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ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at the University of Edinburgh on Thursday and Friday, 7/8 April 1994, when the following papers were presented.

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Seasonal variation in the thermoregulation of the Pygmy shrew, *Sorex minutus*: can true resting metabolic rate be measured in post-absorptive shrews? By R.M. McDEVITT[1] and J.F. ANDREWS[2], [1]*Department of Zoology, University of Aberdeen, Aberdeen AB9 2TN and* [2]*Department of Physiology, Trinity College Dublin, Dublin 2, Republic of Ireland*

Oxygen consumption (ml/min) of *Sorex minutus*, was measured in winter-, summer- and spring-acclimatized animals at a range of ambient temperatures (T_a), from 6-36° with food provided *ad libitum*. The thermoneutral zone (TNZ) was between 22-30° depending on season. O_2 consumption varied significantly with season ($P \leq 0.001$) and T_a ($P \leq 0.001$). The maximum O_2 consumption, recorded at the lowest T_a of the study (6°) did not differ significantly with season. However, resting metabolic rate (RMR), recorded with the TNZ was significantly lower in winter ($P \leq 0.05$) compared with the other seasons. RMR was reduced by between 51-70% compared with the maximum O_2 consumption, depending on season (see Table). An important factor in the acclimatization of winter shrews may be conserving energy by maintaining a lower RMR.

	TNZ	RMR (ml/min)		VO_2 at 6°		Mass (g)		
Season	(°)	Mean	SD	Mean	SD	Mean	SD	(*n*)
Winter	25-30	0.619*	0.131	1.206	0.176	3.4	0.4	(18)
Summer	22-30	0.664	0.107	1.165	0.112	3.5	0.6	(12)
Spring, male	25-28	0.723	0.090	1.212	0.113	5.3	0.7	(10)
Spring, female	25-28	0.750	0.120	1.060	0.083	4.9	0.8	(8)

Mean value was significantly different from other seasonal groups: * $P \leq 0.05$.

Torpor in response to low ambient temperature and food deprivation has never been recorded in any soricine shrew species (Genoud, 1988). However, two species of soricine shrew, *Notiosorex crawfordi* (Lindstedt, 1980) and *S. sinuosus* (Newmann & Rudd, 1978) have shown some degree of hypothermia at high T_a when food was available. This is believed to be a water conservation strategy (Lindstedt, 1980). In the present study, O_2 consumption in summer shrews decreased at T_a above the TNZ to a minimum at 36° of 0.360 (SD 0.076) ml/min, a reduction of 55%, suggesting that some form of metabolic downregulation at high T_a was occurring, though perhaps not hypothermia. There was significant seasonal variation in the heat tolerance of *S. minutus*. Both winter (*n*#6) and spring shrews (*n*#12) had high mortality rates, between 33-50% at 30° and 28° respectively, but summer acclimatized shrews (*n*#12) survived at temperatures up to 36° without any mortality.

We investigated the hypothesis that BMR cannot be measured in shrews because inactivity and post-absorption in shrews are mutually exclusive states due to their short starvation tolerance (Hanski, 1984). Our results showed that there was no significant decrease in O_2 consumption in post-absorptive shrews compared with fed shrews, from any season, at any ambient temperature. We believe that this can be explained by *S. minutus* increasing foraging activity in response to food deprivation, with a resultant increase in O_2 consumption. These data have implications for all studies on basal metabolism carried out in post-absorptive small endotherms.

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Long photophase is not a sufficient stimulus to reduce thermogenic capacity in winter-acclimatized short-tailed field voles (*Microtus agrestis*) during long-term cold acclimation. By J.R. SPEAKMAN and R.M. MCDEVITT, *Department of Zoology, University of Aberdeen, Aberdeen AB9 2TN*

The major cues for acclimatization of thermogenic capacity in free-living endotherms have been postulated to be either ambient temperature (T_a) or photoperiod (Heldmaier *et al.* 1989) or a combination of both (Heldmaier *et al.* 1981). Thermogenic capacity increases in the winter during the process of acclimatization (Klaus *et al.* 1988). We wanted to see if long photophase would reverse the stimulatory effect of cold-exposure in animals that were already cold-acclimatized. To do this, we compared seasonal variation in the thermogenic capacity of interscapular brown adipose tissue (BAT) in short-tailed field voles (*Microtus agrestis*) and then examined how this changed in winter-acclimatized voles kept in a long photoperiod and sustained cold.

The thermogenic capacity of BAT from winter-(*n*#8) and summer-(*n*#6) acclimatized voles and cold-acclimated voles was investigated by examining changes in mass of interscapular BAT, the ratio of white adipose tissue (WAT) to BAT, the concentration of the uncoupling protein (UCP) in whole depots (μg) and in mitochondrial mass (μg/mg) and the activity of cytochrome c oxidase (EC 1.9.3.1) in the BAT depots (mmol/min). The concentration of UCP per BAT depot in winter-acclimatized voles (124.3 (SD 75.7) μg) was significantly higher ($P \leq 0.05$) than in summer-acclimatized voles (28.5 (SD 8.8) μg). Uncoupling protein concentration per mitochondrial mass was also significantly higher ($P \leq 0.05$) in winter voles (11.4 (SD 3.0) μg/mg) compared with summer voles (6.4 (SD 1.1) μg/mg). However, there was no significant difference in the level of cytochrome c oxidase activity between the winter (8.2 (SD 6.2) mmol/min) and summer (7.2 (SD 5.7) mmol/min) voles.

In addition to the control voles, which were not exposed to the cold or long photoperiod, 4 groups of winter-acclimatized voles (*n*#6 in each group) were exposed to 5° for 10, 20, 50 and 100 d in a 14 h light:10 h dark (14L:10D) photoperiod. The resting metabolic rate measured at thermoneutrality (BMR) was measured in each of the cold-exposed voles at the appropriate time point of exposure. Body mass, BAT mass, WAT mass and BMR were significantly positively related to the length of time cold exposed up to 100 d ($P \leq 0.05$). There was a significant inverse relationship between the ratio of WAT to BAT mass and the duration of cold exposure ($P \leq 0.05$). After 100 d in the cold, UCP concentration had increased to 172 (SD 50.7) μg and UCP per mitochondrial mass was 9.25 (SD 0.65) μg/mg. However, there was no significant relationship between UCP concentration, either per depot or in mitochondrial mass of the BAT, with the length of time cold exposed. The level of cytochrome c oxidase activity increased significantly ($P \leq 0.05$) from control levels to a maximum after 10 d in the cold (25.4 (SD 2.5) mmol/min) but decreased from 10 d onwards to 14.2 (SD 2.3) mmol/min which was not significantly different from the control level.

Our data suggest that in winter acclimatized *M. agrestis*, a 14L:10D photoperiod is not a sufficient stimulus to reduce thermogenic capacity during cold acclimation. Indeed some changes in the indirect parameters reflecting thermogenesis, notably the increase in BMR and the decrease in the ratio of BAT to WAT mass, indicated that despite the long photophase the thermogenic capacity was slightly further enhanced during the cold acclimation.

We would like to thank P Trayhurn and M. Thomas for assessing BAT thermogenic capacity.

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Reproductive condition and food intake of Soay sheep born on a 6-month photoperiod when exposed to continuous 12 h light:12 h dark illumination. By R. N. B. KAY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

In sheep, seasonal cycles of reproduction, growth, appetite and moult depend on an endogenous circannual cycle which is attuned to change in day length, as signalled by melatonin secretion. Studies on the photoperiodic control of the breeding cycle of the ram are summarized by Lincoln (1978). What is not yet clear is whether the endogenous circannual cycle is genetically controlled, or is established early in life by the annual cycle experienced by the foetus (exposed to maternal melatonin and other placenta-penetrating hormones) and newborn animal.

To examine this question, three ram and three ewe lambs of the conspicuously seasonal Soay breed were studied. They had been born to ewes subjected to a 6-month sinusoidal photoperiodic cycle throughout pregnancy and thereafter (Argo, 1986); minimum daylength (6 h) occurred on 22 December and 22 June, maximum (18 h) on 22 March and 22 September. The lambs were individually penned in a blacked-out room and given a complete pelleted diet based on barley and barley straw. Measurements started in October 1984 when the lambs were 9 months old and continued until December 1986. Voluntary food intake, and in the rams testis length, inguinal sexual flush and scrotal hair moult were recorded. The 6-month cycle of day length was changed at the second 'autumn' equinox (week 32) to a 12 h light:12 h dark pattern (12 L:12 D) which continued to the end of the experiment (week 117).

Rutting behaviour occurred in the rams twice, starting at about weeks 18 and 44, some 20 weeks after each onset of declining day length. It was accompanied by a 40% increase in testis length, marked inguinal flushing and a 50% decline in food intake, and about 10 weeks later moulting occurred. About 26 weeks after the start of the 12 L:12 D illumination the rams began to show rather irregular fluctuations intermediate between the rutting and sexually quiescent conditions. There was no sign of a 6-month cycle. The pattern of food intake by the ewes was similar to that of the rams but less pronounced.

There was, therefore, no indication that the lambs had acquired an endogenous 6-month cycle during their foetal and neonatal life. However, the rams showed a sudden decline in food intake culminating in a fully developed rut by week 109, 65 weeks after the preceding rut. Whether this arose because of an inherited circannual rhythm, or because they had sensed the rams in an adjoining room which were in rut at this time, is uncertain.

I wish to thank Dr C. McG. Argo, Mr E. D.Goodall and Mr J. McIntosh for their assistance.

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Seasonality of mortality: variation by disease category, age, latitude and national economic status. By A.S. DOUGLAS, *Department of Medicine and Therapeutics, University Medical School, Aberdeen AB9 2ZD*

In Westernized Societies in both the Northern and Southern hemispheres, there are more deaths in winter than in summer (Quetelet, 1842; Sakamoto-Momiyama, 1977). A study was made of the size of the seasonal variation and the timing of the peak (acrophase). The variables investigated were the disease categories, age, latitude and national economic status. The data are from European National Statistics Offices and the World Health Organization (WHO). The statistical method was cosinor analysis, which requires the data to fit a single sine curve. Multiple regression analysis was completed between monthly data. Analysis gives the multiple correlation coefficient, its statistical significance (p) and the position in the year where the fitted sinusoidal regression line has its highest value. Amplitude is the extent of the seasonal variation and is given as a percentage above the mean value. United Kingdom data show that amplitude for deaths due to all disease categories is 13.6% ($p< 0.001$), for cardiovascular disease is 15.4% ($p< 0.001$), for respiratory disease is 31.2% ($p< 0.001$), for tumours is 2.3% ($p< 0.001$) and for the remaining diseases is 9.8% ($p< 0.001$). The acrophase is in January to February except for tumours when the peak is in November to December.

There is a winter/summer rhythm of post perinatal infant deaths with an amplitude of 26.1% ($p< 0.001$). This gradually wanes to be replaced in the teens by a summer peak of 18.6% ($p< 0.05$) which is present only in males. From 40 years of age onwards a winter peak is re-established, with an amplitude of 6.6% ($p< 0.01$) which increases to 23.7% ($p< 0.001$) in those over 90 years of age. WHO and European data covering seventy-four countries demonstrate that amplitude is least at the equator and near the Pole, and greatest at mid latitudes such as in the Mediterranean countries. The amplitude for mortality caused by all disease categories and by respiratory disease in Italy is 11.9% and 34.3%, in Switzerland it is 7.8% and 31.5%, in Finland it is 6.2% and 23.8% and in Iceland it is 0.6% and 17.4% respectively. All p values are < 0.01 to 0.001 except for the amplitude for mortality caused by all diseases in Iceland, which does not reach significance.

National economic status also influences the seasonality of mortality. Egypt has the peak of mortality in the summer with an amplitude of 10.9% ($p< 0.05$). At the same latitude wealthy Libya has a winter peak with an amplitude of 18.9% ($p< 0.05$).

In summary, respiratory death has a much larger variation than mortality caused by all diseases or cardiovascular disease. Surprisingly, death due to tumours has little seasonality. With increasing age the amplitude also increases. In youth the peak is in summer. The greatest amplitude is in mid latitude decreasing towards the Pole and the equator. National poverty causes a summer peak in lieu of winter.

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Lipoprotein lipase and insulin-sensitive glucose transporter (GLUT4) gene expression in tissues of lean and obese *(ob/ob)* mice exposed acutely to cold. By A. NESTOR and P. TRAYHURN, *Division of Biochemical Sciences, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Lipoprotein lipase (LPL; *EC* 3.1.1.34) is a key enzyme in the metabolism of lipids. It hydrolyzes triacylglycerols in chylomicrons and very-low-density lipoproteins, thereby supplying tissues with free fatty acids. A number of hormones play a role in the regulation of LPL, in particular insulin, and in the case of brown adipose tissue, noradrenaline. In this study we have investigated the effects of acute cold exposure on the level of the mRNA for LPL in tissues which utilize or store fatty acids. Cold-exposure was used to stimulate substrate flux, the principal aim being to examine the tissue-specific regulation of the expression of the LPL gene in response to the acute stimulation of energy expenditure in lean and obese (*ob/ob*) mice. LPL mRNA levels were examined in brown adipose tissue (BAT), white adipose tissue (WAT), and the heart. Concomitant changes in the expression of the insulin-sensitive glucose transporter, GLUT4, and the BAT-specific uncoupling protein (UCP) were also examined.

Male, 8-week-old, mice of the 'Aston' strain were housed at 22°, and on the morning of the experiment half were acutely exposed to cold (4° for 2 h). Tissues were removed, frozen in liquid N_2, and stored at -80°. Total RNA was extracted from the tissues, electrophoretically fractionated on a 1.4% agarose gel, and blotted onto a charged nylon membrane (Rayner *et al.* 1994). A new method was used to detect mRNA on Northern blots, employing antisense digoxigenin-labelled oligonucleotides specific for each mRNA, in association with a chemiluminescent detection system (Boehringer). The probes, 30 or 32 bases in length, were designed using the 'Oligo' programme, with high T_m values and a high ratio of G+C residues. The oligonucleotide designed to detect LPL mRNA in mice was a 30-mer (5'-GCCAGCAGCATGGGCTCCAAGGCTGTACCC-3'). The sequences of the GLUT4 and UCP probes were as described previously (Rayner *et al.* 1994; Trayhurn & Duncan, 1994). Hybridization conditions and detection of the mRNA by chemiluminescence were also as previously (Trayhurn & Duncan, 1994). Membranes were exposed to film, and the signal quantified by densitometry.

Results are expressed relative to controls (controls = 1), for six mice per group. In BAT of lean mice there was a cold-induced increase in mRNA levels with both LPL (2.1 times; $P<0.01$) and UCP (2.7 times; $P<0.001$), in agreement with earlier studies (Reichling *et al.* 1988; Mitchell *et al.* 1992). Cold exposure also resulted in an increase in LPL mRNA in the heart (2.1 times; $P<0.05$), while LPL mRNA levels in WAT decreased significantly in the cold (0.68 times; $P<0.05$). In contrast to LPL, there was no significant change ($P>0.05$) in the level of GLUT4 mRNA in either BAT, heart, or WAT of cold-exposed animals. In obese *(ob/ob)* mice, while UCP mRNA levels increased in the cold (2.0 times; $P<0.01$), there was no significant change in LPL mRNA (0.74; $P>0.05$), in contrast to lean animals. Again in contrast to the lean, the obese showed a significant decrease, rather than an increase, in LPL mRNA level in the heart (0.74 times; $P<0.05$). GLUT4 mRNA levels were not significantly changed in tissues of cold-exposed obese animals; nor was there any significant change in LPL mRNA in WAT.

These results suggest that the expression of the LPL gene is differentially regulated between tissues of lean mice, and between lean and obese, in response to the increase in fatty acid utilization induced by acute exposure to cold. In the lean, LPL mRNA levels increased in tissues in which fatty acid utilization is stimulated (BAT, heart) but not in the tissue (WAT) from which net efflux occurs. Increases in LPL mRNA are not evident in obese mice, despite the cold-induced expression of the UCP gene.

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Cellular antioxidant defence systems in smokers compared with non-smokers: effect of vitamin E supplementation. By K.M.Brown, P.C.Morrice, G.G.Duthie and J.R.Arthur, *Division of Biochemical Sciences Rowett Research Institute, Aberdeen.*

During normal metabolism, free radicals generated by a partial reduction of oxygen are potentially injurious to cells, in particular the membrane lipids. However, cellular defence systems exist to modulate free-radical activity thus decreasing the potential for tissue damage. Antioxidant enzymes have a crucial role in cellular defences and their activity may be induced by regulatory mechanisms which respond to O_2 metabolite concentration. Smoking may induce an oxidative stress with which antioxidant defence systems cannot cope. We have therefore examined antioxidant defence systems in erythrocytes of smokers and non-smokers and assessed whether smokers may benefit from high intakes of vitamin E, a major lipophilic free-radical scavenging antioxidant.

One hundred males with no current disease, fifty of whom had never smoked and fifty who had smoked >15 cigarettes/d for at least 10 years were allocated to four treatment groups in a 2 x 2 factorial design (smokers v. non-smokers and placebo v. vitamin E supplementation). For 10 weeks each subject took one capsule/d of either 280 mg DL-α-tocopherol acetate or a visually identical placebo (hydrogenated coconut oil with negligible vitamin E content). Ethical permission was granted by The Joint Ethical Committee of Grampian Region and University of Aberdeen.

Erythrocyte antioxidant enzyme activities were elevated in smokers (S) compared with non-smokers (NS). Table below

	Superoxide dismutase (mg/gHb)		Catalase (k/gHb)		Glutathione peroxidase (U/gHb)		Glutathione reductase (U/gHb)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NS	938	91	1276	337	20.5	2.3	2.95	0.8
S	1095**	71	1487***	157	28.0**	2.4	3.85***	0.6

Mean value was significantly different from that for NS. ** $P<0.01$, *** $P<0.001$.

Erythrocytes of smokers also showed a greater susceptibility to hydrogen peroxide-induced lipid peroxidation than those of non-smokers ($P<0.001$). Prior to vitamin E supplementation erythrocyte α-tocopherol concentrations in smokers and non-smokers were 7.75 μg/g haemoglobin (Hb) and 10.3 μg/g Hb. After 10 weeks of vitamin supplementation these concentrations increased to 15.2 μg/g Hb in smokers and 14.5 μg/g Hb in non-smokers ($P<0.001$). Moreover, vitamin E supplements increased erythrocyte catalase (EC 1.11.1.6) concentrations in both smokers and non-smokers ($P<0.001$), and erythrocyte glutathione peroxidase (EC 1.11.1.9) and glutathione reductase (EC 1.6.4.2) activities in non-smokers ($P<0.001$). There was a concomitant fall in superoxide dismutase (EC 1.15.1.1) activity ($P<0.001$) and total glutathione concentration ($P<0.01$). Furthermore, in both smokers and non-smokers there was a significant decrease by 58% in the susceptibility of erythrocytes to peroxidation ($P<0.001$). The results suggest that smoking induces an up-regulation of antioxidant enzyme activity which cannot cope with the increased free-radical load from tobacco smoke. Although vitamin E improves resistance to erythrocyte lipid peroxidation the associated alteration in antioxidant enzyme regulation may have clinical implications which are as yet not understood. Consequently, supplementation of smokers and non smokers with pharmacological doses of vitamin E must be approached with great caution.

Amylase-rich flour improves the digestibility of cereal-based weaning foods. By L.T. WEAVER[1], B. DIBBA[2], B. SONKO[2], T.D. BOHANE[2], and S. HOARE[2], [1]*Department of Human Nutrition, University of Glasgow, and* [2]*MRC Dunn Nutrition Unit, Cambridge CB4 1XJ, and Keneba, The Gambia*

Traditional Gambian weaning foods are gruels based on cereal flour (maize, rice, millet, sorghum and wheat), and it is the starch within them that provides most energy (Lancet, 1991). However they are frequently watery and of low energy density. The addition of energy in the form of fat and carbohydrate leads to thick, viscous gruels, which are difficult to ingest. Partial digestion with amylase rich flour reduces their viscosity while retaining their energy density (Weaver, 1994).

Our aim was to measure the digestion and absorption of a maize-based weaning food before and after amylase digestion in malnourished Gambian children using a ^{13}C breath test. Eleven children (six boys, five girls) aged 7-17 months (mean 11.5 months) received isovolumetric and isoenergetic quantities of a naturally ^{13}C-enriched maize-based weaning food, followed by the collection of breath samples at 30 min intervals for 6 hr. $^{13}CO_2$ enrichment was measured by isotope ratio mass-spectrometry. Children were enrolled at the weekly welfare clinic and the study was performed with the informed consent of their parents. All children studied had already begun to consume some weaning food.

Each child acted as his or her control, undergoing two tests (test 1, without amylase rich flour; test 2, with amylase rich flour), in random order. The test meal consisted of maize flour, beet sugar, groundnut oil and water (energy density approximately 4184 KJ (100 kcal)/100 g. Germinated maize (amylase-rich) flour (10 g) was added to 1 kg of cooked weaning food to digest it partially. Quantity of food ingested was measured by test weighing the feeding cup and bib before and after each test feed.

Saliva samples were collected from each child, and milk samples from each mother, for the measurement of amylase concentration.

The children ranged in weight from 6.40 to 11.45 kg (mean 8.54 kg), and their weight-for-age Z-score ranged from 0.7 to -2.0 SD (mean -0.8 SD).

There were no differences in the mean food intakes between tests: 13.24 (SD 5.81) g/kg for test 1, and 12.55 (SD 6.18) g/kg for test 2.

There was a 40% increase in total percentage ^{13}C recovery from mean 13.7 (SD 3.7)% for test 1, to 19.7 (SD 7.2)% for test 2 ($P<0.01$). There were no differences in time to peak ^{13}C recovery. There were no differences in concentrations of amylase in saliva: 43.14 (SD 27.83) IU/g protein for test 1 and 59.80 (SD 54.57) IU/g protein for test 2, or for breast milk: 0.70 (SD 0.48) IU/ml for test 1 and 0.76 (SD 0.45) IU/ml for test 2.

The ^{13}C breath test is a convenient non-invasive method for the indirect measurement of cereal starch digestion in early life. We conclude that partial digestion of supplementary weaning foods with amylase-rich flour may improve the nutrition of children, not only by reducing the viscosity of feeds, thereby increasing their ingestibility, but also by improving digestion and absorption.

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Weaver, L.T. (1994). International Journal of Food Sciences and Nutrition 49 (In the Press).

Comparison of adult and neonatal fermentation of simple and complex carbohydrate. By A.M. PARRETT, C.A. EDWARDS, and E. LOKERSE, *Department of Human Nutrition, Glasgow University, Yorkhill Hospitals, Glasgow G3 8SJ*

Previous work has shown marked differences between the faecal flora of breast-fed and formula-fed babies (Balmer & Wharton, 1989). These differences may be one explanation for the increased incidence of diarrhoea in formula-fed babies (Howie *et al.* 1990). Carbohydrate which enters the colon is fermented to short-chain fatty acids (SCFA) which are rapidly absorbed preventing osmotic diarrhoea. The faecal SCFA of breast-fed and formula-fed babies also differ (Edwards *et al.* 1994). In this study the fermentation capacity of formula-fed and breast-fed babies for a range of simple and complex carbohydrates was estimated using an *in vitro* model.

Fresh faeces were obtained from seventeen normal babies (aged 2-20 weeks, ten breast-fed, seven formula-fed). The stool samples were processed within 1 h of passage in basic salts medium (Adiotomre *et al.* 1990). Each culture contained 3.2% faeces, 100 mg carbohydrate (lactose, raftilose, soya-bean polysaccharide, and low molecular weight guar gum), incubated anaerobically at 37° for 24h. The cultures were than assayed for pH, SCFA, and lactic acid by GLC. The results were compared with those obtained with similar cultures inoculated with faeces from five normal adult volunteers. Results are expressed after subtraction of values obtained without carbohydrate. Infant faecal cultures were compared with those of adult faecal cultures by Kruskal-Wallis and Mann Whitney U tests.

Babies produced less SCFA than adults for all carbohydrates tested. Formula-fed babies were more efficient at fermenting the carbohydrates than the breast-fed babies although this was not statistically significant. More SCFA were produced from oligosaccharides than from complex carbohydrates. Lactic acid was produced by all cultures but more was produced from the oligosaccharides. Butyrate production was lowest in the cultures of faeces from breast-fed babies ($P < 0.05$).

The fermentation characteristics of formula- and breast-fed babies reflect bacterial populations, formula-fed babies having more bacteroides than breast-fed babies (Balmer & Wharton 1989).

	SCFA concentration (mmol/ml)					
	Adults		Breast-fed		Formula-fed	
	Median	Range	Median	Range	Median	Range
Blank						
Total SCFA	16.9	14.5-22.3	16.9	5.6-27.9	12.1	0.9-19.1
Lactate	0	0-4.3	0	0-2.56	0	0-0
Lactose						
Total SCFA	50.1	31.2-73.7	53.5	0-63	49.7	34.0-78.7
Lactate	0	0-44.7	33.7	10.8-56.5	19.6	0-34.9
Raftilose						
Total SCFA	51.7	32.1-94.7	35.5	3.6-48.9	44.8	16.9-91.5
Lactate	29.2	0-43.8	3.94	0-27.5	0	0-4
Soya-bean polysaccharide						
Total SCFA	42.4	28.5-77.2	12.3**	6.9-40.2	20.6**	11.5-34.2
Lactate	0	0-2.3	3.9	0-3.2	0	0-0.6
Guar Gum						
Total SCFA	51.7	43.4-66.7	6.5**	0.1-57.3	20.2	5.4-70.0
Lactate	0	0-1.1	0.34	0-9.8	0	0-1.8

** $P < 0.01$ compared with adult; *** $P < 0.001$ compared with adult.

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Digestion in potoroine marsupials: forestomach fermenters or metabolic generalists? By I. R. WALLIS, University of New England, Armidale, NSW, Australia

Small herbivores have high mass-specific nutrient requirements and must select more concentrated diets than those eaten by large herbivores (Demment & Van Soest 1985). Many researchers studying herbivores equate the presence of volatile fatty acids (VFA) and a pH of 5-7 in the foregut with the animal obtaining most of its requirements through microbial metabolism (e.g. Kinnear *et al* 1979). From an energetic perspective, fermenting a low-fibre diet may be wasteful compared with enzymic digestion. However, apart from producing energy-rich nutrients, the forestomach may also store food and its microbes may detoxify plant chemicals and improve the quality of ingested protein. These latter roles are more likely in small concentrate-selecting herbivores such as potoroines (rat-kangaroos) which feed mainly on roots, tubers, gums, hypogeous fungi and seeds.

In balance studies with potoroines fed on grain/oat-hull diets, fibre digestibilities were low and variable (Wallis, 1990). However, the slow passage through the gut refutes any suggestion that limited digesta retention restricts the breakdown of fibre. Instead, it appears that potoroines are either incapable of digesting large amounts of fibre or that some other factor is inhibiting cellulolysis e.g. low pH caused by the rapid fermentation of carbohydrates and/or poor buffering capacity.

To test these hypotheses, I fed potoroines on pelleted diets with limited amounts of soluble carbohydrates. The animals refused pure lucerne (*Medicago sativa*) pellets so I compromised and measured dry matter (DM) and neutral- and acid-detergent fibre (NDF, ADF) digestibilities in diets of 750g lucerne and 250g maize/kg. The results were compared with data from *Macropus robustus* (Freudenberger, personal communication) fed on a diet of similar composition but using a different batch of lucerne (Table).

Genus	*Macropus*	*Aepyprymnus*	*Bettongia*	*Potorous*
n	8	3	5	2
Digestibility (%) DM	63.5	58.8	57.8	56.8
NDF	39.3	59.6	57.9	55.5
ADF	29.3	36.0	37.8	36.0

Results show that all three potoroines can digest fibre efficiently, as efficiently as a large macropodid. Furthermore, the low variation (CV approximately 9%) for fibre digestibility implies that previous variability was diet related. Another finding of interest relates to the reintroduction of the basal (grain-based) diet. In ruminants, a sudden switch to concentrates may have catastrophic effects through the proliferation of facultative organisms that produce copious lactic acid (Allison *et al.* (1975). I am unaware of similar findings in macropodids but, as a precaution, the basal diet was introduced by mixing it in a 1:2 ratio with the lucerne diet. The potoroines selected the basal ration and refused the lucerne, even though it had constituted their entire ration for the previous month.

In a second experiment, four *Aepyprymnus* digested 61% of the DM but only 31% and 23% of the NDF and ADF respectively in a pelleted diet of 650g lucerne and 350g maize/kg. This was followed by DM, NDF and ADF digestibilities of 71, 31 and 29% when they ate a diet of 350 g lucerne and 650g maize/kg. Only the DM digestibility differed significantly ($P<0.05$) between diets.

It seems that the foregut environment of potoroines is extremely labile and that cellulolysis is related inversely to the level of soluble carbohydrates in the diet. This agrees with Beale (1992) who concluded, from parotid salivary composition, that potoroines do not rely on foregut fermentation. Instead, the foregut is best viewed in the context of the flexibility it gives the animal, as a storage organ, as a regulator of digesta flow, and as a provider of microbial protein and VFA.

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Changes in body composition and basal metabolism in physically active elderly men: a longitudinal study. By L.A. MURRAY, J.J. REILLY, M. CHOUDHRY and J.V.G.A. DURNIN, University of Glasgow Department of Human Nutrition, Yorkhill Hospitals, Glasgow, G3 8SJ

Loss of fat-free mass (FFM) and decline in basal metabolic rate (BMR) appear from cross-sectional studies to be features of "normal ageing". Longitudinal studies are somewhat scarce, based exclusively in the USA, and depend on methods for assessment of body composition which are either difficult to interpret (creatinine output) or associated with systematic errors with advancing age (total body K). Furthermore, considerable variability in changes in BMR and body composition might be expected due to differences between individuals or groups in factors such as physical activity, aerobic capacity and disease. In the present study twenty two healthy elderly men (mean age, in 1993, 69 (sd 3) years) underwent measurement of BMR (Douglas Bag) and body composition (sum of four skinfolds), height and weight, in 1987, and the measurements were repeated using the same techniques 6.5 years later. We previously established that the skinfold method was free of bias in this age group (Reilly *et al.* 1993). All subjects were in good health and had no disease or drug treatment which would have substantially altered BMR or body composition. In 1993 the habitual physical activity of all subjects was assessed using the Nottingham Activity Questionnaire, and results compared with the Nottingham values (Dallosso *et al.* 1988) for community elderly.

The group was considerably more active than samples of age- and gender-matched subjects from the Nottingham study, particularly in walking (mean 17 (sd 10) h/week) and "physically active leisure" (mean 7.5 (sd 5.7) h/week). Of the variables measured, only height showed a statistically significant decline (paired *t*, $P<0.05$) from 1987-1993. Changes in absolute BMR, BMR expressed per kg body-weight, fat-mass, fat-free mass (FFM) and percentage total body fat, analysed longitudinally, were not statistically significant (paired *t*, $P>0.05$) over the 6.5 year period. Means of the individual changes in BMR and FFM were in the expected direction but of relatively modest magnitude: -0.4 (sd 1.5) kJ/kg per d BMR; -0.2 (sd 2.7) kg FFM . In a number of individual cases changes were in the opposite direction to those expected, and in many cases were probably within the limits of detection given the precision of the techniques. For physically active elderly men in good health the results suggest a decline in BMR of no more than 1-2% per decade which can be explained mainly by a modest decline in FFM.

We conclude that age-related changes in BMR and body composition are somewhat limited in healthy, active groups of elderly men in this age range. Factors other than age *per se* are likely to be of greater importance in producing the changes in body composition and basal metabolism associated with ageing.

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Fruit and vegetable consumption of 10-11 year old children in Tayside. By WENDY L. WRIEDEN
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A simplified food frequency questionnaire was used to assess the fruit and vegetable consumption of over 2000 primary school children aged 10-11 years from schools throughout Tayside, Scotland in September 1992. Pupils were asked how often they ate a range of fruit and vegetables (including potatoes). Results are given below for chips (Ch), green vegetables (GV) covering lettuce, cabbage, broccoli, peas and runner beans, carrots (Car), oranges and grapefruit (O&G), bananas (Ban), apples (App) and pure orange juice (POJ). Frequencies of consumption were grouped to give never or less than once a week (< once pw), 1-3 times a week (1-3 pw) and 4-6 times a week or every day (4 or more pw). A comparison was made between the urban area of Dundee (D) and the combined rural areas (R) of Angus, and Perth and Kinross (excluding Perth city). The significance of the differences in proportions between the areas was tested using the chi-squared statistic. This gives the probability *P* (quoted below) that the results would occur at random.

	% of children consuming			Comparison of D v. R	
Fruit or vegetable	<once pw	1-3 pw	4 or more pw	Higher % consumers	Chi-square
Ch	21	52	27	D	$P<0.001$
GV	39	35	26	R	$P<0.01$
Car	50	38	12	R	$P<0.01$
Ban	38	37	25	D	$P<0.05$
O&G	37	37	26	D	$P<0.01$
App	19	37	43	-	NS
POJ	36	25	39	-	NS

NS, not significant

Apples and chips were the most popular of the fruit and vegetables and were eaten at least once a week by over three-quarters of the children. However it was found that 32% of Dundee children ate chips four or more times a week compared with only 23% of children in the rural areas. In addition, 44% of Dundee children rarely or never ate green vegetables compared with 37% of rural children. Carrots seemed to be generally unpopular but this was more so in Dundee where 55% of children rarely ate them. There was a higher percentage of regular consumers of bananas and citrus fruits in Dundee. For bananas, 65% of Dundee children ate these at least once a week compared with only 59% of the rural children. For oranges and grapefruit, again 65% of children in Dundee claimed to eat these at least once a week compared with 61% in the rural areas. This may be due to the poor availability of fresh fruit in the rural areas coupled with the fact that Dundee is well supplied with stores offering fruit at competitive prices.

The results suggest that many children eat far fewer fruit and vegetables than the 3-4 portions recently recommended (Scottish Office, 1993). The next stage of this study will be to estimate the extent of this deficiency.

W.L.W. acknowledges technical assistance from Mrs C.A. Taylor.

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Dietary intakes of female lone parents in London: energy and micronutrients. By C. CALVERT, C. RUSHTON and E.A. DOWLER, Centre for Human Nutrition, London School of Hygiene and Tropical Medicine, 2 Taviton Street, London WC1H 0BT

A 3 d weighed dietary survey was carried out during 1992/3 on a random sample of 200 lone parent households in London. Individual dietary records, weights and heights were obtained on the parent and, where possible, all other members of the household. Extensive interviews investigated dietary patterns and health beliefs, shopping and budgeting strategies and household economic circumstances. Good dietary records were obtained from 131 households and the data were analysed using COMPEAT 4 (LIFELINE Nutritional Services Ltd) and transformed to natural logarithms where necessary. We present mean data on energy (MJ), non-starch polysaccharides (NSP), Fe, Ca, vitamins A, C, E, and folate, and the percentage of the dietary reference values (DRV; Department of Health, 1991) for female parents only (n 126). The Table includes data for those in receipt of Income Support, a means-tested benefit (Group A) compared with those with other sources of income, in the majority, their own earnings (Group B).

			Intake Total (n 126)	Intake Group A (n 85)	Intake Group B (n 41)	% DRV Total (n 126)	% DRV Group A (n 85)	% DRV Group B (n 41)
Energy	(MJ/d)	Mean	7.49	7.29	7.91	85	84	88
		SE	0.19	0.23	0.35	2.2	2.8	3.5
NSP	(g/d)	Mean	10.5	9.7**	12.33	59	54**	68
		SE	0.43	0.45	0.8	2.4	2.5	4.8
Iron	(mg/d)	Mean	10.2	9.3***	12.1	72	65***	89
		SE	0.36	0.38	0.6	2.9	2.7	6
Calcium	(mg/d)	Mean	700	690	721	100	99	103
		SE	0.28	33.7	40.7	3.7	4.7	5.6
Vitamin A	(μg/d)	Mean	991	792	1412	165	133	234
		SE	138	103	372	23	17	60.5
Vitamin C	(mg/d)	Mean	63	55**	78	158	140**	196
		SE	4.35	5.23	7.6	10.8	13	18.2
Vitamin E	(mg/d)	Mean	6.0	5.6*	6.9	142	141	144
		SE	0.27	0.3	0.5	5.7	7.3	9.5
Folate	(μg/d)	Mean	190	179*	214	95	89*	107
		SE	7.6	8.5	14.8	3.8	4.3	7.4

Significantly different intakes between those on Income Support and those not on Income Support, by independent T test:
* P <0.05, ** P <0.01 *** P < 0.001.

Although energy intakes of those receiving Income Support were no different from those not receiving Income Support, intakes of NSP, Fe, vitamins C and E were significantly lower in those whose main sources of income were means-tested state benefits. Forty-four women had Fe intakes below the lower reference nutrient intake, most of whom were in receipt of Income Support (P<0.001).

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Dietary vitamin K (phylloquinone) intake in Scottish men. By S. FENTON[1], C. BOLTON-SMITH[1], D. HARRINGTON[2] and M.J. SHEARER[2], [1]Cardiovascular Epidemiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY and [2]Department of Clinical Haematology, Guys' Hospital, London SE1 9RT

The importance of vitamin K to health has recently been highlighted by the reports linking intramuscular vitamin K prophylaxis at birth to childhood cancer (Golding et al. 1990) and data which suggest that vitamin K may be a significant factor in osteoporosis (Knapen et al. 1989, Douglas, 1993). Up to now dietary vitamin K intake has not been adequately characterized in any population group due to the lack of basic food compositional data. Newly analysed vitamin K food compositional data have been integrated into the analysis program for the food frequency questionnaire (FFQ) employed in the Scottish MONICA Optional Antioxidant Vitamin Study (Bolton-Smith et al. 1991) in order to estimate usual intake levels in this Scottish population. Men aged 40-54 were recruited in Aberdeen and north Glasgow in 1988. A total of 191 completed a food frequency and personal health questionnaire and also provided a non-fasted blood sample for biochemical analyses which included serum vitamin K (by HPLC with electrochemical detection in redox mode), cholesterol and triacylglycerol. Dietary and serum vitamin K values are given in the Table by population sub-group.

	Dietary vitamin K			Serum vitamin K	
	n	µg/d	sd	µmole/L	sd
Whole population	191	61	24	1.43	1.16
Aberdeen	93	64*	25	1.65**	1.17
Glasgow	98	59	23	1.22	1.12g
Non-smokers	117	66**	23	1.56	1.22
Smokers	79	54	25	1.24	1.07
Manual	102	55***	22	1.31	1.08
Non-manual	82	69	25	1.57	1.24

Difference between paired groups for dietary and serum values by ANOVA, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Manual Non-manual refers to occupational social class category.

The lower dietary intakes of vitamin K in Glasgow, smokers and the manual occupational group reflect the relative poor quality of the diet in these groups with respect to green vegetables and high quality vegetable oil, the main sources of dietary vitamin K. Dietary vitamin K intake was not significantly correlated with total energy intake, however energy adjusted vitamin K intake correlated more closely with plasma vitamin K levels (expressed as the triacylglycerol:vitamin K ratio; r 0.26, $P<0.001$) than did the µg/d (r 0.19, $P=0.009$). The significant correlation between dietary and serum vitamin K levels suggests that the estimated daily intakes of vitamin K from the FFQ are representative of actual consumption. These values are lower than the interim recommendations for adults of 1µg/Kg body-weight (Committee on Medical Aspects of Food Policy 991), but are consistent with the known lower intakes of antioxidant vitamins from fruits and vegetables in Scotland compared with the rest of the UK and other countries.

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Fruit and vegetable intake in the West of Scotland. By A.S. ANDERSON, K. HUNT and G. FORD, MRC Medical Sociology Unit, 6 Lilybank Gardens, Glasgow G12 8QQ

Dietary recommendations from around the world are consistent in recommending high intakes of fruits and vegetables for the promotion of health and prevention of diet-related diseases (World Health Organisation,1991). The underlying mechanisms by which fruit and vegetable intake may influence diseases such as coronary heart disease and cancer are largely unknown, although it has been postulated that high levels of antioxidant vitamins, dietary fibre and substances such as flavonoids (of which fruits and vegetables are a good source) may be involved.

To identify nutrient patterns associated with fruit and vegetable consumption, the dietary intake of a community sample of people in early and late middle age participating in the West of Scotland Twenty-07 study (Macintyre *et al.*, 1989) were examined by a food frequency questionnaire (Yarnell *et al.*, 1983).

Only 2.7% of the sample reported eating the WHO recommendation of five portions (approximately 400 g) of fruits and vegetables daily and 36.8% reported consuming less than half this amount. Analysis according to categories classified as 'minimal' (0 - 2.49 portions), 'low' (2.50 - 4.99 portions) and 'moderate' (5.00 + portions) showed clear rises in intakes of vitamin A, vitamin C and non-starch polysaccharide (NSP) with increasing fruit and vegetable intake for men and women. Fe and Ca intakes were highest in the moderate consumers and lowest in the minimal consumers. Likewise the percentage of energy derived from fat was lowest in the category with the highest intake of fruit and vegetables. There were no significant differences in energy intake between the three categories. Additionally, fruit and vegetable intake was associated with consumption of a number of other food items which might account for differences in nutrient intake (Anderson *et al.*, 1994).

Nutrient intakes in minimal, low and moderate consumers of fruit and vegetables

Nutrient	Gender	Minimal Mean SD	Low Mean SD	Moderate Mean SD	Statistical significance of difference between groups
Retinol equivalents (ug)	Male	1270±995	1795±1240	2705±1890	***
	Female	1280±1220	1645±1355	2195±1405	***
Vitamin C (mg)	Male	50.4±20.1	78.6±24.7	113.0±29.7	***
	Female	47.3±19.9	80.2±26.6	114.7±25.9	***
NSP (g)	Male	12.2±4.5	18.3±5.8	24.2±7.1	***
	Female	11.2±4.0	17.6±5.7	24.5±7.5	***

Significance of one-way analysis of variance: *** $P < 0.001$.

It is concluded that intakes of fruit and vegetables by middle-aged adults living in the West of Scotland are far below WHO recommendations. The association between fruit and vegetable intake and a number of other nutrients suggests that an assessment of intake of these food items may provide a valuable pointer to key features of dietary quality.

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Longitudinal changes in the snack food choices of teenagers living in the West of Scotland. By A.S.ANDERSON, H. SWEETING and P. WEST. MRC Medical Sociology Unit, 6 Lilybank Gardens, Glasgow G12 8QQ

The Scottish Diet report (Scottish Office, 1993) has highlighted the consumption of "snack foods" in adolescents as one of a number of problems in achieving a healthy diet. In particular, the high intakes of confectionery, soft drinks and savoury snacks and low intakes of fruit in children and adolescents have been criticized. However, few studies have examined snack food consumption in Scottish adolescents and how this changes on leaving school when there may be more freedom from parental food choice and more available money (e.g. for those in employment).

To identify the longitudinal changes in snack food choices of teenagers the dietary inventories completed by a community sample of adolescents aged 15 in 1987 and then aged 18 in 1990 participating in the West of Scotland Twenty-07 study (Macintyre *et al.*, 1989) were examined.

For almost half the sample in 1987 (aged 15), fruit (in summer), crisps, confectionery, biscuits and sweetened drinks were consumed daily. Cakes and fruit (in winter) were consumed daily by a much smaller proportion. This frequency of consumption (apart from fruit) was higher than that reported for the 35-year-old cohort in the same study in the same year (Anderson *et al.*, 1993).

In 1990 (at age 18) there were significant reductions in the frequency of consumption of all snack items except for sweetened drinks.

Year	Age (years)	Food item	*n*	> daily (%)	Once daily (%)	Most days (%)	1-2/ week (%)	<1/week never (%)	Statistical significance of diffs. 1987-1990
1987	15	Fruit (summer)	842	23.3	25.1	25.5	19.1	7.0	
1990	18	Fruit (summer)	842	21.6	21.7	26.0	19.6	11.0	**
1987	15	Fruit (winter)	840	6.2	16.3	22.1	34.5	20.8	
1990	18	Fruit (winter)	840	6.8	12.3	18.8	30.5	31.7	***
1987	15	Crisps etc	834	16.3	31.1	29.7	15.6	7.3	
1990	18	Crisps etc	834	15.6	26.5	31.5	18.9	7.4	*
1987	15	Confectionery	838	19.2	27.7	28.2	18.0	6.9	
1990	18	Confectionery	838	16.3	22.9	27.4	23.2	10.1	***
1987	15	Cakes	832	2.4	9.4	17.5	40.0	30.6	
1990	18	Cakes	832	1.7	6.0	12.4	34.0	45.9	***
1987	15	Biscuits	832	22.5	26.2	28.7	15.1	7.5	
1990	18	Biscuits	832	13.0	18.4	26.0	24.4	18.3	***
1987	15	Sweetened drinks	836	33.7	24.6	23.6	12.6	5.5	
1990	18	Sweetened drinks	836	37.9	21.8	19.9	11.4	9.1	NS

Significance of Z statistic (Wilcoxon matched-pairs signed rank test): P<0.05, **P<0.01, ***P<0.001.

These findings suggest that between ages 15 and 18, adolescents reduce their frequency of consumption of commonly eaten snack foods. These changes may be accounted for by increased quantities of snacks (but eaten less frequently), greater consumption of commonly eaten meal items or greater consumption of novel snack items. Additionally, significantly more alcohol is consumed at age 18 compared with age 15 which may compensate for decreases in snack foods.

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Water turnover rates in sedentary and active middle-aged men. By J.B. LEIPER, A. CARNIE and R.J. MAUGHAN, *Department of Environmental and Occupational Medicine, University Medical School, Aberdeen AB9 2ZD*

Prolonged exercise, even in a cool environment, will induce sweating and will therefore cause the body to lose water. In order to maintain euhydration additional water in the form of food or fluids must be ingested. The effect of exercise on water turnover in endurance-trained middle-aged men was examined. Six endurance-trained and six sedentary healthy males were recruited for this study, which was carried out in May and June. Subjects in the active group (AG) had a mean (SD) age of 41 (6) years, height of 1.73 (0.05) m and body mass of 63.8 (7.3) kg. Subjects in the sedentary group (SG) were matched to the AG with respect to age (42 (SD 7) years; $p= 0.75$), height (1.74 (SD 0.06) m; $p= 0.83$) and body mass (71.3 (SD 10.9) kg; $p= 0.19$). Two of the AG subjects each had a rest day during the 7 d study period and the median (range) daily distance run by the group was 8.5 (0-23) miles. Individuals in the SG undertook less than 60 min strenuous exercise over the study period. All subjects were requested to maintain their normal pattern of eating, drinking and daily lifestyle. On one evening before retiring to bed, subjects micturated, then ingested 10.0004 (0.0003) g deuterium oxide (D_2O) which was diluted with a drink of their choice. The subjects were instructed to rinse the container of D_2O several times and to ingest the contents. A portion of urine from the next urine sample excreted was collected and the deuterium (D_2) concentration of this sample was measured. In all cases this sample was passed next morning. The remainder of the urine passed at this time and all the urine excreted until the subject next retired to bed was collected and pooled. Urine collection was continued over a total of seven consecutive days. Following vacuum distillation of the morning urine sample, the D_2 concentration of the aqueous fraction was determined in duplicate by infra-red spectroscopy. The D_2 measured in the first morning sample was used to calculated total body water (TBW), and the D_2 concentration in the subsequent morning samples was used to estimate daily water turnover assuming that TBW content remained constant for each subject. The total daily urine output including the sample volume used for D_2 measurement was recorded. The daily non-urine water loss was estimated as the difference between calculated water turnover for that day minus the volume of urine excreted over the same time period.

Mean (SD) TBW volume of the AG (39.3 (4.9) l) was similar to that of the SG (39.9 (3.9) l; $p= 0.80$); however, expressed as a percentage of body mass, TBW content was greater in the AG (62.8 (SD 3.6)%) than the SG (55.5 (SD 6.4)%; $p= 0.037$) reflecting differences in body fat. Median (range) body water turnover (ml/24 h) was greater in the AG (4600 (941-16585)) than in the SG (2702 (871-8737); $p= 0.001$). Water turnover rates each day were fairly constant in the AG ($p= 0.33$) and in the SG ($p= 0.17$). The daily urinary loss (ml/24 h) was greater in the AG (2457 (range 477-6290) than in the SG (1346 (range 758-2471); $p= 0.001$). The volume of urine excreted each day was fairly constant in the AG ($p= 0.85$) and in the SG ($p= 0.88$). The calculated non-urine daily water loss (ml/24 h) was similar in the AG (2071 (range 457-10295) and in the SG (1373 (range 113-6272); $p= 0.08$). This study suggests that water turnover rates are faster in exercising than in sedentary middle-aged men, but the difference between the two groups in daily water loss is due mainly to an increased urinary output in the AG rather than a difference in exercise-induced sweat rates. The sensation of thirst in man, although a poor predictor of acute dehydration is considered to be a sensitive mechanism for the long-term maintenance of euhydration. In the small population studied, a volume of fluids considerably greater than that required to rehydrate after exercise appears to be routinely ingested.

Body composition estimation when height is unavailable. By T. S. HAN and M. E. J. LEAN, *Department of Human Nutrition, Glasgow Royal Infirmary, University of Glasgow, Glasgow G31 2ER.*

Body mass index (BMI) and other indices of body composition which include height have been used as measures of stature. Measuring height of the critically ill, elderly, and subjects with musculo-skeletal injury is difficult. Measurements on supine subjects differ significantly from standing height (Watt *et al.* 1994). Limb lengths may offer useful height prediction. Bassey (1986) found a correlation coefficient of 0.74 between demi-arm span and height.

Body height, two-arm spans between finger tips (AS-t) and between webspaces (AS-w), and average lower leg length (LLL), the distance from the floor to the mid-point of a ruler placed on the patellae with lower legs flexed at an angle of 90°, were made on seventy-eight men, mean age 44 (range 17 to 67) years and eighty-one women, mean age 43 (range 22 to 64) years.

Body height was correlated with average LLL (r 0.89 for men and 0.86 for women), AS-t (r 0.80 for men and 0.83 for women), and AS-w (r 0.75 for men and 0.79 for women). All measurements were highly repeatable with coefficients of variation under 1% for duplicates. When adjusted for age, average LLL was the best predictor of height with explained variance (r^2) and standard error of the estimate (SEE) 80.5 % (29 mm) for men and 76.2 % (32 mm) for women.

These measures were then used instead of height, with body weight (BWt) to develop indices to predict BMI (BWt/height^2) and with age to predict percentage body fat (BF%) by densitometry performed in sixty men, mean age 40 (range 17 to 67) years and mean BF% 21.2 (range 5 to 44) % BWt and seventy-five women, mean age 42 (range 22 to 64) years and mean BF% 34.5 (range 16 to 52) % BWt. The results are shown in the Table.

	BMI		BF%	
	r^2 (%)	SEE (kg/m^2)	r^2 (%)	SEE (% BWt)
BWt/LLL				
Males	94.7	1.1	64.3	5.0
Females	94.8	1.1	72.1	4.6
BWt/(AS-t)^2				
Males	93.1	1.2	61.9	5.1
Females	91.0	1.5	71.7	4.8
BWt/(AS-w)^2				
Males	91.9	1.3	66.1	4.8
Females	92.3	1.3	70.8	4.7
BMI				
Males			62.6	5.2
Females			74.6	4.4

LLL and arm spans are useful when height measurement is unavailable. They are easy to measure for both observer and subject, highly reproducible between observers, and give high prediction with low error for height, BMI and an estimation comparable to BMI for BF% when used with weight and age.

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Measuring the body composition of elderly subjects: a comparison of methods. By J.J. REILLY, L.A. MURRAY, J. WILSON and J.V.G.A. DURNIN, University of Glasgow Department of Human Nutrition, Yorkhill Hospitals, Glasgow G3 8SJ

There are few data on differences between methods of assessment of body composition in elderly subjects. Studies of younger adults suggest that methodological differences are of some practical significance at the individual level. Changes in body composition and fat distribution with advancing age mean that methodological differences might be expected to be more serious in the elderly. In the present study the following methods of estimating body fatness (percentage of body weight, BF%) were compared in the same healthy elderly subjects (nineteen males, sixteen females, mean age 70 years): densitometry (D); skinfold thickness; (SFT); total body water (TBW); bioelectrical impedance (BIA) using an age-specific regression equation and software provided by the manufacturers (Cranlea & Company Limited); body mass index (BMI) using regression equations derived from Dutch elderly (Deurenberg *et al.* 1991). A further twenty-five healthy elderly subjects (eight males, seventeen females) were unwilling or unable to participate in measurement of D and TBW, and comparisons were made between the other three methods. Differences between methods were analysed according to the method of Bland & Altman (1986).

Estimates of BF% from the various methods tended to be highly correlated with each other. However, the correlations were somewhat misleading because differences in estimates of BF% between methods were marked at the individual level, and some distinct biases were apparent. Specifically, the age specific regression equations for prediction of BF% from BIA (Deurenberg *et al.* 1990) and BMI (Deurenberg *et al.* 1991) systematically overestimated BF% relative to the other methods. Biases between estimates of BF% derived from D, SFT, BIA (manufacturers equation), and TBW were less marked and indicated little evidence of systematic differences between these methods in elderly subjects.

Individual differences between methods were slightly greater than those reported in two studies of younger adults (Fuller *et al.* 1992; McNeill *et al.* 1991) and this may reflect differences between and within elderly subjects in the extent to which the underlying assumptions of these two-component methods are valid.

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Weaning practice in Glasgow. By S.A.H. SAVAGE, J.J. REILLY, C.A.EDWARDS, and J.V.G.A. DURNIN, University of Glasgow Department of Human Nutrition, Yorkhill Hospitals, Glasgow G3 8SJ

Current advice (Department of Health and Social Security, 1988) suggests that "very few infants will require solid food before the age of 3 months". However, there is increasing concern over the prevalence of weaning before 3 months and its possible implications for health. A cohort of 128 infants (forty-nine breast-fed, seventy-nine formula-fed) from North and West Glasgow, recruited by postcode to represent the normal population of the city, are currently participating in a longitudinal study of infant growth from birth to 2 years. In this cohort, median age for introduction of solids was 11 weeks (range 4-35 weeks). In 10% of the sample, solids were introduced by 8 weeks rising to 52% by 12 weeks of age. Introduction of solids occurred significantly earlier in formula fed infants compared with breast fed infants (10.8 (sd 4.2) weeks *v.* 12.8 (sd 2.9) weeks ; $p<0.01$, t test) and in first babies (10.7 (sd 2.5) weeks) compared with second or later babies (12.2 (sd 4.5) weeks; $P<0.05$). Mothers under 20 years of age were more likely to introduce solids early (8.2 (sd 2.6) weeks *v.* older mothers 11.8 (sd 3.8) weeks; $P<0.01$).

A questionnaire was given to ninety-eight mothers to identify the factors influencing weaning practice. In 34% of mothers no formal advice or information on weaning was reported. In the remaining 66% information had been given and the most common source was the Health Visitor (77% of cases). Other information sources included reading books and leaflets (32%), and advice from doctors/nurses/hospital (6%). There was no significant difference in the mean age of weaning between mothers who had received formal information and those who had not, however, there was a significantly greater proportion of those women with no formal information weaning before 10 weeks ($P <0.05$, chi square test). Even when advice had been given it was clear that other factors were more important in determining age of weaning. In 74% of cases the principal reason given was the perception that the child was not satisfied.

In 104 of the 128 infants (81%) the first solid food introduced was a commercial cereal preparation. Baby rice was the most popular (66%) then baby cereal (9%) and rusks (5%). Fruit and vegetable purées (recommended by the COMA panel of Child Nutrition) were introduced as the first food by only six mothers (5%). Feeding cows' milk is not recommended before 1 year (Wharton, 1990). Of the 128 infants eighty-two (64%) had been fed cows' milk before 9 months.

We conclude that the current government recommendations for weaning practice are not being followed by substantial proportions of the population of the West of Scotland, many mothers do not appear to be receiving adequate advice and those who do receive advice often ignore it.

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Resistant Starch in young Children. By K. VERITY, and C.A. EDWARDS. *Department of Human Nutrition, Glasgow University, Yorkhill Hospitals, Glasgow G3 8SJ*

The digestibility of weaning foods in the Third World is often of concern. However little is known of the digestibility of foods in young childhood in the UK. Babies in the UK are weaned from milk onto a variety of weaning foods at about 3-4 months of age (although a significant proportion are weaned much earlier). First weaning foods are well liquidized and at 7-9 months more structured food is given. As the infant develops teeth and locomotor function, the young child moves from well chopped or minced food which needs little chewing to whole foods. Little is known of the digestibility of foods during this period of changing food intake and chewing ability. Starch in food is either rapidly digestible, slowly digestible or resistant to human pancreatic enzymes (Englyst *et al.* 1992). The definition of resistant starch (as accepted by the EEC concerted action Euresta) is the starch or starch breakdown products that enter the colon. Resistant starch can be divided into three types : physically inaccessible starch in fibre rich foods, intact starch granules, and retrograded starch (Englyst *et al.* 1992). If there is inadequate chewing the amount of type 1 resistant starch may increase. Starch which enters the colon is fermented by the colonic bacteria to short-chain fatty acids (SCFA) and gases. Some of the factors which inhibited digestion of the starch in the small intestine will also delay the fermentation of starch in the colon. Starch which escapes this fermentation will appear in faeces.

In the present study we have measured the faecal starch content in nine young children aged from 5 to 37 months of age eating their normal diets. The children were weaned at 10-20 weeks. Fresh faecal samples were collected from the children within an hour of being passed. They were immediately frozen for subsequent analysis. The stools were freeze-dried and then assayed by a method developed by H. Englyst and S.M. Kingman for residual starch in fermentation studies (modified version of Englyst *et al.* 1992): dispersion by boiling and treatment with KOH, hydrolysis with amyloglucosidase (*EC* 3.2.1.3*)* and measurement of glucose by glucose oxidase reagent (Merck Ltd, Lutterworth, U.K. Faecal SCFA were measured by GLC of ether extracts. In vitro fermentation of starch and glucose by the same faecal samples was measured after 24h in a basic salts medium with tryptone and 100mg carbohydrate. Results obtained from incubations with no carbohydrate were substracted from test values (Parrett *et al.* 1994).

All samples tested contained small but measurable amounts of starch. Using average values for stool output, (Weaver *et al.* 1988) this would be equivalent to a maximum of 0.9g starch per day. However, this is starch which has escaped both digestion and fermentation and may represent a much greater amount of starch entering the colon. Faecal starch was significantly correlated to age ($r=-0.667$, $p<0.05$) but did not relate to faecal butyrate or the ability of the faecal sample to ferment starch.

Infant	Age (months)	Weaning age (weeks)	Faecal starch (g/100g)	Faecal butyrate (moles/mole total)	Faecal water %	Starch+ Fermentation index
1	5	12	15	37	72	17.3
2	7	20	7.5	9.7	74	15
3	9	17	7	26.3	67	26.3
4	9	12	13.3	5.9	88	1.2
5	11	10	10.6	13.6	84	85.7
6	13	12	13.5	16.8	74	25
7	14	12	9	1.4	78	100
8	26	12	8.7	22.4	78	113
9	37	13	3	15.6	69	86.7

+ Starch fermentation index = (SCFA Starch - SCFA Blank) / (SCFA Glucose - SCFA Blank)

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Selenoprotein gene expression in the thyroid. By G. BERMANO[1], F. NICOL[1], J. A. DYER[2], R. A. SUNDE[2], J. E. HESKETH[1] and J. R. ARTHUR[1], [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB and* [2]*University of Missouri-Columbia, Mo 65211, USA*

Selenocysteine-containing proteins, which occur in rat thyroid, include cytosolic glutathione peroxidase (GSH-Px), phospholipid hydroperoxide glutathione peroxidase (PHGSH-Px) and type I iodothyronine 5' deiodinase (5'IDI). The two selenoperoxidases are thought to regulate intracellular hydrogen peroxide and lipid hydroperoxide concentrations, whereas 5'IDI is involved in the metabolism of thyroid hormones.

Selenium (Se) availability to cells regulates both activity and concentrations of selenoproteins and thus controls the functions of the trace element (Arthur, 1992). However, the responses of individual selenoproteins to dietary Se supply are very different both within and between tissues. In contrast to loss of Se in the liver, the thyroid gland is able to retain Se when dietary supplies are limiting. We have therefore investigated the regulation of selenoprotein synthesis in the thyroid by determining enzyme activities and mRNA levels.

Two groups of rats were offered either a Se-deficient diet (0.003 mg Se/kg diet) or a Se-adequate diet (0.104 mg Se/kg diet) for 6 weeks from weaning. Total thyroid RNA was isolated and subjected to successive Northern hybridizations using cDNA probes specific for GSH-Px, PHGSH-Px and 5'IDI. Finally samples were hybridized with cDNA for 18S rRNA to allow corrections for loading of the filters. Hybridization was quantified by direct measurement of specifically-bound probe using a Packard. Radioactivity-Imager. Enzyme activities were determined by standard techniques. Results, expressed as a percentage of Se-adequate control, are shown in the table (mean values with standard errors, SE).

	GSH-Px				PHGSH-Px				5'IDI			
	+Se		-Se		+Se		-Se		+Se		-Se	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Enzyme activity (%)	100	5	70***	13	100	16	93	11	100	7	116	9
mRNA (% control)	100	22	98	23	100	20	167*	32	100	13	207**	28

Mean values are significantly different from Se-adequate control, *$P<0.02$, **$P<0.01$, ***$P<0.001$.

Thyroid glands from Se-deficient rats contained similar amounts of GSH-Px-mRNA, 67% more PHGSH-Px mRNA and 107% more 5'IDI. mRNA than the thyroids from the Se-adequate rats. Thyroid PHGSH-Px and 5'IDI activities were not significantly affected, while GSH-Px activity decreased in thyroids from Se-deficient rats.

The increased levels of 5'IDI and PHGSH-Px mRNA may be the mechanism by which the thyroid is able to preserve the selenoprotein activities in Se deficiency (Table). This contrasts with liver which suffers up to 90% decreases in 5'IDI and PHGSH-Px activities and over 99% loss of GSH-Px-activity in Se deficiency and emphasizes the importance of adequate dietary Se intake for normal thyroid function.

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Selenoenzyme activity in the brain stem. By J. H. MITCHELL[1], F. NICOL[1], G. J. BECKETT[2], and J. R. ARTHUR[1], [1]*Division of Biochemical Sciences, Rowett Research Institute, Aberdeen AB2 9SB and* [2]*University Department of Clinical Biochemistry, Royal Infirmary, Edinburgh EH3 9YW*

In euthyroid animals and man approximately 80% of plasma 3,3',5 triiodothyronine (T3), the biologically active thyroid hormone, is derived from the deiodination of thyroxine (T4) in liver and kidney. Thus, plasma T3 and T4 concentrations are regulated by the activity of the Se containing type I iodothyronine deiodinase (ID-I). Additionally, a non-Se-containing type II iodothyronine deiodinase (ID-II) in pituitary, brain and brown adipose tissue regulates the local supply of T3 (Arthur & Beckett, 1993). The brain contains ID-I and ID-II activities and requires an adequate supply of T4 for normal development and function. Here we describe some effects of Se and/or I deficiency on ID activities in the brain.

Male, Hooded Lister, weanling rats of the Rowett strain were divided into eight groups and fed one of the four following diets: (1) Se- and I-adequate controls (+Se+I), (2) Se-deficient (-Se), (3) I-deficient (-I), (4) Se- and I-deficient (-Se-I). After 8 weeks, ID-I, ID-II and glutathione peroxidase (*EC* 1.11.1.9; GSHPx) activities were determined in the brain stem by standard techniques (Nicol *et al.* 1994) and are shown in the Table.

	+Se+I		-Se		-I		-Se-I	
Brain stem	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
ID-I pmol/h per mg	23.62	2.41	7.66*	5.66	ND***		ND***	
ID-II fmol/h per mg	18.08	5.94	ND***		ND***		ND***	
GSHPx units per mg	0.069	0.011	0.061	0.013	0.094	0.028	0.040	0.003

ND, no activity detected. Mean value was significantly different from that for +Se+I, $^{*}P<0.05$, $^{***}P<0.001$.

In Se deficiency in the thyroid gland and liver there is a preferential supply of Se to IDI at the expense of GSHPx (Beech *et al.* 1993). In contrast in both Se and I deficiencies, cytosolic GSHPx activity is maintained at the expense of ID-I activity in the brain stem. Additionally phospholipid hydroperoxide GSHPx activity was not significantly affected by 6 weeks Se deficiency in rat brain stem (+Se 9.521 (SE 0.791), -Se 7.214 (SE 0.927) munits/mg protein). Thus, in Se and I deficiencies there is maintenance of antioxidant activity through GSHPx activities, which may take priority over maintenance of thyroid hormone metabolism since ID-I and ID-II activities decrease.

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The effect of excessive amounts of branched-chain amino acids on amino acid utilization in growing pigs. By S. LANGER and M.F. FULLER, *Rowett Research Institute, Aberdeen AB2 9SB*

The optimal utilization of protein and minimization of amino acid excess are currently of great interest. Better adjustment of amino acid supply to the requirements can improve the performance of the animal and also minimize wastage. Usually, requirements are expressed as minimum values and the influence of excess of amino acids has not generally been considered.

In diets limiting in one amino acid, protein quality is dependent only on the amount and the utilization of the limiting amino acid. Any difference in protein utilization between two diets supplying equal amounts of a limiting amino acid must therefore relate to the utilization of that amino acid.

A N balance experiment was designed to investigate the importance and mechanism of amino acid imbalance, especially amongst the branched-chain amino acids (BCAA, leucine, valine and isoleucine) and the effects of BCAA on methionine metabolism. Four diets with balanced amino acid pattern (Wang & Fuller, 1989) or limiting (-20%) in methionine, valine or isoleucine were supplemented with leucine (100% excess) or BCAA (each 100% excess unless limiting) and given to growing pigs (30-40 kg). All diets were isonitrogenous and isoenergetic. The utilization of N (N retained/N digested) of each diet was measured.

Influence of leucine or BCAA excess on nitrogen utilization in diets limiting in methionine, isoleucine or valine

Limiting amino acid	Amino acid in excess					
	None		Leucine		BCAA§	
	Mean	SE	Mean	SE	Mean	SE
None	0.77	0.015	0.75	0.015	0.76	0.014
Methionine	0.67*	0.014	0.72†	0.016	0.72†	0.016
Valine	0.68*	0.014	0.66	0.015	0.70‡	0.017
Isoleucine	0.67*	0.016	0.61†	0.014	0.66‡	0.014

* Values significantly ($P<0.05$) different from non-limiting diet without excess (row 1).
† Values significantly ($P<0.05$) different from corresponding limiting diet without excess (column 1).
‡ Values significantly ($P<0.05$) different from corresponding diet with excess leucine (column 2).
§ Without the limiting amino acid.

Protein quality was significantly ($P<0.05$) and equally reduced by the 20% reduction of methionine, valine or isoleucine. Adding leucine to an isoleucine-limiting diet decreased N utilization significantly ($P<0.05$). This was largely reversed by simultaneous addition of valine with the excess leucine. Valine-limiting diets showed a similar trend. These changes probably result from the BCAA antagonisms described by Harper *et al.* (1984).

In methionine-limiting diets an excess of either leucine alone or all three BCAA increased N utilization.

The effect of amino acid excess depended upon which amino acid was deficient and which was in excess. It seems to be necessary to consider both.

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Role of coprophagy in the utilization of microbial lysine by rats. By D. TORRALLARDONA, C.I. HARRIS, E. MILNE and M.F. FULLER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

In previous experiments with rats (Torrallardona *et al.* 1993*a*,*b*) there was substantial absorption of lysine synthesized by the gastrointestinal microflora. The present study was designed to assess how much of this absorption might be due to coprophagy.

Four rats (C) were housed in cages with slatted floors, in which their movements were not restricted and they had direct access to their own faeces. Another four rats (N) were housed in tubular anti-coprophagic cages similar to those used by Metta *et al.* (1961), in which they could move comfortably but could not turn round to reach their own faeces.

Both groups were fed for 6 d on a low-protein diet containing fermentable carbohydrates and $^{15}NH_4Cl$. At the end of the experiment they were killed; the gastrointestinal tract was removed and the carcass of each animal was homogenized. Lysine was isolated by ion-exchange chromatography and its ^{15}N-enrichment was measured by isotope ratio mass spectrometry. The microbial fraction of the faeces was separated by successive centrifugation and the ^{15}N-enrichment of lysine was measured. The total amount of body lysine was also determined. The absorbed microbial lysine was estimated from the content and enrichment of whole body and microbial lysine as described previously (Torrallardona *et al.* 1993*a*,*b*).

Treatment	Lysine ^{15}N-enrichment (ape)		Body Lys content (g)	Absorbed microbial Lys (mg/d)
	Body	Microbial		
Coprophagic (C)	0.0066	0.457*	2.7	7.12*
Non-coprophagic (N)	-0.0002*	0.681	2.6	-0.15*
SEM	0.00050	0.0314	0.05	0.131
P	<0.005	0.017	0.606	<0.001

ape, atom percent excess.
* One missing value (i.e. *n* 3).

Body lysine enrichment in rats prevented from coprophagy (N) was not significantly different ($P=0.47$) from that previously found in rats given unlabelled NH_4Cl (0.0000 (SEM 0.00009); Torrallardona *et al.* 1993*b*) but in coprophagic rats (C) the ^{15}N-enrichment of body lysine was significantly higher. Microbial lysine was significantly less enriched in group C than in group N; this could be due to the dilution of the dietary ^{15}N with unlabelled faecal N.

The weight gain (adjusted for intake) in group C was significantly higher ($P<0.01$) than group N (15.5 v. 3.1 g/6 d).

These results suggest that in rats microbial lysine is utilized through coprophagy.

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Variation in probable individual feed intake of lactating cows at grass given calcined magnesite-containing feed as either nuts or cobs. By ELIZABETH McELENEY and R.G.HEMINGWAY, Veterinary Animal Husbandry Department, Glasgow University Veterinary School, Bearsden, Glasgow G61 1QH

Compound feeds providing magnesium to group-fed animals at grass should be consumed uniformly. Whilst, for pregnant ewes, Kendall *et al.* (1980) found that cobs were eaten with less variation than trough-fed nuts, it could not be certain that this was not due to giving relatively larger amounts of cobs than nuts.

Twenty-seven lactating Hereford x Friesian cows at autumn grazing were given, in consecutive 7 d periods, (a) 1 and then 2 kg cobs (35 mm x 30 mm x 20 mm, 0.99 g Cr from chromic oxide and 23.4 g Mg/kg dry matter (DM)) spread on the ground (0.25 m wide with 1 m length/cow) followed by (b) 1 and then 2 kg nuts (15-20 mm x 7.5 mm diameter, 1.09 g Cr and 7.1 g Mg/kg DM) in raised troughs (0.23 m wide with 0.66 m length/cow). The cows were previously trained to the feeds which were given at 07.30 hours when all the cows were gathered in the feeding area. Samples of faeces (per rectum) and blood were obtained on day 7 of each period. The nuts were eaten more rapidly than the cobs. Three cows obviously ate no nuts given at either rate and their faeces contained no Cr.

		Faecal Cr (g/kg DM)		Plasma Mg (mmol/l)		Eating time
Feed	kg	Mean	CV%	Mean	CV%	(min)
Cobs	1	0.22	19.6	0.75	8.8	7
	2	0.49	28.4	0.92	8.2	20
Nuts	1	0.29	40.0	0.77	9.6	<5
	2	0.43	61.1	0.91	10.5	15

The much lower coefficients of variation (CV%) found for faecal Cr concentrations when cobs were given at either rate on the ground indicated a more uniform intake relative to giving nuts in troughs. For both cobs and nuts the CV% for the faecal Cr concentrations tended to increase at the 2 kg rate of feeding. This is in contrast to the findings of Kendall *et al.*(1980) who found that the CV% of faecal Cr concentrations fell with both increased feed allocation and increased trough space allocation. Mean plasma Mg concentrations were normal but were increased at the higher rate of feeding. The relatively low CV% reflected that all values were in the upper range.

When the faecal Cr concentrations for each cow as it consumed each feed were ranked in order, very significant rank-order correlations (ROC) were found for individual cows when given the various feed allocations. When 2 kg nuts were matched with those for (a) 1 kg cobs the ROC was 0.62 ($p<0.001$), for (b) 2 kg cobs the ROC was 0.60 ($p<0.001$) and for (c) 1 kg nuts the ROC was 0.64 ($p<0.01$). The ROC between giving 2 kg cobs and 1 kg cobs was 0.77 ($p<0.001$). The erratic movements of the cows around the trough at feeding time when only 1 kg nuts was given resulted in non-significant correlations with 2 kg cobs (ROC 0.37) and with 1 kg cobs (ROC 0.43).

It is concluded that giving calcined magnesite in cobs spread on the ground ensures a more uniform intake than giving comparable amounts of feed in troughs but some individual cows always eat more (or less) of all the feeds provided.

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Mercury-zinc interactions in the marginally zinc deficient rat. By J. PRICE[1], J. H. BEATTIE[1], I. BREMNER[1], M. GABRYSZUK[2] and S. G. WOOD[1], [1]Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB and [2]Institute of Genetics and Animal Breeding, Mrokóv, Poland 05-551

Recent studies have indicated that tissue accumulation of mercury (Hg) from an inorganic dietary source is influenced by dietary zinc (Zn) intake in the rat (Kulkova *et al* 1993). Although tissue Hg concentrations were increased markedly when dietary Zn intake was decreased from adequate to marginal, the reasons for this interaction are uncertain. We have therefore investigated whole-body retention of Hg given in the diet or injected intravenously in order to differentiate between Zn effects on absorption and systemic metabolism of Hg.

Male, Hooded Lister rats weighing 250 g were offered an albumin-based, semi-purified (SP) diet containing 40 mg Zn/kg DM for 2 weeks. In the first study, thirty six rats were then allocated at random to six equal sized groups and offered, *ad libitum*, the SP diet containing 6, 8 or 40 mg Zn/kg DM without or with added Hg (0 or 10 mg Hg/kg DM as $HgCl_2$) and slaughtered for determination of tissue Hg concentration after 4 weeks. A further three groups offered the 6, 8 and 40 mg Zn/kg diets without added Hg were given $^{203}HgCl_2$ intravenously (tail vein; 75 kBq) after 4 weeks and radioactivity was monitored by whole-body counter for 1 week. In the second study, thirty six rats in six groups were offered SP diets containing 6, 8 or 40 mg Zn/kg DM without or with added Hg (10 mg/kg DM) for 2 weeks, then given $^{203}HgCl_2$ (250 kBq) in a reduced amount of feed on day 15 and radioactivity was monitored for a further week. Kidney Hg concentrations after 4 weeks and whole-body retention of ^{203}Hg 1 week after administration of the radioisotope are shown in the Table.

Diet Zn	Diet Hg	Kidney Hg		Whole-body retention of ^{203}Hg after 7 d (% of initial dose)			
		(μg/g DM)		Intravenous ^{203}Hg		Diet ^{203}Hg	
(mg/kg DM)	(mg/kg DM)	Mean	SD	Mean	SD	Mean	SD
6	0	0.75	0.32	70.60	3.83	11.38	2.26
8	0	0.37	0.08	69.35	1.22	8.83	1.60
40	0	0.24	0.03	70.79	1.96	3.28	0.81
6	10	61.82	2.20			7.92	2.62
8	10	58.86	12.89			7.42	0.91
40	10	24.05	6.46			3.52	1.35

Within columns, values with unlike superscripts were significantly different (ANOVA; $P<0.05$)

Feed intake and growth rate were not affected by dietary Zn or Hg intake. However, the concentrations of Hg in kidneys from Hg-supplemented rats with marginal intakes of Zn (6 and 8 mg Zn/Kg DM) were over two-fold greater than those of rats with an adequate dietary supply of Zn (40 mgZn/kg DM). Although Zn intake did not influence whole body retention of ^{203}Hg given intravenously, retention of the radioisotope from the diet was significantly greater when dietary Zn was low indicating that the interaction between Zn and Hg occurs at the level of absorption rather than systemically. Since marginal Zn deficiency occurs in man, these findings may have important implications for populations living in areas affected by industrial Hg contamination.

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A simplified approach to measuring mRNA on Northern blots using chemiluminescence-based detection with digoxigenin end-labelled oligonucleotides. By P. TRAYHURN, J.S DUNCAN, M.E.A. THOMAS, A. NESTOR and D.V. RAYNER, *Division of Biochemical Sciences, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Examination of the expression of specific genes is now central to a growing number of studies in nutrition, as in other areas of biology. Gene expression is generally determined through measurement of the level of an mRNA on Northern blots, using a cDNA labelled with ^{32}P. With a view to establishing a simplified, non-radioactive procedure for Northern blotting, we have combined the use of antisense oligonucleotides as probes for an mRNA with chemiluminescence detection, through labelling with the digoxigenin (DIG) system (Boehringer). This approach obviates the need to isolate and purify plasmids, and avoids the problems associated with ^{32}P (safety, stability, waste disposal). The strategy of using DIG-labelled oligonucleotides with chemiluminescence detection has been applied to several mRNA species, providing a rapid and convenient procedure for Northern blotting in nutritional studies.

To design an antisense oligonucleotide as a probe for a specific mRNA, sequences were obtained from the European Molecular Biology Laboratory (EMBL) database, through the Daresbury 'SEQNET' facility. Oligonucleotides, 30-35 bases in size (sufficient to ensure specificity), were designed with the aid of the 'Oligo' programme (MedProbe). The design was based on obtaining a high proportion of G+C residues (which form triple 'H' bonds), a high melting temperature (T_m), and low levels of self-complementarity and dimerization. The uniqueness of selected sequence regions was tested against the EMBL database ('Fasta' programme). The chosen oligonucleotides were synthesized (R & D Systems Europe) with a single DIG ligand at the 5'-end, or at both the 3' and 5' ends.

Total RNA was extracted from tissues (e.g. skeletal muscle, heart, brain, brown fat, white fat), fractionated electrophoretically on a 1.4 % agarose gel, and blotted onto charged nylon membranes (Trayhurn & Duncan, 1994). Hybridization conditions were as described previously (Trayhurn & Duncan, 1994). After hybridization of a DIG-labelled oligonucleotide to the target mRNA, and stringency washes, a polyclonal antibody conjugated to alkaline phosphatase (Boehringer) was used to detect DIG. The alkaline phosphatase catalyses the breakdown of chemiluminescence substrates, with the emission of light which is located on X-ray film. Lumigen PPD™ (Boehringer) and CSPD™ (Tropix) have both been used as substrates. Membranes were exposed to film for up to 4 h.

DIG-labelled oligonucleotides have been designed and successfully used as probes, in conjunction with chemiluminescence detection, for a number of mRNA species. These include mRNA encoding the facilitative glucose transporters (GLUT 1-5), the mitochondrial uncoupling protein (UCP), lipoprotein lipase, the β_3-adrenoceptor, and ß-actin. A poly-A^+ selection has not been required, total RNA being satisfactory. Exposure to film has generally been for 1-2 h, although with some mRNA a few minutes has sufficed. A 32-mer oligonucleotide (5'-CGGACTTTGGCGGTGTCCAGCGGGAAGGTGAT-3'), for example, detected the 1.5 kb UCP mRNA in 20 µg total RNA from rat brown fat within a few minutes exposure to film; the same mRNA was identified in just 250 ng of total RNA after a 4 h exposure. Similarly, a 32-mer oligonucleotide (5'-GACTCTTTCGGGCAGGCCCCTCCAGGTTCC-GG-3') has detected the 2.7 kb mRNA for the insulin-sensitive glucose transporter, GLUT4 (rat/mouse), within 20 min of exposure using 10 µg total RNA from those tissues in which glucose transport is stimulated by insulin (skeletal muscle, heart, brown fat, white fat), but not in other tissues (Rayner *et al.* 1994).

DIG-labelled oligonucleotides, coupled with a chemiluminescence substrate, can provide a rapid, simplified, non-radioactive approach to detecting an mRNA on Northern blots. This approach may be particularly useful to laboratories requiring accessible procedures for examining nutrient effects on gene expression.

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Non-enzymic glycosylation of haemoglobin in genetically obese (*ob/ob*), heterozygous (*ob/+*) and normal mice. By J.L. MORTON[1], D.A. PEIRIS[1], D. GUNASEKERA[1] and B. WINTON[2], 1 Department of Life Sciences, University of Buckingham, Buckingham MK18 1EG and 2 Department of Chemical Pathology, Milton Keynes Hospital, Milton Keynes MK6 5LD

The non-enzymic glycosylation of tissue proteins brought about by raised blood glucose concentrations has been implicated in the ageing process and the development of diabetic complications. This is thought to be due to the accumulation of advanced glycosylation end-products (AGE) which interfere with normal protein function and turnover. Blood haemoglobin is a convenient indicator of glycosylation status, as glycosylated haemoglobin (GlyHb) can be readily measured.

The genetically obese (*ob/ob*) mouse is hyperphagic and hyperglycaemic. The present study was undertaken to determine whether heterozygous animals possessing only one *ob* gene exhibit an increased susceptibility to protein glycosylation. If this was the case then the *ob/+* mouse could be a valuable model for glucose-induced protein damage in the absence of other diabetic and obesity induced abnormalities.

GlyHb, fasting blood glucose and body-weight were recorded at 8, 12 and 20 weeks in groups of obese, heterozygous and normal lean mice (Table). The heterozygous mice ("littermates") were derived from *ob/+* x *ob/+* crossing. After removal of *ob/ob* animals the remainder of the litter was *ob/+*:+/+(2:1). Ten animals were chosen at random from this population. There was a 0.92 probability that at least half of these animals were *ob/+*. GlyHb was measured by affinity chromatography using immobilized boronic acid, with displacement and elution of GlyHb by sorbitol.

The weight, fasting plasma glucose concentration or concentration of GlyHb in the littermates group did not differ significantly from the lean animals, although there is a suggestion that a steady-state proportion of GlyHb (approximately 1.5%) was reached at an earlier age in the littermates group. The obese animals grew rapidly, but they were not significantly hyperglycaemic in the fasted state at 20 weeks. The concentration of GlyHb was, however, significantly increased at 20 weeks.

The results indicate that mice with a single *ob* gene do not definitely show an increased blood GlyHb, although a steady state may be reached earlier than in lean animals. In obese mice the blood GlyHb shows a significant increase in the absence of a concomitant rise in plasma glucose, showing its value as an indicator of impaired glucose tolerance. We conclude that the heterozygous *ob/+* mouse is not likely to be a valid model for increased protein glycosylation.

Group of animals	Age (weeks)	n	Weight (g)	Fasting glucose (mmol/l)	GlyHb (%)
			Mean SE	Mean SE	Mean SE
Lean	8	10	26.5 1.3	7.60 0.24	1.05 0.08
	12	10	33.9 1.2	6.66 0.18	1.47 0.07
	20	10	37.4 1.5[a]	7.76 0.22	1.52 0.25
Littermates	8	10	27.6 1.5	6.57 0.24	1.58 0.27
	12	10	35.0 1.5[ab]	6.32 0.45	1.41 0.11
	20	10	37.6 1.7[ab]	7.57 0.32	1.58 0.30
Obese	8	9	47.8 1.7[c]	7.85 0.44	1.57 0.15
	12	8	68.6 2.8[abc]	7.59 0.60	1.68 0.13
	20	8	93.4 3.6[abc]	11.25 0.42	3.82 0.25[abc]

[a] Significantly different from value at 8 weeks, $P<0.05$, Tukey multiple comparison test. [b] Significantly different from value at previous time period, $P<0.05$, Tukey multiple comparison test. [c] Significantly different from value of lean animals at the same age, $P<0.05$, Tukey multiple comparison test.

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Determination of the effect of dietary polyunsaturated fatty acid on the content of triacylglycerol molecular species in pig backfat. By C.O. LESKANICH[1], R.C. NOBLE[1] and C.A. MORGAN[2], [1]*Department of Biochemical Sciences, SAC(Auchincruive), Ayr KA6 5HW and* [2]*Department of Genetics and Behavioural Sciences, SAC(Edinburgh), West Mains Road, Edinburgh, EH9 3JG*

Increases in the polyunsaturated fatty acid (PUFA) content of pig backfat have generally resulted in unacceptably soft fat. In particular, the level of linoleic acid (18:2*n*-6) has been inversely correlated with pig fat hardness (r -0.78; $P \leq 0.001$) with a resulting maximum backfat 18:2*n*-6 level of 15% total fatty acids being recommended (Whittington *et al.* 1986). However, in pigs fed on a soya-bean/fish oil diet this level has been far exceeded in the absence of noticeable fat softening (Morgan *et al.* 1992; Leskanich *et al.* 1993). As the physical properties of fat are not explained solely by a consideration of fatty acid composition, these findings could be explained by differences in the structural characteristics of the constituent triacylglycerols.

Twenty-four Large White x Landrace male pigs were randomly assigned to six groups and were given different levels and types of fat *ad lib* including tallow, soya-bean oil (SO) and fish oil (FO) for 6 weeks before slaughter. Diets contained the following (per kg diet): 50 g tallow (control); 25 g SO (SO1); 25 g SO plus 10 g FO (SOFO1); 50 g SO (SO2); 50 g SO plus 10 g FO (SOFO2) and 75 g SO (SO3). The major component of SO was 18:2*n*-6 present at 54.1 g/100 g fatty acids, whilst FO contained significant amounts of eicosapentaenoic acid (20:5*n*-3) and docosahexaenoic acid (22:6*n*-3). After slaughter, the distribution of molecular species of triacylglycerols in the outer backfat was measured by silver ion HPLC and GLC according to Christie (1988). Separation of intact triacylglycerols occurred on the basis of differences in unsaturation. A total of fourteen fractions were identified. The fatty acid composition of the adipose tissue was determined by GLC. Firmness of shoulder fat was determined by penetrometry. Thickness of backfat (P2) was measured. Statistical comparison was by one-way analysis of variance.

Neither the total lipid content of outer backfat nor P2 fat thickness was affected by dietary treatment ($P > 0.05$). Levels of 18:2*n*-6, 20:5*n*-3 and 22:6*n*-3 were affected by dietary treatment (all $P \leq 0.001$). Levels of long-chain PUFA were markedly increased in the FO groups. A significant effect of diet was observed in triacylglycerols with the following fatty acid configurations, where S is saturated, M is monoenoic, D is dienoic, T is trienoic, P is pentaenoic, H is hexaenoic fatty acids: SSS ($P \leq 0.006$), SSM, SMM, MMM, MMD+SDD, MDD, MMT+SDT+DDD, MDT (all $P \leq 0.001$), SMP ($P \leq 0.002$), MMP+SMH ($P \leq 0.007$) and MDH ($P \leq 0.01$) fractions. Comparison of triacylglycerol molecular species between SO1 and SOFO1 and between SO2 and SOFO2 revealed significant effects only in SMP, MMP+SMH and MDH fractions. The content of 18:2*n*-6 was not different between SO1 and SOFO1 groups or between SO2 and SOFO2 groups. However, SOFO1 and SOFO2 groups produced a more firm shoulder fat than SO1 and SO2, respectively ($P \leq 0.07$). The fat from SOFO1 was 23% firmer than that from SO1. Firmness of shoulder fat from SO1 and SO3 was below and that from SOFO1 and SOFO2 was above, the critical value assigned by Dransfield & Kempster (1988). The results indicate that presence of long-chain triacylglycerol species did not induce increased softness in shoulder fat but rather had a hardening effect.

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