g sucrose, as well as 43·2 g of other simple sugars (particularly from dairy products, fruit and glucose syrups used as ingredients in foods). It is not now possible to quantify the contribution from such foods in earlier years.

The additional contributions that could be made by sweets and alcoholic drinks are also shown. These foods are not included in the NFS and have been estimated from national supply data. Soft drinks could provide a further 11 g sugars/person per d. If the sugars from these items are added to total household sugars, then the overall intake is 137 g/d. Nevertheless, the sucrose in all these foods is little more than that from packet sugar alone in the 1960s.

	Carbohydrate intake (g/person per d)					
	Sucrose	Glucose	Fructose	Lactose	Other*	Starch
Packet sugar	31·8	0	0	0	0	0
Milk, cream and cheese	0·8	0·2	0·1	15·5	0·2	0·1
Meat, fish and products	0·1	0·1	tr	0·1	0·2	5·4
Potatoes	0·6	0·4	0·3	0	0	19·2
Other fresh and processed vegetables	2·8	1·4	1·2	tr	0·2	10·5
Fruit and fruit products	5·2	4·3	5·5	0	0·2	0·4
Bread and flour	0·7	2·5	0·2	tr	0·1	67·0
Cakes, biscuits and other cereal products	10·8	1·2	0·9	0·6	0·6	36·6
Other foods	4·4	3·1	2·2	0·7	1·1	1·9
Totals	**57·0**	**13·2**	**10·4**	**16·9**	**2·7**	**141·1**
Chocolate and sugar confectionery	16·1	2·1	0·3	1·2	1·3	0
Alcoholic drinks	0·1	1·0	0·4	0	3·2	0

*Other sugars include maltose and galactose, but not sugar alcohols.

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Ministry of Agriculture, Fisheries and Food (1951–1989). *Annual Reports of the National Food Survey Committee*. London: H.M. Stationery Office.

Diet and plasma lipids in a group of vegetarians and omnivores. By L. S. ROE, M. THOROGOOD and J. I. MANN, *Department of Community Medicine and General Practice, University of Oxford, Oxford*

As part of a prospective study of diet and health, 5500 4 d diet records and 3300 blood samples were collected from vegetarians and health-conscious omnivores. 'Vegetarians' were recruited through the Vegetarian Society and through media appeals, and the omnivore comparison group was formed of meat-eating acquaintances of vegetarian participants. The omnivores are thus not necessarily representative of the UK population, but are likely to be very similar to the vegetarians in social class and smoking, drinking, and exercise habits. The diet records are A4-sized booklets which include instructions for describing the foods eaten and recording portion sizes in household measures. The booklets also include photographs of several portion sizes of common foods, and questions about types of food consumed regularly. The records cover two weekend days and two weekdays. A sample of 208 of these records was analysed in order to examine the relation between nutritional intake and serum lipids. Twenty-six male and twenty-six female vegans, who eat no animal products, were matched on age and sex to the same number of lacto-ovo vegetarians, fish-eating vegetarians and omnivores.

The average age of these subjects was 42 years.

	Body mass index (kg/m^2)	Energy (MJ/d)	Total fat (g/d)	Saturated fatty acids (g/d)	Polyunsaturated fatty acids (g/d)	Polyunsaturated: saturated fatty acids	Dietary fibre (g/d)	Plasma cholesterol (mmol/l)
Vegans	22·0	9·4	88·2	17·2**	28·2**	1·8**	49·0**	4·92
Lacto-ovo vegetarians	22·5	9·6	97·6	33·6	20·9	0·7	36·6	5·34
Fish-eating vegetarians	22·0	9·7	102·0	33·4	21·8	0·7	33·4	5·65
Omnivores	22·9	9·5	98·3	34·9	17·3	0·5	30·9	5·93

$^{**}P<0·01$.

Although total fat intake did not vary between the diet groups, vegan participants had significantly lower intakes of saturated fat and higher intakes of polyunsaturated fat than the other groups. Dietary fibre intakes were also significantly higher in vegans. Total plasma cholesterol and low-density lipoprotein cholesterol were lowest in the vegans, higher in the vegetarian groups, and highest in omnivores. High-density lipoprotein cholesterol was slightly elevated in the fish-eating vegetarians, reflecting a trend found in the larger group from which the sample was drawn (Thorogood *et al.* 1987).

Over all of the diet groups, total plasma cholesterol was correlated significantly with intake of saturated fat (r 0·32), polyunsaturated fat (r −0·25), and dietary fibre (r −0·26). The relation between plasma cholesterol and dietary intakes was strongest in female participants. Most cross-sectional studies have failed to find a significant relation between nutritional components and plasma lipids. This study suggests that the examination of a wide range of diets, the inclusion of female subjects, and a more detailed method of dietary recording are important methodological factors.

Thorogood, M., Carter, R., Benfield, L., McPherson, K. & Mann, J. I. (1987). *British Medical Journal* **295**, 351–353.

What do 'vegetarians' eat? By ALIZON DRAPER and ERICA F. WHEELER, *Centre for Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT* and JANET LEWIS, *Ministry of Agriculture, Fisheries and Food, 65 Romney Street, London SW1P 3RD*

A group of 137 subjects who had changed their diet to become 'vegetarian' (i.e. not vegetarian for ethnic or religious reasons) was recruited in Greater London, of whom 127 completed a 3 d weighed dietary intake measurement. They comprised thirty-seven who usually avoided fish or meat or both (demi-vegetarians), fifty-two who usually ate no animal foods other than milk and eggs (lacto-ovo vegetarians) and thirty-eight who ate no animal foods at all (vegans). Recruitment was done through local radio, specialist shops and magazines, and personal introductions. Nutrient intakes were computed using the Ministry of Agriculture, Fisheries and Food database, derived from food tables (Paul & Southgate, 1978), with the addition of new recipes and specialist foods where necessary. The Table shows intakes of energy-yielding constituents and dietary fibre. Analysis of variance showed some significant effects of sex and vegetarian group, but no interactions.

	Intake/d											
	Demi-vegetarian				Lacto-ovo vegetarian				Vegan			
Sex . . .	Male		Female		Male		Female		Male		Female	
No. in group . . .	13		24		16		36		18		20	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy (MJ)	9·86	3·91	7·98	2·59	9·40	2·47	7·67	2·45	9·20	3·09	7·35	2·66
Protein*† (g)	82	39	59	20	66	21	56	17	65	27	47	18
Fat* (g)	100	51	85	34	93	35	77	36	85	43	67	42
Fatty acids (g)												
Saturated*†	37·3	23·5	15·9	15·9	32·6	16·2	25·1	13·9	18·0	11·5	15·7	7·9
Polyunsaturated†	20·2	12·4	22·5	12·4	21·4	9·6	20·2	12·6	30·5	15·6	23·9	16·2
Carbohydrate (g)	268	122	221	71	280	86	221	66	289	99	243	85
Dietary fibre*†‡	35	20	30	10	34	13	33	12	44	16	36	17
Alcohol*†	17·4	24·4	8·8	14·4	12·2	25·2	12·3	25·2	11·6	18·6	3·3	8·9

*Significant effect of sex (ANOVA): $P<0·05$.
†Significant effect of vegetarian groups (ANOVA): $P<0·05$.
‡Southgate (1978).

The main food groups contributing to energy intake were cereals (32–40%), milk products (11–12%, not vegans), and fruits and nuts (11–19%). Fats and oils contributed less (15%) to fat intake than did cereals (19–25%) and milk products (15–18%, not vegans). Cereals were also the major contributor to protein (29–42%) and fibre (41–44%) intakes.

Paul, A. A. & Southgate, D. A. T. (1978). *McCance and Widdowson's The Composition of Foods*. London: H.M. Stationery Office.
Southgate, D. A. T. (1978). *American Journal of Clinical Nutrition*, **315**, 107–110.

Who are 'vegetarians' and what do they think about food? By ALIZON DRAPER, NINA MALHOTRA and ERICA F. WHEELER, *Centre for Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

Anecdotal evidence suggests that avoidance of some or all animal foods is increasing. A group of 137 'vegetarians' was recruited in Greater London, of whom thirty-seven were demi-vegetarians (D), fifty-two lacto-ovo vegetarians (L), and thirty-eight vegans (V) (Draper *et al.* 1990). A pretested question schedule was administered, covering their attitudes, opinions and practices in relation to food, health and related aspects of lifestyle.

Women outnumbered men (D 65%, L 69%, V 53%). There was a predominance of the professional and managerial social classes, Registrar General's groups 1–2 (58%, whole group) and of graduates (51%). Vegans were a conspicuously younger group, having adhered to their diet for correspondingly shorter periods of time. Vegans were more likely to be unpartnered (D 45%, L 65%, V 72%), to live alone (D 11%, L 31%, V 30%), and to belong to an organization directly connected with their diet (D 0%, L 28%, V 55%). They were more likely to modify other aspects of lifestyle (D 68%, L 81%, V 96%) and to cook their own food (D 30%, L 44%, V 63%). Vegans were less likely to compromise by eating animal foods in any social context (D 62%, L 14%, V 5%). Most of them (89%) had adhered to some kind of vegetarian diet before becoming a vegan. Reasons for adopting the diet included moral/ethical considerations (D 27%, L 22%, V 17%) and personal preference (D 22%, L 15%, V 6%). Most of the group (93%) viewed meat as harmful. Reasons included presence of antibiotics, etc. (D 32%, L 30%, V 33%), high fat content (D 35%, L 32%, V 26%) and revulsion to eating a 'dead' substance (5%). White meats were considered less harmful than red (63%). Most of group D considered fish to be an acceptable food, and all rated fish as 'lower' animals.

The whole sample had very strong feelings about health: 99% felt that diet was important in maintaining good health; 94% took care about what they ate; 67% felt that their health had improved since becoming vegetarian, which was associated with group (V 87%, L 61%, D 51%). Health itself was conceptualized in holistic terms, encompassing mental and spiritual qualities as well as the purely physical. Fibre and vitamins were poorly defined in terms of orthodox medical knowledge, but moral and symbolic qualities were found to be projected onto these items. This may partly explain the high use of dietary supplements in the sample.

Attitudes regarding food revealed a preference for it to be as unprocessed and as natural as possible. The word natural was a constantly recurring word in responses to all topics and carried symbolic connotations of goodness, purity and wholeness. Inevitably, the food industry and food additives were viewed with great cynicism: 96% felt that most additives were unnecessary. Organic produce was considered superior by 93%.

Vegetarianism expresses more than a pragmatic decision about dietary choice; it articulates a coherent and rational set of attitudes and beliefs which extend from food and health to abstract and philosophical issues such as ethics and man's relationship to the natural world.

Draper, A., Wheeler, E. F. & Lewis, J. (1990). *Proceedings of the Nutrition Society* **49**, 60A.

Dietary differences between social class groups in the Scottish Heart Health Study. By C. BOLTON-SMITH, W. C. S. SMITH, M. WOODWARD, C. A. BROWN and H. TUNSTALL-PEDOE, *Cardiovascular Epidemiology Unit, Ninewells Hospital and Medical School, Dundee DD1 9SY*

Heart disease mortality is higher in the low social classes, but there is little information available on dietary differences between social class groups. The Scottish Heart Health Study (Smith *et al.* 1987) included food frequency questionnaire data and social class status by occupation (Office of Populations, Censuses and Surveys, 1980) for 10 359 men and women aged 40–59 years. Analysis of the questionnaires (Yarnell *et al.* 1983) by non-manual (N) and manual (M) groupings revealed significant differences in nutrient intakes.

	Men		Women	
Daily intake	Non-manual (*n* 2193)	Manual (*n* 2873)	Non-manual (*n* 2323)	Manual (*n* 2597)
Energy (kJ)	9154***	10 363	7168***	7507
Protein (g)	84·3***	90·3	74·3	74·9
Fat (g)	85·6***	95·0	74·0***	80·5
Carbohydrate (g)	255***	295	188**	197
Alcohol (g)	19·1***	23·9	6·9***	5·4
Fibre (g)	22·0***	20·7	20·5***	18·0
α-Tocopherol (mg)	10·0***	9·4	8·7***	8·3
Vitamin C (mg)	56·7***	50·1	56·6***	48·1
β-Carotene (μg)	3344**	3145	3556***	3267
Vitamin A (μg)	1244**	1312	1264	1282
Protein† (% of energy)	15·6***	14·8	17·6***	17·0
Fat (% of energy)	35·0**	34·6	38·6***	40·2
Carbohydrate (% of energy)	43·3***	44·3	40·9	40·7
Alcohol (% of energy)	6·1	6·4	3·0***	2·2
P:S	0·324	0·308	0·305***	0·281

P:S, polyunsaturated:saturated fatty acid ratio.

By analysis of variance, after log or square root transformation as appropriate, non-manual group significantly different from manual group: $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.

† % Energy given inclusive of alcohol.

Higher energy intakes in M compared with N women were mainly due to an 8·8% increase in fat, with a shift in the source of fats away from margarines containing polyunsaturated fatty acids, meat and sweets/puddings to meat products, 'other' margarines and butter. For M compared with N men, the higher energy intakes were largely due to a 15·7% increase in carbohydrate, with a shift away from cereals, rice/pasta, milk and sweets/puddings to bread, alcohol and table sugar. The lower vitamin intakes in the M groups were related to less regular consumption of fresh fruit and vegetables.

Both dietary fat and, more recently, antioxidant vitamins (Gey, 1986), have been implicated in the aetiology of cardiovascular disease. Thus, these differences in eating habits between social class groups may contribute to disease patterns. Community nutrition education programmes should therefore take into account the particular dietary aberrations of the group targetted.

Gey, K. F. (1986). *Bibliotheca Nutritio et Dieta (Basel)* **37**, 53–91.

Office of Populations, Censuses and Surveys (1980). *Classification of Occupations*. London: H.M. Stationery Office.

Smith, W. C. S., Crombie, I. K., Tavendale, R., Irving, J. M., Kenicer, M. B. & Tunstall-Pedoe, H. (1987). *Health Bulletin* **45**, 211–217.

Yarnell, J. W. G., Fehilly, A. M., Milbank, J. E., Sweetnam, P. M. & Walker, C. L. (1983). *Human Nutrition: Applied Nutrition* **37A**, 103–112.

Age trends in nutrient intakes for non-manual and manual occupational groups: the Scottish Heart Health Study. By C. BOLTON-SMITH, W. C. S. SMITH, M. WOODWARD and H. TUNSTALL-PEDOE, *Cardiovascular Epidemiology Unit, Ninewells Hospital and Medical School, Dundee DD1 9SY*

Age is an important factor in many disease processes. This may be a result of accumulated 'risk' over the years, to physiological factors associated with ageing, or to changes in lifestyle with age. All three processes may contribute to the increased risk of heart disease with age. Heart disease mortality is also higher in the low social classes. Dietary differences between social class groups have been noted (Braddon *et al.* 1988; Bolton-Smith *et al.* 1990) but the effects of age on nutrient intake have not been previously reported.

Dietary differences between four 5-year age groups, from 40 to 59 years, for non-manual (N) and manual (M) categories (Office of Populations, Censuses and Surveys, 1980) were examined as part of the Scottish Heart Health Study (Smith *et al.* 1987). Food frequency questionnaires (Yarnell *et al.* 1983) were collected from an average of 624 men and women for each age and social class group.

		Men				Women			
Age (years) . . .		40–44	45–49	50–54	55–59	40–44	45–49	50–54	55–59
Energy (kJ/d)	N	9267	9305	8979	9083	7063	7151	7273	7189
	M	10811**	10685	10116	9920	7574	7544	7448	7461
Protein† (% of energy)	N	15·5	15·5	15·6	15·7	17·6	17·7	17·7	17·5
	M	14·6**	14·6	14·9	14·9	17·0	17·0	16·8	17·0
Fat (% of energy)	N	34·1**	34·8	35·5	35·6	37·7**	38·4	38·7	39·4
	M	33·8**	33·9	35·0	35·6	39·6**	40·1	40·4	40·8
Carbohydrate (% of energy)	N	43·6	43·4	43·1	43·2	41·3	40·7	41·0	40·5
	M	44·2	44·6	44·4	44·1	40·6	40·5	40·9	40·6
Alcohol (% of energy)	N	6·8**	6·3	5·8	5·4	3·4**	3·2	2·6	2·6
	M	7·5**	7·0	5·8	5·4	2·7**	2·4	1·9	1·6
Fibre (g/d)	N	22·0	22·7	20·8	20·8	19·3	20·5	19·9	20·5
	M	20·8	21·2	20·7	20·2	17·8**	17·8	20·9	18·6

Significant differences with age (ANOVA) on log or square root transformed data as appropriate: $^{**}P<0{\cdot}01$.
† % Energy inclusive of alcohol.

The reduced energy intake in M men with age paralleled a significantly lower protein, carbohydrate and alcohol consumption (ANOVA, all $P<0{\cdot}01$). The percentage energy from fat increased with age, and the percentage energy from alcohol decreased with age for all groups. Nutrient densities for vitamins A, C and E were stable or elevated with age for both sexes.

It is not clear whether the dietary differences with age reported here reflect an individual's change in diet with age, or reflect trends in eating habits/dietary awareness in birth cohorts.

Bolton-Smith, C., Smith, W. C. S., Woodward, M., Brown, C. A. & Tunstall-Pedoe, H. (1990). *Proceedings of the Nutrition Society* **49**, 62A.

Braddon, F. E. M., Wadsworth, M. E. J., Davies, J. M. C. & Cripps, H. A. (1988). *Journal of Epidemiology and Community Health* **42**, 341–349.

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Sex, age and social class differences in attitudes towards consumption of high-fat foods. By R. SHEPHERD and G. TOWLER, *AFRC Institute of Food Research, Colney Lane, Norwich NR4 7UA*

Recommendations for a reduction in fat intake (COMA report, Department of Health and Social Security, 1984) might be achieved by modifying people's attitudes and beliefs, but this requires a clear theoretical framework within which to measure and relate attitudes, beliefs and behaviour (Shepherd & Stockley, 1987). Previously reported differences in attitudes between demographic groups may have been clouded by unequal sampling (Shepherd & Stockley, 1987).

Two hundred and forty subjects were quota sampled to give equal numbers from two age groups, two categories of social class and both sexes. Subjects completed a questionnaire on consumption of four food types, contributing highly to dietary fat; this was based on the Ajzen & Fishbein (1980) attitude model. For each food type, there was one question on usual behaviour, one on intention to consume the food during the following week, three attitude items, six salient belief items (from structured pre-interviews), and six corresponding evaluation items. Each item was scored −3 to +3.

Scores for the components of the model were calculated by summing attitude responses (scores can vary −9 to +9) and the products of belief evaluations (scores can vary −54 to +54). Correlations between belief values and attitudes varied from 0·50 to 0·64, for attitudes *v.* intention from 0·29 to 0·64 and for intention *v.* behaviour from 0·46 to 0·70 (df = 238, all $P<0·001$). Analyses of variance were carried out on each component for each food group with factors of sex, age and social class (Table).

		Sex		Class		Age		
Food type		Male	Female	abc1	c2de	15–44	Over 44	SE
Meat	Belief value	12·4	9·9	8·7	13·6*	4·0	18·3***	1·7
	Attitude	3·9	1·9**	1·6	4·2***	1·5	4·2***	0·5
	Intention	5·9	5·6	5·6	5·8	5·3	6·1**	0·2
	Behaviour	3·8	3·8	3·8	3·8	3·6	4·1***	0·1
Meat products	Belief value	2·5	−1·7†	−3·6	4·5***	−2·4	3·2*	1·8
	Attitude	2·3	−0·1***	−0·4	2·5***	0·0	2·1**	0·5
	Intention	5·3	4·1***	4·3	5·1*	4·4	5·0†	0·2
	Behaviour	3·1	2·6**	2·7	3·1*	2·8	2·9	0·1
Dairy products	Belief value	16·0	11·6†	10·5	17·2**	9·3	18·3***	1·6
	Attitude	5·2	4·9	4·3	5·8**	4·7	5·4	0·4
	Intention	6·6	6·5	6·6	6·5	6·6	6·5	0·1
	Behaviour	5·0	4·9	5·1	4·8*	5·1	4·9†	0·1
Fried foods	Belief value	−1·9	−7·7**	−9·1	−0·5***	−6·3	−3·3	1·5
	Attitude	−2·7	−5·6***	−4·6	−3·7†	−4·7	−3·7†	0·4
	Intention	4·2	2·8***	3·2	3·9*	3·5	3·6	0·2
	Behaviour	3·0	2·4***	2·7	2·8	2·8	2·7	0·1

†$P<0·10$, *$P<0·05$, **$P<0·01$ ***$P<0·001$.

Using the structured attitudes model, along with a quota sample, showed a clear relation between expressed belief values, attitudes, intention and self-reported behaviour. In general the females, higher social class and younger subjects showed more negative attitudes towards consumption of these types of food.

This work was supported by a grant from the Commission of the European Communities.

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Beliefs and attitudes toward healthier eating among women attending a maternity hospital. By ANNIE S. ANDERSON, *Department of Obstetrics, University of Aberdeen Medical School, Foresterhill, Aberdeen AB9 2ZD* and R. SHEPHERD, *AFRC Institute of Food Research, Colney Lane, Norwich NR4 7UA*

In order to identify beliefs and attitudes surrounding healthy food choices which could be targetted in maternity education programmes, ninety-five consecutive pregnant (50) and post-natal (45) women attending Aberdeen Maternity Hospital completed a detailed self-administered questionnaire on factors affecting food selection. Assessment items were based on Fishbein & Ajzens' (1975) expectancy value model which examines the relationship between behavioural intention and direct attitudes (feelings), direct subjective norm (perceived pressure from others), estimated attitudes (measured as a function of beliefs multiplied by the outcome evaluation of these beliefs) and estimated subjective norm (measured as a function of normative beliefs multiplied by motivation to comply).

Healthier eating was defined as 'increasing intakes of fibre-rich foods such as bread, potatoes, cereals and fruits, and decreasing intakes of foods high in fat such as dairy products, meat products and fried foods'. These foods were specifically targetted following a weighed dietary survey study which identified them as the most important contributors to dietary carbohydrate and fat in the population under study (Anderson *et al.* 1988).

More than half the group indicated a positive intention to try healthier eating (mean score +1·61, range −2 to +3). Behavioural intention (to try healthier eating) correlated significantly with the attitude component (r 0·500, $P<0·001$, β 0·433) and with the subjective norm component (r 0·340, $P<0·001$, β 0·182).

Responses were analysed by sub-dividing the total group into a high-scoring group (i.e. women who scored above the mean) and a low-scoring group. Significantly better results were found in the high-scoring group for belief items relating healthier eating to health maintenance ($P<0·002$) and eating the same as the rest of the family ($P<0·015$). Normative items relating perceived pressure from doctors ($P<0·010$) and other family members ($P<0·000$) to healthier eating were also significantly higher in the high-scoring group.

In conclusion, belief items relating healthier eating to maintaining good health and family concerns were identified for promotion in ante-natal education programmes. Normative items relating to the role of doctors and family in promoting healthier diets were also identified as important considerations. These findings suggest that efforts to change dietary habits could usefully focus on the belief and attitude variables.

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Meeting the dietary goals: how and at what cost? By JANET CADE and SUSAN BOOTH, *Community Medicine, South Block, Southampton General Hospital, Southampton SO9 4XY*

In order to meet the UK dietary goals for healthy adults changes in the usual diet will need to be made. Guidance has not always been clear as to what foods or combination of foods should be eaten to meet the goals. How achievable are the goals in practise and how much will the diet cost?

We analysed the diets in three English towns of a representative sample of 2340 men and women, aged 35–54 years, who had completed a 1 d food record in household measures (Cade *et al.* 1988). The majority of the subjects consumed more fat, more sugar and less carbohydrate and fibre than recommended. Nutrient intakes for those meeting the goals were compared with those who did not meet them, for example men and women who met the fat goal and saturated fat goal had lower energy intakes and higher fibre and vitamin C intakes than those who did not meet the goals.

When the goals were combined only 7% (eighty-one men and eighty-eight women) met all three fat goals (COMA Report, Department of Health and Social Security, 1984) and only 10% (106) men and 5% (55) women met both the carbohydrate and fibre goals (National Advisory Committee on Nutrition Education, 1983). The twenty-eight subjects who met a combination of the goals for total fat/saturated fat/polyunsaturated:saturated fat ratio/carbohydrate or sugar/fibre (the combined-goals group) were compared with a similar number of subjects randomly selected from subjects whose nutrient intakes were within the 25th and 75th percentiles and so deemed to be average. Nutrient and food intakes were compared. The combined-goals group ate more cereals, wholemeal and brown bread and less white bread than the others. They also ate less high-fat dairy products, less fatty meat and fried fish and more fruit and potatoes (including chips) than the average group.

The food diaries from the two groups were costed using prices from a supermarket in November 1988. Where possible the supermarket's own brand or the cheapest alternative was costed, except where a specific product had been described. The average daily cost of the food intake for the combined-goals group was (excluding alcohol) £2·03 and for the average group £1·86. Although not statistically significant, if a difference in cost of this size could be demonstrated in a larger study, then multiplied for a family of four for a week the extra cost of the diet is £4·76, which may put the cost of a diet that meets the goals out of reach of certain groups. However, it was also possible to eat a diet meeting the goals which was substantially cheaper than that of the average group.

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The interaction of vitamin A deficiency and rotavirus infection. By FARUK AHMED[1], DAVID B. JONES[2] and ALAN A. JACKSON[1], [1]*Department of Human Nutrition* and [2]*Department of Pathology, University of Southampton, Southampton SO9 3TU*

Vitamin A deficiency is the most common specific dietary deficiency in developing countries (Pirie, 1983). Rotavirus infection, one of the major causes of viral diarrhoea in infants and children (World Health Organization scientific working group, 1980), damages the epithelium of the intestinal tract (Starkey *et al.* 1986). Since vitamin A is essential for maintaining the integrity of epithelia, we have investigated the interaction of vitamin A deficiency and rotavirus infection in the mouse.

Weanling, male Porton mice were fed on a control diet *ad lib.*, a vitamin A-deficient diet *ad lib.*, or pair-fed the control diet to the intake of the vitamin A-deficient group. Vitamin A deficiency was induced by giving the deficient diet for 63–70 d. On day 77, mice were dosed orally with 30 μl EDIM rotavirus and examined 1 week later.

	No. of goblet cells/villus				Antibody levels (units)‡		
	Non-infected		Infected		Non-infected	Infected	
Group	Mean	SE	Mean	SE	Mean§	Mean	SE
Control	12·6	0·2	13·3	0·3	352	1299	236
Pair-fed	12·7	0·3	12·8	0·3	216	1100	76
Vitamin A deficient	9·9*	0·3	9·8*	0·5	384	594†	217

Significantly different from control value (Student's *t* test): *$P<0·01$.

Significantly different from the corresponding control and pair-fed groups (Wilcoxon rank sum test): †$P<0·05$.

‡One unit was defined as the antirotavirus activity present in 100 μl of a standard serum diluted $1:10^6$.

§Mean of pooled serum from each group.

The control and pair-fed animals had histologically normal villi, but rotavirus infection in the vitamin A-deficient animals caused drastic changes to the mucosa, with almost complete destruction of the tips of the villi. The number of goblet cells per duodenal villus was significantly lower in the deficient mice compared with the control or pair-fed animals. The serum level of total antibody specific to rotavirus was measured by ELISA. The vitamin A-deficient animals had significantly lower levels than the control or pair-fed mice.

This study shows that there is an interaction between vitamin A deficiency and rotavirus infection at the level of the gastrointestinal tract. Vitamin A deficiency leads to a reduced resistance to rotavirus infection which may be attributed to both an impaired mucosal barrier and defective antibody production.

The study was supported by the Commonwealth Commission, London.

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Effects of protein deficiency on blood cell and tissue metallothionein-I concentrations in rats. By J. N. MORRISON, ANNE M. WOOD and I. BREMNER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The concentration of metallothionein-I (MT-I) in blood cells is increased in anaemic rats but decreased after restriction of food intake (Robertson *et al.* 1989). These results suggested that the level of erythropoietic activity controls MT-I concentrations in blood cells. Since protein status also influences erythropoietin production, a study has been made of the effects of protein deficiency on MT-I production in blood, liver and kidneys of rats.

Groups of five male Hooded Lister rats aged 5 weeks were fed for 19 d on a semi-purified diet containing 50(LP), 120(MP) or 200(HP) g protein, as egg albumen/kg. MT-I was measured in blood cells and plasma, liver and kidneys by radioimmunoassay.

Growth rates in both groups of protein-deficient rats, LP and MP, were significantly less than in the control rats (HP). Concentrations of MT-I were also significantly less in the blood cells and kidneys of the LP rats than in the control animals. However, there was a consistent trend for the concentrations in the MP rats to be greater than in the control rats. In contrast, MT-I concentrations were increased in the liver and plasma of the LP rats but there were no differences between the MP and HP animals.

Haematological indices were measured in a parallel experiment and it was found that reticulocyte counts in the LP, MP and HP rats were equivalent to 11, 15 and 1% respectively of the erythrocytes. It seems therefore that the effect of protein deficiency on blood cell MT-I levels depends on the degree of protein restriction and on the associated changes in erythropoietic activity.

MT-I concentration in protein-deficient rats

(Mean values with their standard errors for five rats per group)

Dietary protein (g/kg) . . .	LP 50		MP 120		HP 200	
	Mean	SE	Mean	SE	Mean	SE
Body-wt (g)	118	4	197	5	256	5
Blood cells (ng/ml blood)	22	1	53	5	43	2
Plasma (ng/ml)	8	1	4	1	4	1
Liver (μg/g)	25	3	8	1	2	1
Kidney (μg/g)	23	2	75	9	56	3

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Plasma ^{65}Zn kinetics in zinc-deficient and endotoxin-treated rats. By N. M. LOWE and M. J. JACKSON, *Department of Medicine, University of Liverpool, PO Box 147, Liverpool L69 3BX*

The diagnosis of marginal zinc deficiency is difficult due to the lack of a suitable accessible tissue which is sensitive to Zn status and because a reduction in the plasma or serum Zn concentrations may be caused by either Zn depletion or 'stress'. Stable isotope studies in man have suggested that the size of certain exchangeable body 'pools' may be influenced by body Zn status (Jackson *et al.* 1988), and we have therefore studied the kinetics of short-term ^{65}Zn turnover in rats of varying Zn status or 'stressed' with *Escherichia coli* endotoxin.

Groups of weanling female rats were fed on either an albumin-based Zn-deficient (Zn−) diet (Zn content 5μg/g) or the same diet supplemented with Zn (Zn+) (Zn content 181 μg/g). Further groups of weight-matched animals were fed on a standard laboratory chow and given intraperitoneal injections of either *E. coli* endotoxin (3 mg/kg body-weight), (Strain 0127: B8 Butanol extract; Sigma, Poole, Dorset) or an equivalent volume of isotonic saline. At 24 h post-endotoxin or saline, and after 6 weeks of feeding the Zn+ or Zn− diets animals were anaesthetized with sodium pentobarbitone (60 mg/kg body-weight) and a cannula placed in the carotid artery. ^{65}Zn chloride (100 μl, 2·5 μCi/rat) was then injected via the femoral vein and blood samples removed at various times post-injection. The decay curve of plasma ^{65}Zn specific activity was analysed using a two-compartment model (Shipley & Clark, 1972).

The Zn-depleted diet induced a fall in the plasma and tissue Zn concentrations in Zn− compared with Zn+ animals (mean (SD)) (plasma: Zn− 427 (204) cf. Zn+ 1274 (223) μg/l; bone: Zn− 34·4 (7·8) cf. Zn+ 222·9 (9·1) μg/g) while endotoxin-injected animals also had a reduced plasma Zn concentration (endotoxin: 903 (221) cf. saline: 1222 (195) μg/l). Calculation of the size of the initial pool with which the ^{65}Zn had equilibrated revealed a significant fall in the Zn− rats (17·5 (11·4) cf. 40·3 (20·6) μg/kg; $P<0·01$), but the initial exchangeable pool size in the endotoxin-treated animals was higher than in saline-injected controls (saline: 49·8 (13·8) cf. endotoxin: 72·8 (34·6) μg/kg; $P<0·05$).

These results support the hypothesis that kinetic studies of body Zn turnover may provide useful additional information on the Zn status of animals when used in conjunction with measurements of plasma Zn concentrations.

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Absorption of iron and zinc from human milk in suckling and weanling rats. By M. M. BRENNAN, A. FLYNN and P. A. MORRISSEY, *Department of Nutrition, University College, Cork, Irish Republic*

The ontogeny of micronutrient absorption processes in early life is poorly understood in humans and other animal species. We have previously outlined a method for simultaneous studies on iron and zinc absorption in rat pups (Brennan *et al.* 1989). This report outlines age-related changes in Fe and Zn absorption from human milk in young rats.

Pooled mature human milk was extrinsically labelled with both ^{59}Fe (1 μCi/ml) and ^{65}Zn (1 μCi/ml) and 0·6 ml milk was given by gavage to 16- and 22-d-old rats, previously fasted for 16 h. Animals were killed 6 h later and stomach, small intestine (SI), caecum–colon and liver removed. Small intestines were perfused with 6 ml 0·15 M-NaCl and divided into three segments of equal length. ^{59}Fe and ^{65}Zn in tissues were determined in a well gamma counter using a channels ratio method.

For ^{65}Zn the proportion of dose recovered in the caecum–colon was much greater and absorption, retention in SI tissue, transfer to carcass and uptake into the liver were much lower in 22-d-old than in 16-d-old rats. For ^{59}Fe the proportion of dose recovered in the caecum–colon was much greater and absorption was much lower in 22-d-old than in 16-d-old rats. However, there was little difference between the two ages in carcass or liver uptake due to a much greater retention of ^{59}Fe in SI tissue, particularly the ileum, at 16 d of age.

Uptake (% dose) of ^{65}Zn and ^{59}Fe from human milk in rats

	^{65}Zn				^{59}Fe			
	16 d (*n* 5)		22 d (*n* 6)		16 d (*n* 5)		22 d (*n* 6)	
Organ/tissue	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Stomach	1·2	0·1	0·9	0·2	0·5	0·1	0·5	0·2
Duodenum	8·9	0·5	2·4**	0·4	1·2	0·1	0·9	0·1
Jejunum	12·6	2·4	1·8**	0·3	6·6	2·0	0·9*	0·3
Ileum	17·9	2·8	2·5**	0·4	35·3	4·4	0·8*	0·2
SI total	39·5	3·2	6·6**	1·1	43·1	4·3	2·6***	0·5
SI perfusate	4·2	1·2	1·0	0·2	9·7	3·2	0·2*	0·1
Caecum–colon	2·9	1·3	57·0**	6·3	8·0	3·2	48·7**	6·8
Liver	18·1	0·6	10·8**	1·8	3·3	0·4	3·4	0·4
Absorbed†	89·5	1·8	40·9**	2·3	81·8	2·8	50·6**	6·6
Carcass‡	50·0	1·7	35·4*	5·9	38·7	1·6	48·0	6·8

Significantly different from 16 d: *$P<0·05$, **$P<0·01$, ***$P<0·001$.
†Absorbed = 100 − (stomach + SI perfusate + caecum–colon)(%).
‡Carcass = absorbed − SI total.

These results show that there is a considerable decrease in absorption of Fe and Zn from human milk in the rat between 16 and 22 d of age, coinciding with the transition from a diet of maternal milk only to one of chow and water. Food residues were found to be present in the SI of the 22-d-old animals, which may have influenced the uptake of Fe and Zn, but the effects could also have been due to an improvement in the nutritional status for Fe and Zn or to developmental changes in the gastrointestinal tract.

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A critical assessment of leucocyte zinc as an index of Zn status in chronically ill hospitalized elderly patients. By HELEN F. GOODE[1], N. D. PENN[2], J. KELLEHER[1] and B. E. WALKER[1], *Departments of* [1]*Medicine* and [2]*Medicine of the Elderly, St James's University Hospital, Leeds LS9 7TF*

The elderly are at risk of becoming nutritionally depleted for a number of reasons. There is no specific recommended zinc intake for the elderly, and studies on the trace element content of hospital meals have shown Zn intakes of half that recommended for younger people (Thomas *et al.* 1986). Metabolic balance studies have revealed that Zn intakes are adequate in healthy, free-living elderly people in contrast to housebound and hospitalized elderly patients (Stratford *et al.* 1988).

Plasma Zn is unreliable as the only index of Zn status, and is particularly difficult to interpret in the presence of reduced plasma albumin concentrations. Leucocyte Zn has been suggested as a good index of Zn status, but since leucocytes consist of a variety of sub-populations of differing morphology, half-life, function and Zn content (Goode *et al.* 1989), measurement of the Zn content of pure cell populations should be more reliable.

The Zn status of twenty-five chronically ill, hospitalized elderly patients (mean age 81 years) was assessed. Plasma Zn was measured using flame atomic absorption, and mononuclear cell (MNC) and polymorphonuclear cell (PMNC) Zn concentrations were measured by graphite furnace atomic absorption (Goode *et al.* 1989). Plasma albumin was measured using bromocresol green.

The mean (SD) plasma Zn concentration was 9·38 (2·8) μmol/l, which was significantly lower than that of twenty-three young, healthy controls (12·7 (1·4) μmol/l, $P<0·001$). Plasma albumin was also reduced (31·8 (5·9) g/l) and correlated significantly with plasma Zn (r 0·44, $P<0·05$). Mean PMNC Zn in the elderly subjects was 1·12 (0·36) nmol/mg protein which was not significantly different from that of controls (1·26 (0·28) nmol/mg protein). However, six patients (24%) had PMNC Zn concentrations on or below the lower limit of normal (≤0·70 nmol/mg protein, control mean −2SD). Mean MNC Zn was 1·86 (0·60) nmol/mg protein in the elderly patients, which was similar to that of the control group (2·00 (0·59) nmol/mg protein). None of the patients had an MNC Zn concentration below the normal range. No correlations were found between plasma, MNC or PMNC Zn concentrations.

In separate studies we have shown that in human experimental Zn depletion, PMNC Zn concentrations fall and in surgical patients an excellent correlation exists between PMNC and muscle Zn. We therefore suggest that 24% of the hospitalized elderly patients may be Zn depleted. Marginal Zn deficiency can cause depressed cellular immunity, leading to an increased susceptibility to infections and poor wound healing. We are currently involved in a Zn supplementation trial of patients with low PMNC Zn levels. However, it must be noted that PMNC Zn concentrations may also be controlled by other complex cellular mechanisms unrelated to Zn status.

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The relationship between leucocyte and muscle zinc in surgical patients with and without gastrointestinal cancer. By HELEN F. GOODE[1], J. KELLEHER[1], B. E. WALKER[1], R. I. HALL[2] and P. J. GUILLOU[2], *Departments of* [1]*Medicine* and [2]*Surgery, St James's Hospital, Leeds LS9 7TF*

Patients with gastrointestinal cancer are at risk of nutritional deficiences, including zinc, through decreased intake, increased losses or increased requirements. In addition, the stress associated with surgery causes redistribution of circulating Zn. Plasma Zn is unreliable as the only index of Zn status and is particularly difficult to interpret when plasma albumin levels are low (Walker *et al.* 1973). Leucocyte Zn has been suggested as a reliable marker of Zn status and has been shown to correlate with abdominal muscle Zn (Jones *et al.* 1981). However, leucocyte sub-populations differ in morphology, function, half-life and Zn content (Goode *et al.* 1989). The Zn content of pure cell populations should, therefore, be subject to less variation than total leucocyte Zn.

We measured abdominal muscle, plasma, leucocyte (WBC), mononuclear cell (MNC) and polymorphonuclear cell (PMNC) Zn concentrations in two groups of patients undergoing abdominal surgery. Ten patients had gastrointestinal cancer; the other ten had a variety of benign conditions.

Plasma Zn was reduced in both groups of patients, concurrent with low plasma albumin concentrations (r 0·66, $P<0·05$). Mean (SD) PMNC Zn was 0·86 (0·27) nmol/mg protein in patients with cancer and 0·83 (0·22) nmol/mg protein in those without cancer, which were both significantly lower than in healthy controls (1·26 (2·8) nmol/mg protein, $P<0·001$). Five patients with cancer and four without had PMNC Zn levels on or below the lower limit of normal ($\geq$0·70 nmol/mg protein). In all patients, both MNC and WBC Zn levels were similar to control values. No difference in muscle Zn concentrations was observed between cancer and non-cancer patients whether the results were expressed in terms of wet weight, dry weight or protein content. Correlations were found between muscle Zn expressed per mg protein and PMNC Zn (r 0·89, $P<0·001$), and also between muscle Zn expressed per mg dry weight, and PMNC Zn (r 0·48, $P<0·05$). WBC Zn correlated with muscle Zn expressed per mg protein (r 0·48, $P<0·05$) but just failed to reach significance when expressed in terms of dry weight (r 0·45, $P = 0·056$). MNC Zn did not correlate with muscle Zn regardless of the method of expressing the results.

The results confirm the relationship between muscle and leucocyte Zn and further pinpoint this to a very close correlation with PMNC Zn concentrations. Nine patients (45%) had low PMNC Zn levels which were unaffected by the presence of gastrointestinal cancer. Zn deficiency causes poor wound healing and impaired immunity, and hence pre-operative Zn supplementation should be considered in such patients.

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Neutrophil zinc is related to the severity of hepatic damage in patients with liver disease.

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Patients with liver disease are at risk of zinc deficiency through decreased intake, increased losses, decreased absorption and impaired hepatic uptake (Keeling *et al.* 1981). Leucocyte Zn has been described as a good indicator of Zn status (Keeling *et al.* 1980). Assay of the Zn content of pure white cell populations should be more reliable, however, since leucocyte sub-populations vary not only in function, half-life and morphology, but also in Zn content (Goode *et al.* 1989).

We measured plasma, neutrophil, mononuclear cell and erythrocyte Zn to assess Zn status in thirty patients with liver disease, in thirteen of whom this was of alcoholic origin. Patients were graded according to their hepatic functional reserve (Pugh *et al.* 1973), which takes into account the degree of ascites and encephalopathy, and biochemical indices of hepatic function. Ten patients were grade C (severe), eight were grade B (moderate) and twelve were grade A (mild). Results were compared with those of twenty-three healthy controls.

Plasma Zn (mean (SD)) was reduced in all patients (8·1 (2·5) μmol/l compared with 12·7 (1·4) μmol/l in controls, $P<0·001$) but was accompanied by decreased albumin levels (r 0·86, $P<0·001$). Neutrophil Zn concentrations were directly related to the degree of hepatic damage. In grade C patients, mean neutrophil Zn was 0·86 (0·24) nmol/mg protein, which was significantly lower than grade B patients (1·08 (0·30) nmol/mg protein, $P<0·05$), grade A patients (1·44 (0·43) nmol/mg protein, $P<0·01$) and healthy controls (1·26 (0·28) nmol/mg protein, $P<0·001$). No differences were observed between patients with alcoholic or non-alcoholic liver disease. Erythrocyte and mononuclear cell Zn concentrations were not decreased and were unrelated to hepatic damage.

These results indicate that the decreased mixed leucocyte Zn concentrations previously reported in some patients with liver disease are probably due to reduced neutrophil Zn only, and that assay of Zn in this leucocyte sub-population may give a more reliable assessment of Zn status than mixed leucocyte Zn. The relationship between the severity of hepatic damage and neutrophil Zn suggests that Zn depletion is progressive, and if Zn supplementation is considered, it should be initiated in the early stages of the disease.

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Pugh, R. N. H., Murray-Lion, I. M. & Dawson, J. L. (1973). *British Journal of Surgery* **60**, 646–649.

Contribution of foods eaten outside the home to nutrient intake. By J. M. LOUGHRIDGE, A. D. WALKER, H. SARSBY and R. SHEPHERD, *AFRC Institute of Food Research, Colney Lane, Norwich NR4 7UA*

The National Food Survey (NFS) provides a valuable supply of information on the intake of food and nutrients in the British diet (Derry & Buss, 1984), but is confined to foods brought into the home. The present study examined the nutrient contribution from foods eaten outside the home to the overall diet.

Seventy subjects (thirty-five males and thirty-five females, aged 15–64 years) were selected from a local health centre register, using age and sex as criteria, and asked to keep a record of everything consumed for three consecutive days, using scales and household measures. The males and females in each age group commenced their recorded intake on a different day, so that both weekday and weekend eating habits would be accounted for. Each subject was asked to indicate in their diary whether the food they ate was consumed at 'home' or 'away'. The criteria for this definition was that 'home' foods included all food and drink prepared in the subject's home, even though it may have been eaten out of the home, e.g. packed lunches, sandwiches; 'away' foods included all food and drink bought out of the home, even though it may have been brought into the home to eat, e.g. take-aways, ready-meals, etc.

A very large percentage of both males and females ate some type of food or drink out of the home at some stage over the 3 d; the number of actual meals eaten out per person per week was 2·7 compared with 3·5 from the NFS (Ministry of Agriculture, Fisheries and Food, 1987). The employed ate out on 3·2 occasions per week compared with 1·4 by the unemployed (housewives, schoolchildren and retired subjects were also included in this group).

The contribution by 'away' foods to total intake was 25% for energy, 22% for protein, 25% for fat, 24% for carbohydrate, 26% for sugar and 17% for dietary fibre. Meats, sugars and preserves, alcoholic beverages, fish and chips contributed most highly to nutrient intake away from home. Energy from sugar was significantly higher in away foods, but fibre density and protein as a percentage of energy was higher in foods eaten at home. There was no significant difference in fat and carbohydrate, in terms of percentage energy, between home and away foods. The contribution of energy from alcoholic beverages was removed from the data and the energy precentages from home and away foods reanalysed; there were no major changes, apart from redistribution of the energy from the alcohol between the other nutrients.

Although foods eaten outside the home are not greatly different in terms of nutrient composition from foods eaten in the home, they are potentially significant contributors to total nutrient intake and future research on 'out of home' eating would benefit from inclusion of nutritional analysis.

Derry, B. J. & Buss, D. H. (1984). *British Medical Journal* **288**, 765–767.

Ministry of Agriculture, Fisheries and Food (1987). *Household Food Consumption and Expenditure. Annual Report of the National Food Survey Committee*. London: H.M. Stationery Office.

Dietary intake and thirst in patients with chronic renal failure. By C. A. FARLEIGH and R. SHEPHERD, *AFRC Institute of Food Research, Colney Lane, Norwich NR4 7UA* and J. S. PRYOR, *Norfolk and Norwich Hospital, Brunswick Road, Norwich NR1 3SR*

Patients with chronic renal failure (CRF) generally demonstrate an impaired ability to excrete excess fluid and sodium loads. This results in a gain in body-weight between haemodialysis sessions, and may lead to a rise in blood pressure. As such, most CRF patients are restricted to their daily fluid allowance and consequently experience symptoms of thirst (Wirth & Folstein, 1982).

Twenty-five CRF patients (twelve male, thirteen female) completed a 3 d weighed dietary intake record for the day before (1), the day of (2), and the day following (3) haemodialysis treatment. The mean daily nutrient intake was calculated for each patient; the mean intakes for the male and female patients respectively are shown in the Table.

	Males				Females			
Day . . .	1	2	3	SEM	1	2	3	SEM
Energy (MJ)	7·91	7·42	8·01	0·44	6·24	6·78	5·76	0·34
Protein (g)	67·1	61·4	80·2	5·3	42·7	51·2	43·5	5·1
Fat (g)	82·9	67·3	80·3	7·4	64·8	68·5	60·3	5·5
Carbohydrate (g)	220	240	224	12	185	200	162	9
Sodium (mg)	1978	2181	2809	256	1925	2104	1739	136
Potassium (mg)	2143	2203	2320	202	1505	1713	1228	112
Calcium (mg)	654	623	733	54	510	559	468	42
Phosphorus (mg)	990	906	1084	75	683	772	623	42
Water (kg)	1·37	1·20	1·48	0·10	0·98	1·08	0·97	0·05

Thirst was measured using a validated seventeen-item questionnaire which was administered to each patient on eighteen separate occasions. A standard score was derived for each completed questionnaire.

For analysis a mean value was calculated for each patient for each nutrient from the 3 d, and thirst from six occasions. Analysis of variance of the intake data revealed that there were no significant differences in the dietary intakes of subjects between the 3 d. An expected significant difference was found between the total intakes of male and female patients, males having a greater intake. Patients were identified as being significantly more thirsty on the day following haemodialysis than on the day of haemodialysis, although there were no differences in drinking patterns on these days.

There was no correlation between overall thirst and the mean daily dietary intake of Na or water in this population with means taken over 3 d. However, further investigation into the dynamic changes in thirst with dietary intake is necessary.

The authors acknowledge the support of the East Anglian Regional Health Authority.

Wirth, J. B. & Folstein, M. F. (1982). *Psychosomatics* **23**, 1125–1134.

Is there a North/South divide? Regional variations in the diet of British adults. By MARGARET J. WHICHELOW and SHARON W. ERZINGLIOGLU, *Department of Community Medicine, University of Cambridge Clinical School, Fenner's, Gresham Road, Cambridge CB2 2ES*

The Health and Lifestyle Survey (Cox *et al.* 1987) was carried out on a stratified random sample of 9003 adults in Great Britain. Amongst the information obtained were details of eating and other lifestyle habits. The frequency of consumption of many items of the diet were recorded. The usual choice of type of bread and milk were also noted. Previous analysis had demonstrated that variations in consumption of some foods were related to the sex, age, social class and smoking status of the respondents (Whichelow *et al.* 1988). As the structure of the eleven standard regions differed with respect to these variables, log-linear analysis was used to allow for these factors in comparing the frequent consumption of food items between the regions of Great Britain, with significance reported at the 5% level.

Greater London was taken as the base region against which the other regions were compared. For each of the thirty food items considered, there was at least one region where consumption differed significantly from that in Greater London.

Fresh fruit, fruit juice, salads and green vegetables were consumed significantly less often whilst fattier foods such as chips, crisps, cheese, processed meats and red meats were eaten more often in many regions. Poultry, nuts, 'brown' bread and pasta and rice were consumed more frequently in Greater London than in most other regions, but total bread consumption and the frequent consumption of cakes and puddings or biscuits was greater outside London.

The overall pattern suggested that Greater London was following recommended dietary guidelines (COMA report, Department of Health and Social Security, 1984) more closely than most other regions. The number of food items for which there were significant differences from Greater London were Scotland 21, North 17, North West, Yorkshire/Humberside and Wales 16, West Midlands 15, East Midlands 14, East Anglia and South West 12 and South East 9.

These findings indicate a definite North/South trend in eating habits, even when adjustments for the sex, age, social class and smoking habit differences between the regions are made.

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Constructing a modest-but-adequate food budget for households with two adults and one child aged 1–4 years. By M. NELSON and K. PEPLOE, *Department of Food and Nutritional Sciences, King's College London, London W8 7AH*

This work has been carried out under the aegis of the Family Budget Unit, an ad hoc group of nutritionists, home economists and social scientists whose aim is to construct a 'modest-but-adequate' household budget which is an alternative standard to the current social security scale rates. A food budget standard prescribes the cost of a shopping basket of foods which meet the needs of a household, where 'needs' has both physiological and cultural connotations. 'Modest-but-adequate' implies a diet generous enough to promote well-being, rather than being 'subsistence' in concept.

A modest-but-adequate budget can be constructed using the household as the basic unit. Customary food consumption patterns dictate the core of food purchases, and modifications are introduced to ensure that the purchased diet will provide the sum of the recommended daily amounts for the nutrient intake of all household members and will satisfy Government recommendations for healthy eating. The costing should be based on average not minimum prices, and the budget should be generous enough to allow sufficient flexibility to meet variations in food preferences from one household to the next.

National Food Survey (NFS) data from 1983 provided information on food consumption patterns in households with one man, one woman and one child aged 1–4 years from three income groups ('C', 'D & E2', and median ±15 percentiles), and two expenditure groups, those spending >25% of net household income on food, and those spending >30% (the Orshansky poverty line (Orshansky, 1965)). The median income group food consumption pattern was selected as an appropriate basis for the construction of a modest-but-adequate budget. NFS food purchasing data were adjusted to meet dietary guidelines (National Advisory Committee on Nutrition Education, 1983; Department of Health and Social Security, 1984). Total weekly expenditure was based on Family Expenditure Survey data (October, 1988), and the costing was based on Tesco supermarket food prices (July, 1988). The costs per person per week were £9.64 (home food consumption), plus £1·49 for foods purchased and eaten away from home, plus 73p for sweets and soft drinks, giving a total weekly expenditure for the family of £35.58. Home food expenditure by food category is given in the Table.

Food group	Pence/person per week	Food group	Pence/person per week
Bread	76·7	Eggs	24·8
Cereals	91·0	Potatoes	58·6
Carcass meat	154·2	Vegetables	75·8
Meat products and poultry	86·6	Fruit	76·8
Fish	65·5	Sugar and preserves	18·1
Fats	20·7	Beverages	25·4
Milk	120·0	Other foods	37·2
Cheese	32·9	Total cost	£9·64

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Assessment of the errors in the calculation of metabolic rate from respiratory gas exchange measurements using Weir's (1949) method. By P. I. MANSELL, *Queen's Medical Centre, Nottingham NG7 2UH* (introduced by I. A. MACDONALD)

In 1949 Weir showed that the metabolic rate (M) may be calculated using respiratory gas exchange measurements simply from the difference in percentage oxygen content between inspired and expired air (O_i–O_e). This circumvents the necessity for measuring the respiratory exchange ratio (R) or the rate of protein oxidation. The Weir formula works because the lower respiratory quotient (RQ) for fat compared with carbohydrate oxidation means that, for a constant volume of O_2 consumed O_i–O_e is smaller when fat is oxidized, and this almost exactly compensates for the lower associated heat equivalent of O_2 (H^{eq}). Since the original description of Weir's formula, two difficulties in its usage have arisen.

Firstly, values for H^{eq} and for the respiratory quotient (RQ) for the oxidation of fat and protein have been revised since 1949 (Table).

The error in the calculation of M can be estimated for various combinations of metabolites by calculating the corresponding value for (O_i–O_e) and inserting this figure into the Weir equation. Using revised values of H^{eq} and RQ (Table), M is virtually independent of the contribution to total metabolism made by protein oxidation. The variation in the calculated value of M is, however, 1·2% as R ranges from 0·71 to 1·00, and not 0·4% as originally supposed. The error in the calculation of M can be reduced by rederiving the required equation using the general principles outlined by Weir, although incorporation of a factor dependent on R cannot be avoided. Hence

$$M = 0{\cdot}995 \times \frac{(3{\cdot}799 + 1{\cdot}248R)}{(79{\cdot}07 + 20{\cdot}93R)} \times (O_i\text{–}O_e) \times V$$

where V is the expiratory minute volume.

Secondly, the Weir equation is sometimes applied where significant amounts of metabolites other than dietary carbohydrate, fat and protein are being oxidized (for which its use was not originally envisaged). If additional substrates for oxidation have values of R which are relatively high for their H^{eq} compared with carbohydrate and fat, the Weir equation will provide an underestimate of M, and vice versa. Thus, where oxidation of alcohol contributes 50% to M, the latter may be underestimated by 2·5%, with a similar overestimate for the oxidation of ketone bodies (see Table).

	Weir (1949)			Livesey & Elia (1988)		
	H^{eq}			H^{eq}		
	kJ/l	kcal/l	RQ	kJ/l	kcal/l	RQ
Carbohydrate	21·12	5·047	1·000	21·12	5·047	1·000
Protein	18·67	4·463	0·802	19·48	4·656	0·835
Fat	19·81	4·735	0·718	19·60	4·685	0·710
Alcohol				20·33	4·859	0·667
Acetoacetate				19·78	4·727	1·000

The error in the calculation of M using the Weir equation is greater than originally supposed. Where particular accuracy is required, the error can be minimized using a revised version of the equation incorporating a factor dependent on R. Caution is required in applying Weir's equation where nutrients other than dietary carbohydrate, fat and protein are being oxidized.

Livesey, G. & Elia, M. (1988). *American Journal of Clinical Nutrition* **47**, 608–628.
Weir, J. B. de V. (1949). *Journal of Physiology* **109**, 1–9.

The effect of fasting on plasma corticosterone kinetics in the anaesthetized rat. By C. J. H. WOODWARD[1], G. R. HERVEY[1], R. E. OAKEY[2] and E. M. WHITAKER[1], [1]*Department of Physiology* and [2]*Division of Steroid Endocrinology, Department of Chemical Pathology, University of Leeds, Leeds LS2 9NQ*

An increase in the concentration of plasma corticosteroids is an important component of the metabolic adaptation to fasting. Corticosteroid levels are generally considered to be controlled via the adrenal cortex. During fasting, however, reduced hepatic clearance of hormone may also occur (Herbst *et al.* 1960). In the present study we have attempted to assess the relative contributions of these two mechanisms.

The plasma turnover rate of corticosterone in anaesthetized (halothane) rats was calculated from the rate of disappearance of radioactivity after injection of [1,2,6,7-^{3}H]corticosterone, multiplied by the plasma corticosterone level as determined by radioimmunoassay. Fasting for 2 d significantly reduced fractional turnover rates in both sexes, and increased corticosterone concentrations, but did not alter absolute turnover rates (see Table). The capacity of the liver to metabolize corticosterone was assessed from the activity of hepatic corticosterone 4,5-reductase (*EC* 1.3.1.3, Herbst *et al.* 1960). Fasting reduced enzyme activity by 44 and 60% in males and females respectively. The binding capacity of plasma corticosterone-binding globulin (CBG) was also measured (Martin *et al.* 1977). Fasting caused a 23% decrease in CBG capacity in females but had no effect in males. All the above variables of corticosterone metabolism were greater in females than in males.

	Male				Female			
	Fed		Fasted		Fed		Fasted	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fractional turnover rate (%/min)	5·7	0·3	4·3*	0·4	8·6	0·3	6·8*	0·7
Corticosterone concentration (ng/ml)	344	27	460*	43	769	27	983***	29
Absolute turnover rate (ng/ml/min)	19	2·2	19	2·1	66	3·3	67	6·5
Corticosterone 4,5-reductase (mU/g protein)	517	31	290***	14	1150	85	462***	56
CBG capacity (pmol/ml)	540	21	554	28	1560	41	1207***	48

n, 4–6 rats per group.
*$P<0.05$, ***$P<0.001$ compared with fed group of same sex.

Assuming that the changes measured in enzyme activity reflect alterations in vivo, the results suggest that diminished hormone clearance is a major cause of raised plasma corticosteroid levels during fasting. The results for plasma hormone turnover confirm the importance of decreased clearance rather than increased secretion rate. Finally, reduced levels of plasma CBG in fasted females may influence the metabolic effects of hypercorticosteronaemia.

This study was carried out under a contract with MAFF.

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Martin, C. E., Cake, M. H., Hartmann, P. E. & Cook, I. F. (1977). *Acta Endocrinologica* **84**, 167–176.

Elevated corticosterone-sensitive enzyme activities in obese Zucker rat brain. By S. C. LANGLEY and D. A. YORK, *Department of Human Nutrition, University of Southampton, Southampton SO9 3TU*

The genetic obesity of the Zucker (*fa/fa*) rat is glucocorticoid dependent, with most of the abnormalities associated with the obese syndrome being abolished by adrenalectomy (Holt & York, 1982) and restored by corticosterone replacement (Freedman *et al.* 1986). Glucocorticoids are thought to modulate the expression of the obese phenotype centrally. However, it is unclear whether this is a direct effect of corticosterone, or an effect mediated through changes in corticotropin-releasing factor, or other neuropeptides (York, 1987).

To assess the central activity of corticosterone in obese rats, glial cell glycerol 3-phosphate dehydrogenase (GPDH, cytosolic form) and neuronal tyrosine hydroxylase (TH) were assayed in hippocampus and hypothalamus. These enzymes are known to be induced by glucocorticoids. The hippocampus and hypothalamus are important sites of corticosterone uptake in the brain, and are rich in glucocorticoid receptors. Lean (*Fa/?*) and obese (*fa/fa*) female rats aged 18 d to 8 weeks, fed *ad lib.*, and housed in a room with a 10 h light cycle were studied. GPDH activity was significantly elevated in both the hypothalamus and hippocampus from day 25. Maximal elevations in females were 43% in hippocampus (8 weeks, $P<0{\cdot}05$) and 33% in hypothalamus (6 weeks, $P<0{\cdot}01$). Similar elevations of GPDH (73% hippocampus, $P<0{\cdot}01$ and 47% hypothalamus, $P<0{\cdot}001$) were observed in 32-d-old males.

Adrenalectomy abolished the differences in GPDH activity between lean and obese animals, as shown in Table 1.

The effect of adrenalectomy (ADX) on GPDH activity (nmol/min per mg protein)

		Hippocampus		Hypothalamus	
	n	Mean	SEM	Mean	SEM
Lean, sham	4	159·02†	7·41	135·77†	9·33
Lean, ADX	4	113·69*†	11·02	123·13†	20·68
Obese, sham	4	288·54†	15·31	372·67†	51·62
Obese, ADX	3	121·48***	19·76	108·18***	22·77

Effects of adrenalectomy (one-way ANOVA): *$P<0{\cdot}05$, ***$P<0{\cdot}001$.
Effect of phenotype: †$P<0{\cdot}01$.

In contrast the activity of TH was unaltered in the hypothalamus of obese rats and only increased in the hippocampus (46%, $P<0{\cdot}05$) at 4 weeks of age.

The differences in activity of glucocorticoid-sensitive enzymes could reflect changes in corticosterone uptake, receptor binding or activity. Preliminary studies of specific binding of [^{3}H]corticosterone to cytosolic receptors from whole rat brain indicate similar binding levels in lean and obese rats (349 (SEM 40) and 385 (SEM 86) fmol/mg protein respectively), but data on regional receptor populations are not yet available.

Our results would support the conclusion that the brain of young obese rats may be overstimulated by glucocorticoids but the basis of the effect is not yet clear.

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A comparison of skeletal muscle mass in rabbits as measured by dissection and by creatine dilution. By PETER J. REEDS, *USDA-ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030* and GERALD E. LOBLEY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The use of creatinine excretion as an indirect measure of muscle mass assumes that all urinary creatinine originates from skeletal muscle, and that both the fractional rate of creatinine synthesis and muscle creatine concentration are constant (Picou *et al.* 1977). Seven New Zealand White rabbits were injected intravenously with [1–^{14}C]creatine (1 μCi/kg). Collections of urine (24 h) were made for 3 d and the animals were then killed, eviscerated and skinned. All visible muscle was dissected and representative portions of skin, whole gastrointestinal tract and skeletal muscle were homogenized in 20 vol water. Portions of the homogenates were centrifuged and radioactivity in the supernatants was measured. Creatine in the muscle supernatant was estimated enzymically and isolated by cation exchange chromatography on Dowex-50 (Na^+ form). Creatine was then converted to creatinine by incubation for 3 d at room temperature in 1 M-HCl and precipitated with phosphotungstic acid. The specific radioactivity of the isolated creatinine was calculated. The creatine pool size was estimated as dose remaining minus dose excreted/specific radioactivity of creatine either with (W) or without (WO) a correction for the dose retained in the viscera and skin.

Digesta-free body-wt (g)	Rate of creatine catabolism (%/d)*	Muscle creatine concentration (mg/g muscle)	Muscle mass (g)			Comparison of estimates	
			D	Creatine			
				W	WO	W/D	WO/W
292	2·3	4·7	96	83	92	0·86	1·11
656	2·5	4·2	268	277	288	1·03	1·04
868	2·4	6·2	359	386	436	1·08	1·12
1176	2·1	5·8	552	524	579	0·95	1·10
2782	2·3	6·1	1363	1472	1533	1·08	1·04
2908	2·1	6·0	1568	1609	1652	1·03	1·03
3512	2·0	6·5	1931	1918	1992	0·99	1·04

D, dissection; W, correction for dose retained in viscera and skin; WO, without correction.
*Excretion of label on day 3 as a percentage of dose remaining.

The rate of creatine turnover (2·2 (SE 0·16) %/d) was similar to that observed in rats (Waterlow *et al.* 1982) and infants (Picou *et al.* 1977). Muscle creatine concentration tended to rise with age and the average creatine concentration (5·6 mg/g muscle) was higher than that observed in children (Picou *et al.* 1977) and young rats (Reeds *et al.* 1982). As a result, the rate of creatinine excretion per unit dissectable muscle was approximately double that found previously in infants (Picou *et al.* 1977). Muscle mass by method W was 101 (SE 5)% of dissectable muscle. Method WO overestimated the muscle pool of creatine by 7 (SE 4)%. We conclude that variations in muscle creatine concentrations can significantly affect the accuracy of estimates of muscle mass obtained only from creatinine excretion, and that approximately 93% of the body pool of creatine is located within skeletal muscle.

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A non-invasive method for assessing muscle glycogen stores by whole-body calorimetry.

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A non-invasive method for measuring muscle glycogen stores is required for studies of energy and nutrient balance and for assessing the effectiveness of training and dietary regimens for endurance athletes. Current techniques of muscle biopsy are often unacceptable to experimental volunteers and to elite athletes in particular.

We propose a new technique in which glycogen depletion is monitored during regular repeated periods of exercise in a whole-body calorimeter. It is assumed that glycogen oxidation reduces exponentially during depletion and that the area under an exponential curve fitted to carbohydrate oxidation and extrapolated to infinite time represents the total glycogen stored in the exercised muscles of fasted subjects.

Nine healthy young subjects were studied by this method in 11-m^3 calorimeters. The subjects fasted for 14 h before and during repeated 30 min periods of cycle ergometer exercise at 45% of maximum oxygen uptake, each followed by a 30 min rest period. This protocol continued for seven to nine periods until the subject felt unable to complete the next full exercise period. Exercising energy expenditure ranged from 22·6 to 41·8 kJ/min with a within-subject coefficient of variation of 4·2%. Carbohydrate oxidation was computed from non-protein respiratory quotient values and an exponential was fitted to these results by the method of Feldman (1977). Glycogen oxidation decreased during the exercise from 1058 (SD 315) to 502 (SD 185) mg/min, an average decrease of 53% (range 28–68%). The total glycogen oxidized by each subject is given in the Table. The standard error of the estimate (SEE) for individual estimates averaged only 11%.

Subject no. and sex	Wt (kg)	Available glycogen (g)		Predicted–estimated	SEE (g)
		Predicted	Estimated		
1 Male	70·3	330	447	117	30
2 Male	62·0	290	460	170	53
3 Female	63·7	215	151	−64	21
4 Female	52·6	180	251	71	28
5 Male	68·5	315	496	181	124
6 Male	63·4	280	510	230	71
7 Male	67·6	305	302	−3	12
8 Male	70·0	315	334	19	14
9 Male	81·6	370	345	−25	13
Mean	66·6	289	366	77	41

For most subjects the estimated muscle glycogen closely matched predictions based on published estimates of relative muscle masses and glycogen concentrations in untrained subjects. The relatively poor correspondences of some individual estimates are predictable from their standard errors and do not necessarily invalidate our technique. We conclude that this well-tolerated and non-invasive method is technically feasible and warrants further development and cross validation against muscle biopsy.

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Tserng, K.-Y. & Kalhan, S. C. (1983). *American Journal of Physiology* **245**, E476–E482.

Carbohydrate storage following muscle glycogen depletion as assessed by ^{13}C-labelled glucose. By B. J. SONKO, P. R. MURGATROYD, GAIL R. GOLDBERG, W. A. COWARD and A. M. PRENTICE, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

Carbohydrate (CHO) loading following muscle glycogen depletion is now an established procedure adopted by many endurance athletes. This study used whole-body calorimetry in conjunction with the administration of ^{13}C-labelled CHO in order to study the factors controlling glycogen repletion.

Seven of the subjects who underwent glycogen depletion as outlined in the accompanying abstract (Murgatroyd *et al.* 1990) were studied for a further 17 h while their glycogen stores were repleted. Thirty min after the last exercise each subject received the first of ten high-CHO meals (protein:fat:CHO, 2·6:0·3:97:1, by wt) offered at 30-min intervals. A major component of the repletion diet was Fortical (Nutricia-Zoetermeer, Holland) which contains naturally enriched ^{13}C-glucose. Substrate oxidation was calculated from the calorimeter non-protein respiratory quotients values. Exogenous CHO oxidation was calculated from breath $^{13}CO_2$ enrichment. Stored CHO was calculated as the difference between CHO consumed and that oxidized over the next 16 h. In order to measure the enrichment of the newly deposited glycogen, at the end of the protocol each subject performed three 30 min exercises at 45% maximum oxygen consumption.

			Depletion			Repletion			Post-repletion exercise	
Subject no.	Sex	Wt (kg)	Oxidation 1st exercise (mg/kJ)	Predose oxidation* (g)	(g/kg)	Consumed (g)	Stored (%)	Stored (g/kg)	Oxidation† (mg/kJ)	Exogenous† ^{13}CHO as % of total
1	♂	70·3	34·4	415	5·91	964	66·7	9·16	43·9	67·9
2	♂	62·0	30·2	373	6·02	856	73·9	10·21	38·8	56·4
3	♀	63·7	20·7	226	3·55	528	60·0	4·64	23·2	61·5
4	♀	52·6	37·4	298	5·56	418	69·7	5·52	31·1	40·4
5	♂	68·5	29·3	307	4·48	787	61·7	7·10	42·5	80·6
6	♂	63·4	30·4	399	6·29	968	65·7	10·04	37·4	78·0
7	♂	67·6	29·5	398	5·89	778	69·8	8·02	29·7	61·6
Mean		64·0	30·3	345	5·39	757	66·8	7·81	35·2	63·8
SD		5·9	5·2	70	1·00	211	4·8	2·17	7·5	13·6

*Total CHO oxidation before repletion.
†Average of last two exercises.

CHO consumption averaged 757 g of which 253 g was oxidized and 504 g was stored (Table). The amount stored was not significantly related to body-weight and varied over a wide range when expressed on a mass specific basis (4·64–10·21 g/kg). CHO stored was correlated with the amount oxidized during the depletion phase (r 0·80, $P<0·02$), but was most strongly determined by the amount of CHO consumed during repletion (r 0·97, $P<0·001$). Thus CHO stored represented a very constant proportion of CHO consumed (67% with a coefficient of variation of only 7%) indicating a linear dose-response relationship which was not saturatable within the limits studied. CHO oxidation during the post-repletion exercises was 16% higher than at the beginning of the depletion period, indicating satisfactory repletion. ^{13}C recovery indicated that the proportional contribution of exogenous CHO was high (64%) during these exercises.

We conclude that short-term glycogen storage after muscle glycogen depletion is linearly related to the amount of CHO consumed. Maximum tolerable consumption is therefore advocated in order to achieve maximum muscle glycogen loading.

Murgatroyd, P. R., Wittekind, A., Ceesay, S., Wilson, C., Goldberg, G. R. & Prentice, A. M. (1990). *Proceedings of the Nutrition Society* **49**, 82A.

The effect of chronic food restriction on contractile characteristics of rat skeletal muscle.

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Chronic food restriction has been shown to result in slowing of relaxation rate and shifts in the force–frequency relationship of the rat gastrocnemius muscle, a muscle composed of mixed fibre types (Russell *et al.* 1984). The aim of the present study was to examine the influence of chronic food restriction on the contractile characteristics of rat skeletal muscles chosen to reflect the differing muscle fibre types.

Muscle function was assessed *in situ* under Sagatal anaesthesia in male Wistar rats (initial weight 184 (SE 3) g) following an overnight fast (CONTROL), 21 d growth (FED) and 21 d food restriction at a level of 25% the intake of paired FED animals (FR). Contractile characteristics were assessed in the soleus (type I), extensor digitorum longus (EDL: type II_B) and tibialis anterior (TA: type $II_{A/B}$) during single twitches and stimuli of increasing frequencies up to 200 Hz.

Muscle weight was raised approximately 80% in the FED animals, in parallel to the changes in body-weight ($P<0{\cdot}01$). Despite body-weight loss in the FR animals (11%, $P<0{\cdot}01$), muscle weight was not discernibly altered from CONTROL values. The changes in muscle weight, time to peak tension (TPT), half relaxation time (½RT), peak twitch tension (PT) and maximal tetanic force (F_{max}) are shown in the Table.

The effect of food restriction on rat skeletal muscle contractile characteristics

			Muscle wt (mg)		TPT (ms)		½RT (ms)		PT (N/g muscle)		F_{max} (Ng/ muscle)	
Muscle	Group	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Soleus	CONTROL	10	71·8	4·3	31·6	1·9	53·4	4·3	0·17	0·03	0·67	0·12
	FED	8	132·0**	7·3	36·0	0·9	58·3	6·0	0·15	0·02	0·78	0·05
	FR	6	64·3††	5·9	38·5	2·7	61·5	7·3	0·27	0·07	0·78	0·04
EDL	CONTROL	10	78·2	4·6	14·4	0·5	13·3	0·6	0·26	0·04	0·85	0·06
	FED	8	129·0**	9·1	16·0	0·7	13·5	1·1	0·36	0·05	1·06	0·11
	FR	6	82·2††	3·1	13·6†	0·5	13·8	0·5	0·28	0·06	1·12*	0·12
TA	CONTROL	10	324·6	9·8	13·4	0·8	13·6	0·7	0·19	0·04	0·91	0·09
	FED	8	586·8**	28·1	16·1	0·9	12·8	0·8	0·33	0·06	0·73	0·11
	FR	6	304·5††	16·5	15·5†	0·5	13·9	1·4	0·23	0·06	1·09	0·11

*$P<0{\cdot}05$ *v.* CONTROL; **$P<0{\cdot}01$ *v.* CONTROL by the unpaired Student's *t* test.
†$P<0{\cdot}05$ *v.* FED; ††$P<0{\cdot}01$ *v.* FED by the unpaired Student's *t* test.

In general, no impairment of twitch characteristics were apparent in either FED or FR animals compared with CONTROLS, except a faster time to peak tension (FR *v.* FED 3·7%, $P<0{\cdot}05$) and greater maximal tetanic force (FR *v.* CONTROL 49%, $P<0{\cdot}05$). Contrary to the results of Russell *et al.* (1984), no shift in the force–frequency relationship was detected for the soleus muscle of the FR group when compared with CONTROL or FED animals. However, EDL and TA muscles of the FR group exhibited greater maximal forces at 50 Hz than the CONTROL animals.

Thus, the twitch contractile characteristics of muscle selected in this study were unaltered by growth or food restriction but food restriction appears to show selective alteration of the force–frequency relationship of muscles containing predominantly type II fibres.

Russell, D. McR., Atwood, H. L., Whitakker, J. S., Itakura, T., Walker, P. M., Mickle, D. A. G. & Jeejeebhoy, K. N. (1984). *Clinical Science* **67**, 185–194.

Analysis of isotope ratio disproportionation for evaluation of dietary requirements and of nitrogen metabolism in vivo. By H. SICK, N. ROOS and N. TRUGO, *Institut für Physiologie und Biochemie der Ernährung, Bundesanstalt für Milchforschung, D-2300 Kiel 14, Federal Republic of Germany*

Products of metabolic pathways competing for the same precursor in the same metabolic compartment should exhibit isotope ratio disproportionation (IRD) of the isotopes of the precursor. This can even be observed at the level of natural abundance. We report here on the IRD of the ^{15}N:^{14}N ratio (N-IRD) of two products of liver metabolism: serum albumin and urea. These products are easily accessible for measurement. The N-IRD of these follows in a first approximation simple rules and should allow the calculation of ratios of hepatic protein and urea synthesis.

To validate this approach ten minipigs were equilibrated for 15 months with a semi-synthetic diet (150 g casein/kg) with a ^{15}N:^{14}N ratio according to $\delta\ ^{15}N_{Air} = 7{\cdot}1‰$. The ^{15}N content of serum albumin in these animals increased to $\delta\ ^{15}N_{Air} = 11{\cdot}8‰$ while it decreased in urea to $\delta\ ^{15}N_{Air} = 5{\cdot}0‰$. Thus the N-IRD amounted to $11{\cdot}8‰ - 5{\cdot}0‰ = 6{\cdot}8‰$. (This N-IRD is 100 times larger than the external precision of the isotope ratio measurement.) We equated the nitrogen isotope composition of the precursor in this long-term experiment with that of haemoglobin, because the protein is synthesized in the bone marrow without interference of major amino acid breakdown. This is suggested by the ^{15}N content which was the lowest of a protein so far found in the equilibrated pigs and which corresponded to $\delta\ ^{15}N_{Air} = 10{\cdot}3‰$.

From these data it is possible to calculate the molar ratio of amino acids used for hepatic protein and urea synthesis. In the present experiment, the ratio was about 3·5.

N-IRD was decreased by a higher protein intake (300 g casein/kg diet, N-IRD = 5·6‰) and increased if no protein was given for a 12 d period (N-IRD = 7·3‰). This observation in pigs is further highlighted by nutrition experiments with growing rats fed on iso-energetic diets with different protein contents (0, 50, 100, 200 and 400 g casein/kg). After a 14 d equilibration phase, isotope ratio measurements indicated that N-IRD reached a maximum value (ca. 8‰) at the protein intake level with the highest apparent net protein utilization (non-standardized, 200 g, body-weight), which was 5 g/kg body-weight per d. $\delta^{15}N$ of urea exhibited a minimum under these conditions. The ratio of hepatic protein:urea synthesis calculated from N-IRD analysis formed a maximum at this intake level.

Applicability of IRD analysis for determination of metabolic rates in vivo of specific organs and tissues on the one hand, and for protein evaluation or evaluation of dietary requirements on the other hand, has to be checked by further investigations.

Assessment of body composition by segmental impedance measurements and specific resistivities. By N. J. FULLER and M. ELIA, *Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

In clinical practice it may occasionally be more practical to obtain measurements of impedance from body segments rather than from the whole body. Also, whole-body impedance may be disproportionately influenced by a constituent segment of differing composition. Therefore, the present study was undertaken to evaluate the contribution of various segments to whole-body impedance, to estimate specific resistivities of segments, and to relate these measurements to body composition as assessed by densitometry and anthropometry.

Segmental impedances were measured, using a Holtain analyser, on forty-eight normal subjects (twenty-six male, twenty-two female; age 34·0 (SD 10·2) years; body mass index (BMI) 23·0 (SD 2·5) kg/m^2) and seven obese female subjects (age, 38·6 (SD 10·1) years; BMI, 47·8 (SD 7·6) kg/m^2). Segment lengths and mid-segment circumferences were measured in order to obtain approximate estimates of specific resistivities (impedance × cross sectional area/length, ohm/cm). All the subjects had body composition assessed by skinfold measurements at four sites, and twenty-four subjects by densitometry.

	Impedance (male + female)			Specific resistivity (ohm/cm)					
	ohm			Male		Female		Obese female	
Segment	Mean	SD	%	Mean	SD	Mean	SD	Mean	SD
Fore arm	155	19	25	248	35	294	77	460	100
Upper arm	127	16	21	212	23	263	80	359	66
Trunk	60	15	10						
Upper leg	79	15	13	344	97	388	81	751	304
Lower leg	196	24	31	452	69	480	75	657	146

The distribution of impedance measurements and segmental specific resistivities are given in the Table. The arm (about 4% of body-weight) contributed disproportionately to whole-body impedance (about 46%). It was found that fat-free mass (densitometry) related strongly to height^2/whole body impedance (r 0·98, standard error of the estimate (SEE) 1·95 kg), and to length^2/impedance for individual segments such as the arm (r 0·96, SEE 2·75 kg), leg (r 0·91, SEE 3·80 kg) and trunk plus leg (r 0·90, SEE 3·98 kg). Specific resistivities were considerably greater in the obese subjects than in normal female subjects ($P<0·01$, for all segments), and greater in normal female subjects than in normal male subjects, reaching significance in the upper arm ($P<0·05$). These differences may reflect the relative amounts of fat present in the segments of these groups of subjects. This trend was further demonstrated by the relation of specific resistivity to percentage body fat assessed by skinfold thickness (e.g. for the upper arm; r 0·79, SEE 3·73% fat).

The study indicates that (a) segments make unequal contributions to whole-body impedance; (b) segmental impedance measurements may be used to obtain reasonable estimates of body composition, especially if whole body measurements are impractical; and (c) specific resistivities may provide information about the composition of individual body segments.

Albumin and prealbumin as nutritional indicators. By C. J. SCHORAH[1], P. BLADON[1] and J. K. WALES[2], *Departments of* [1]*Chemical Pathology* and [2]*Medicine, University of Leeds LS2 9JT*

The usefulness of plasma albumin and prealbumin as nutritional indices is uncertain (Whicher & Spence, 1987). We have investigated their relationship to food intake and weight loss in one male and eleven female patients (mean age 45 years) admitted for treatment of obesity with 4 weeks of starvation. Fluid intake was unrestricted and potassium and vitamin supplements (Octovit) were given. The subjects were weighed and had a venous blood sample taken at the beginning, at weekly intervals during and at the end of the period of starvation. Eight patients were also assessed during 4 d of low-energy refeeding (mean daily intakes 2300 kJ (550 kcal), 40 g protein). Prealbumin was measured by immunodiffusion and albumin with bromocresol green.

The results show significant weight loss (11%) during the period of starvation. Albumin did not fall significantly until the third week, but prealbumin had decreased within 7 d. The plasma protein changes were not caused by an acute-phase response (Fleck *et al.* 1985), as assessed by C-reactive protein measurements. Fluid balance showed an average loss of 2 litres during the first week which possibly accounted for some of the weight change, but could have masked some of the fall in prealbumin by haemoconcentration. There were no significant correlations between the amount of weight lost and the change in either albumin or prealbumin.

Although the response in obese patients may be atypical, lack of albumin change and the rapid decrease in prealbumin have been reported in a short starvation study in normal-weight volunteers (Elia *et al.* 1984).

These findings suggest that albumin is an insensitive indicator of either weight loss or decreased food intake. Prealbumin concentrations do not reflect weight change well, but respond rapidly to marked changes in intake. Before we can say if this is of use in assessing response to dietary advice, we must investigate whether prealbumin responds to alterations in protein or energy intake and examine its sensitivity to dietary change.

		Period of starvation (weeks)					Period of refeeding (d)	
		0	1	2	3	4	0	4
Wt (kg)	Mean	103·1	98·1**	95·6**	93·5**	91·8**	90·1	91·3††
	SD	19·9	19·5	18·9	18·5	18·0	14·7	14·6
Albumin (g/l)	Mean	44·8	44·9	43·6	41·3*	40·4*	42·3	41·5
	SD	3·3	3·7	3·7	4·7	3·9	4·0	4·0
Prealbumin (mg/l)	Mean	275	184**	161**	161**	149**	152	181†
	SD	43	28	31	42	28	23	40

Significantly different from starvation week 0 (paired *t* test): **P*<0·05, ***P*<0·001.
Significantly different from refeeding day 0 (paired *t* test): †*P*<0·02, ††*P*<0·001.

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